Mol Biol Rep (2014) 41:4721–4731
DOI 10.1007/s11033-014-3343-y

Polymorphisms in twelve candidate genes are associated with growth, muscle lipid profile and meat quality traits in eleven European cattle breeds

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Received: 27 February 2013 / Accepted: 21 March 2014 / Published online: 10 April 2014

Abstract Current customers' demands focus on the nutritional and sensory quality of cattle meat. Candidate gene approach allows identification of genetic polymorphisms that have a measurable effect on traits of interest. The aim of this work is to identify new molecular markers for beef production through an association study using 27 candidate genes and 314 purebred bulls from 11 European cattle breeds. Twelve genes were found associated with different lipid and meat quality traits, and among these stand out the considerable effect of *CAST* on fatness score, *CGGBP1* on growth traits, *HSPB1* on the percentage of lauric acid (12:0) and phospholipid docosahexaenoic acid (DHA 22:6 n − 3), *RORA* on the ratio of light absorption (K) to light scattering (S) (K/S), and *TNFA* on lightness (L*). Most of these traits are related to post-mortem muscle biochemical changes, which are key factors controlling meat quality and consumers' acceptance. Also, the variations produced on muscle fatty acid profiles, such as those of *AANAT*, *CRH*, *CSN3*, *HSPB1*, and *TNFA*, give insights into the genetic networks controlling these complex traits and the possibility of future improvement of meat nutritional quality.

Keywords Beef cattle · Meat quality · Candidate genes · SNP · *Bos taurus*

The members of GemQual Consortium is listed in Appendix.

Electronic supplementary material The online version of this article (doi:10.1007/s11033-014-3343-y) contains supplementary material, which is available to authorized users.

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Introduction

Cattle meat provides several nutrients necessary for a balanced diet and for health preservation, especially high value proteins, minerals, B-complex vitamins and essential fatty acids, and also can have an important role as a dietary source of n − 3 fatty acids [1, 2]. Besides nutritional quality, consumers' preferences are related to specific attributes such as tenderness, taste and flavour. As current demands focus clearly in the improvement of product quality, in the near future breeders and industry will have to take this into account as a main production objective [3, 4]. Cattle breeding programmes have been very successful in increasing the quantity and efficiency of meat production, but up to now the efforts to improve meat quality through conventional genetic selection have yielded very limited results, mainly due to the relatively high costs and technical difficulties that measuring quality traits imply [5].

Meat quality improvement can be achieved by marker-assisted selection, a tool of very high potential to improve traits that usually have very slow or inexistent genetic progress with traditional phenotypic selection [3, 5, 6]. Thanks to the genomic revolution of the past few years,

Table 1 Phenotypes measured on 314 purebred bulls belonging to 11 cattle breeds

Variable	Description	Units	Data transformation[a]
Growth traits			
Wither high at arrival, 9, 12 and 15 months old	Measured from the highest point of the wither, between the shoulders	cm	Raw data
Pelvis width at arrival, 9, 12 and 15 months old	Measured at trochanters	cm	
Body length at arrival, 9, 12 and 15 months old	Measured from humerus-breastborne articulation to the ischium tuberisity	cm	
Close weight at 9, 12 and 15 month old	Animal weight	kg	
Mean ADG	Average daily gain	kg/day	
Final weight	Slaughter weight	kg	
Physical variables			
μ-calpain activity		U/g meat	Raw data
m-calpain activity		U/g meat	
Calpastatin activity		U/g meat	
Soluble collagen		%	
Total amount of collagen		mg/g meat	
COX	Cytochrome-c oxydase activity	υmol/min g of meat	$\sqrt{Y}$
CS	Citrate synthase activity	υmol/min g of meat	
ICDH	Isocitrate dehydrogenase activity	υmol/min g of meat	
LDH	Lactate dehydrogenase activity	υmol/min g of meat	
MHCI	Myosin heavy chain isoform I	%	
MHCIIA	Myosin heavy chain isoform IIA	%	
MHCIIX	Myosin heavy chain isoform IIX	%	
L*	Lightness: 100 represents white and 0 represents black		Raw data
a*	Positive a* is red. Negative a* is green. The a* axe has no specific numerical limit		
b*	Positive b* is yellow. Negative b* is blue. The b* axe has no specific numerical limit		
Hue angle (H*)	Physical colour. Hue is obtained from following mathematical equation: H = arctan(b*/a*)		
Chroma (C*)	Physical colour: saturation index. Intensity or saturation obtained from the mathematical equation $C* = \sqrt{(a*^2 + b*^2)}$		

Table 1 continued

Variable	Description	Units	Data transformation[a]
WB test: maximum load	Shear force made by the Instron machine with Warner–Bratzler apparatus, a blade that cuts the meat sample with perpendicular fibres. The instrument supply tissurometric tracing with a peak that indicates maximum value of force, necessary to break fibres	N	log(1 + Y)
WB test: toughness	Energy required for breaking down the sample obtained from the area of tissurometric tracing	N/cm	
WB test: maximum load on raw meat	Similar to maximum load but measured on raw meat and related with other textural values	N/cm	
WB test: toughness on raw meat	Similar to maximum load but measured on raw meat and related with other textural values	N/cm	
pH at 3, 24 h, 10 days and at thaw			
Drip loss		%	
Thaw loss	Water loss due to breach of the myofibrils by interstitial freezing water	%	
Cooking loss	Heating at temperatures around 75 °C	%	
Absorbance at wavelengths between 360 and 740 nm			
K/S	Ratio of light absorption (K) to light scattering (S)		
Compression test: maximum load	Force made by the Instron machine to completely compress the sample	N	
Compression test: stress at 20 %	Force made by Instron machine by surface area, when compression rate is 20 % of tota	N/cm^2	
Compression test: stress at 80 %	Force made by Instron machine by surface unit, when compression rate is 80 % of total	N/cm^2	
Sarcomere length	Distance between two consecutive Z lines in the muscular structure	μm	
MFI	Myofibrilar fragmentation index: controlled homogenation of muscle in aqueous solution		
Fatty acids			
Fatness score	Visual fatness cover estimated by UE standard. 1: very low—15: very high	1-15	Raw data
Individually	12:0; 14:0; 16:0; 16ald; 16:1; 18:0; 18ald; t18:1; 9c18:1; 11c18:1; 18:2n − 6; 20:1; 18:3n − 3; 9c11tCLA; 20:3n − 6; 20:4n − 6; 20:5n − 3; 22:4n − 6; 22:5n − 3; 22:6n − 3	mg/100 g muscle	log(1 + Y)
Individual neutral FA (FA_N)	N 12:0; N 14:0; N 16:0; N 16ald; N 16:1; N 18:0; N 18ald; N t18:1; N 9c18:1; N 11c18:1; N 18:2n − 6; N 20:1; N 18:3n − 3; N 9c11tCLA; N 20:3n − 6; N 20:4n − 6; N 20:5n − 3; N 22:4n − 6; N 22:5n − 3; N 22:6n − 3		
Individual phospholipids (FA_P)	P 12:0; P 14:0; P 16:0; P 16ald; P 16:1; P 18:0; P 18ald; P t18:1; P 9c18:1; P 11c18:1; P 18:2n − 6; P 20:1; P 18:3n − 3; P 9c11tCLA; P 20:3n − 6; P 20:4n − 6; P 20:5n − 3; P 22:4n − 6; P 22:5n − 3; P 22:6n − 3		
Grouped			
Saturated (SFA)	12:0 + 14:0 + 16:0 + 18:0		
Monounsaturated (MUFA)	16:1 + trans 18:1 + 9cis18:1 n − 9 + 11cis18:1 + 20:1 n − 9		
Polyunsaturated (PUFA)	18:2 + 18:3 + 20:3n − 6 + 20:4n − 6 + 20:5n − 3 + 22:4n − 6 + 22:5n−3 + 22:6n − 3		
n − 3 PUFA	18:3 + 20:5n − 3 + 22:5n − 3 + 22:6n − 3		
n − 6 PUFA	18:2 + 20:3n − 6 + 20:4n − 6 + 22:4n − 6		
Polyunsaturated/Saturated (P:S1)	(18:2 + 18:3)/(12:0 + 14:0 + 16:0 + 18:0)		
Polyunsaturated/Saturated (P:S2)	(18:2 + 18:3 + 20:3n − 6 + 20:4n − 6 + 20:5n − 3 + 22:4n − 6 + 22:5n − 3 + 22:6n − 3)/(12:0 + 14:0 + 16:0 + 18:0)		
n − 6/n − 3	(18:2 + 20:3n − 6 + 20:4n − 6 + 22:4n − 6)/18:3 + 20:5n − 3 + 22:5n − 3 + 22:6n − 3)		
18:2/18:3			

[a] Eventual phenotypic data transformation to give normal distributions assumed for the linear model

more information and technology are available that can be used with this aim. The full development of these technologies greatly depends on the precise identification of the genes and polymorphisms that have a measurable effect on muscle physiology and on meat quality. Within functional genomics, candidate gene strategy allows focusing the analysis on particular genes involved in key metabolic pathways or physiological processes which are probable to be involved in the traits of interest. Following this approach, in this paper we describe and discuss the association results obtained between 27 candidate genes involved in muscle metabolism and energy homeostasis and different performance measurements in muscle samples from one highly selected dairy breed and ten beef breeds that can be useful in future cattle breeding.

Materials and methods

Animals and feed system

A sample of 314 unrelated bulls belonging to 11 European cattle breeds was used: 26 Holstein, 30 Charolais, 31 Limousin, 18 Simmenthal, 30 Piedmontese, 30 Asturiana de los Valles, 31 Pirenaica, 29 Danish Red, 28 Marchigiana, 31 Asturiana de la Montaña, and 30 Avileña-Negra Ibérica.

Bulls were fed a total mixed ration containing barley and soy bean with appropriate minerals and vitamins. All ingredients were mixed into a form that prevented selection using molasses up to 3–5 % as a binding agent. Metabolisable energy of the ration was 12.5-kJ/kg and straw was available ad libitum to provide fibre. Bi-carbonate was added to the ration to prevent acidosis. This diet was designed to achieve the slaughter weight of 75 % of mature weight for each breed within a window of 13–17 months [7].

Phenotypic data

A comprehensive range of phenotypes were measured which fell into three categories detailed in Table 1: (1) Growth traits measured on the live animal until slaughter described in Albertí et al. [7]; (2) Physical variables including enzymes activity, drip loss, toughness, etc.; (3) Lipid traits in which fat was extracted as described by [8]. Total lipid content was taken as the sum of the neutral lipid and phospholipid fractions. Some additional phenotypes were set as are polyunsaturated fatty acids (PUFA), n − 6/n − 3 ratios and polyunsaturated/saturated (P:S) ratios.

SNP selection and genotyping

Twenty seven candidate genes previously described as being involved in muscle development, metabolism and structure were selected from the literature and 31 SNPs chosen from the GenBank® database (http://www.ncbi.nlm.nih.gov). Whenever possible, non-synonymous polymorphisms or those located in 5′ or 3′ untranslated regions (UTR) and exons were used to search for associations. Polymorphisms belong to one of the following categories:

Polymorphisms previously associated to different production or meat quality traits (12): calpain (*CAPN1*) ss77832259 [9, 10]; calpastatin (*CAST*) g.2959 G<A AF159246 [11]; corticotropin releasing hormone (*CRH*) g.22 C<G AF340152 [12]; kappa casein (*CSN3*) g.12947 G<A AY380229, g.13100 C<A AY380229, g.13120 A<G AY380229 [13]; fatty acid desaturase 1 (*FADS1*) ss63322537 (unpublished data); growth hormone receptor (*GHR*) 4962 g.T<A AM161140 [14]; β-lactoglobulin (*LGB*) g.5864 C<T Z48305 [15]; pro-opiomelanocortin (*POMC*) g.437del1 J00021 [16]; retinoic acid receptor-related orphan receptor C (*RORC*) g.3290 T<G DQ667048 [17]; thyroglobulin (*TG*) g.1696 C<T M35823 [18].

Polymorphisms from GenBank® database (18): arylalkylamine *N*-acetyltransferase (*AANAT*) ss62584155; ATP-binding cassette (*ABCA1*) ss28451692; acyl-CoA: cholesterol acyltransferase-2 (*ACAT2*) ss65658764; caveolin-3 (*CAV3*) ss62797050; CGG triplet repeat-binding protein 1 (*CGGBP1*) ss65141556, ss65141555; crystallin alpha B (*CRYAB*) ss62086225; DnaJ (Hsp40) homologue subfamily A member 1 (*DNAJA1*) ss65351307; fat-inducing transcript 2 (*FIT2*) ss61961642; homeobox containing 1 isoform 1 (*HMBOX1*) ss62392772, ss62392070; heat shock 27 kDa protein 1 (*HSPB1*) ss63015930; insulin induced gene 2 (*INSIG2*) ss62463931; ras-related associated with diabetes (*RAD*) ss62428567; RAR-related orphan receptor alpha (*RORA*) ss65549854; 40S ribosomal protein S28 (*RPS28*) ss38325285; selenoprotein T precursor (*SELT*) ss64953664; SWI/SNF-related matrix-associated actin-dependent regulador of chromatin subfamily A member 4 (*SMARCA4*) ss38322880.

Polymorphism inferred by GenBank sequence alignment (1): tumor necrosis factor alfa (*TNFA*) ss244244313.

Polymorphisms were genotyped through Multiplex-Capillary Primer Extension as described by Sevane et al. [19]. Table S1 shows the multiplex and Primer Extension primers and PCR conditions for those polymorphisms not previously recorded. Replication of SNP genotyping was performed in 5 % of the samples for repeatability purposes and Mendelian inheritance was checked in four trios (sire-dam-bull) for reliability.

Statistical analysis

Many phenotypic data had to be transformed to comply with normality conditions underlying the linear model, either by $\log(1 + Y)$ or $\sqrt{Y}$ transformation (Table 1).

SNPs with minor allele frequency (MAF) <0.05 were excluded from the association analysis to avoid bias of the data (Table S2). Linear regression analysis was then applied to test associations between genotypes and phenotypes using R programming (http://www.r-project.org) and the *lme4* statistical package, which fits linear models and generalized linear mixed models (GLMM) to data [20]. The main assumptions in this study were that the SNP effect on any of the traits is completely additive and there is no interaction between SNP genotype and breed (some preliminary analyses allowing interaction between breed and SNP effect were carried out, the results were unreliable as expected from the relatively small number of records within each breed, and thereafter no interaction between SNP genotype and breed was assumed).

The effect of the SNP on each of the traits was estimated by including them as a covariate into a linear model. The model used in this study was:

$$y = breed + farm\_season + g\alpha + e$$

where y is the trait in question, *breed* is the effect of breed, *farm_season* is the combined effect of farm and slaughter date, g is the SNP genotype, and α is the additive effect of the SNP. Traits were analyzed by groups: growth traits, physical variables, total lipids, phospholipids, and neutral lipids.

In order to correct for multiple testing in each group a permutation analysis was carried out to calculate the experiment-wise significance threshold within each trait (F Th) [21]. For each permutation, SNP genotypes were randomised across all animals. The effect of each SNP was then estimated and maximum F statistic across all SNP was used to calculate the distribution of the null hypothesis. A total of 10,000 permutations were used to calculate the null distribution from which the 5 % experiment-wise significance thresholds were inferred.

Gene pathway annotations

DAVID Bioinformatics Resources (http://david.abcc.ncifcrf.gov/), which consist of an integrated biological knowledgebase and analytic tools aimed at systematically extracting biological meaning from gene or protein lists, were used to analyze the associated cattle genes by enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms and to obtain a functional annotation table [22].

Results and discussion

Thirty-one polymorphisms belonging to 27 different genes were analyzed, and after elimination of 6 markers with MAF less than 0.05 (Table S2), 12 SNP located in 12 candidate genes were found associated with different growth, physical and lipid traits through linear regression analysis (Table 2). Significant associations (when F Reg > F Th) are shown as well as suggestive (when F Reg > 8), which should be further validated. Frequencies of the analysed SNPs per breed are shown in Table S2, and mean and standard deviation for the traits associated to different genes in Table S3. Table S4 shows the gene functional and pathway annotations currently available from DAVID Bioinformatics Resources for some of the associated genes. Only five genes are included in some biological pathway (*CAPN1*—Apoptosis, Alzheimer's disease–, *CAV3*—Focal adhesion–, *HSPB1*—MAPK signalling pathway, VEGF signalling pathway–, *POMC*—Melanogenesis, Adipocytokine signalling pathway–, *TG*—Autoimmune thyroid disease). Apart from these functional networks, the genes analyzed here play also roles in the biology of muscle and adipose tissue (Table 3 summarises published gene associations).

Although some of the effects were considerable (*AANAT* on phospholipid 14:0, *CAST* on fatness score, *CGGBP1* on growth traits, *HSPB1* on percentage of 12:0 and phospholipid 22:6 n − 3, *RORA* on K/S, and *TNFA* on L*), most of the associations found in this study had an overall low effect (Table 2), which may be explained by several reasons: (i) the polymorphism examined was not causative but in linkage disequilibrium with the associated trait; (ii) the traits are polygenic and the genes detected only explain a part of the total effect; (iii) the relatively few individuals within each population and the different breeds included in the analysis may miss some positive results. Hence, all these handicaps lower the success of this candidate gene approach and can explain the lack of some associations that may have been expected based on the literature; however, the results obtained here allow to apprehend the issues that should be addressed when starting this kind of association studies.

Genes affecting lipid profile in muscle

The results obtained for *HSPB1* gene point at a novel role of this gene on muscle fatty acid profile. The T allele of *HSPB1* SNP ss63015930 was associated with the increase of n − 6 PUFA, such as phospholipid linoleic acid (LA 18:2n − 6) or the ratios n − 6/n − 3 and linoleic acid/linolenic acid (18:2 n − 6/18:3 n − 3), whereas the C allele seems to increase the percentage of palmitoleic acid (16:1), lauric acid (12:0), phospholipid conjugated linoleic acid cis-9,trans-11 (CLA 9c18:1), and phospholipid DHA (22:6 n − 3). In particular, it is worth highlighting the effect of the CC genotype on 12:0 and 22:6 n − 3, which increases these lipids by 8.2 and 8 % respectively,

Table 2 Significant and suggestive associations between SNPs and different growth, physical variables and lipid traits

Locus symbol	GenBank dbSNP[a]	Trait associations[b]	Mean	Stand. Dev.	F Th[c]	Allele[d]	F Reg[e]	SE[f]	p value[g]	Effect[h]	Effect/s.d.[i]
AANAT	ss62584155 Intron 2	pH 3 h	6.422	0.319	10.046	T	11.501*	0.001	0.0008	0.004	0.013
		FA P W 14:0	1.263	1.000	10.701		9.711	0.008	0.002	0.027	0.027
		FA P % 14:0	0.252	0.161	10.958		8.914	0.003	0.0031	0.009	0.056
CAPN1	ss77832259	A 420 48 h	3.710	0.405	10.364	C	11.165*	0.003	0.0009	0.011	0.027
	Exon 9 (316aa)	K/S 440 48 h	8.754	1.691	10.509	G	10.045	0.007	0.0017	0.021	0.012
	Gly → Ala										
CAST	g.2959 G<A	Fatness score	6.404	2.208	10.306	A	8.063	0.108	0.0048	0.306	0.139
	3′UTR										
CAV3	ss62797050 3′UTR	pH 24 h	5.669	0.172	9.926	C	9.637	0.001	0.0021	0.002	0.012
		K/S 390 48 h	9.184	1.363	10.410		10.112	0.004	0.0016	0.014	0.010
		A 390 48 h	5.012	0.686	10.418	T	10.370	0.004	0.0014	0.012	0.017
CGGBP1	ss65141555	9 m pelvis width	43.807	4.162	10.347	A	10.737*	0.363	0.0012	1.188	0.285
	Exon 1 (69aa)	9 m wither height	114.895	7.289	10.349		8.432	0.608	0.0048	1.767	0.242
	Lys → Arg										
CRH	g.22 C<G	FA W 20:4 n − 6	40.140	9.711	10.692	C	10.803*	0.006	0.001	0.018	0.002
	Exon 2-NS (4aa)	FA P W 20:4 n − 6	39.431	9.406	10.633		9.009	0.006	0.003	0.017	0.002
	Pro → Arg										
CSN3	g.13100 C<A	FA N % 14:0	2.837	0.463	10.653	C	9.969	0.004	0.0018	0.014	0.030
	Exon 8 (148aa)										
	Asp → Ala										
HSPB1	ss63015930	pH thaw	5.576	0.089	9.886	T	17.627*	0.0004	0.00004	0.002	0.023
	5′ near	n − 6/n − 3	11.202	3.359	10.643		13.375*	0.009	0.0003	0.032	0.010
		18:2/18:3	23.063	8.230	10.624		9.792	0.010	0.0019	0.033	0.004
		FA P % 18:2 n − 6	25.332	4.978	10.641		11.294*	0.007	0.0009	0.024	0.005
		FA P W 18:2 n − 6	118.784	28.849	10.622		9.512	0.010	0.0022	0.030	0.001
		FA % 16:1	3.053	0.725	10.741	C	9.777	0.007	0.0019	0.022	0.030
		FA % 12:0	0.049	0.017	10.709		8.480	0.001	0.0039	0.002	0.118
		FA P % 9c18:1	15.466	4.214	10.596		8.834	0.010	0.0032	0.029	0.007
		FA P % 22:6 n − 3	0.178	0.088	10.680		8.190	0.003	0.0045	0.007	0.080
POMC	ss77832219	LDH	929.982	121.083	10.299	T	8.001	0.279	0.0053	0.624	0.005
	Exon 3-S	Cook loss 10d	25.775	2.239	9.711	C	8.524	0.005	0.0041	0.015	0.007

Table 2 continued

Locus symbol	GenBank dbSNP[a]	Trait associations[b]	Mean	Stand. Dev.	F Th[c]	Allele[d]	F Reg[e]	SE[f]	p value[g]	Effect[h]	Effect/s.d.[i]
RORA	ss65549854	% SUMFA	93.223	1.410	11.352	G	14.750*	0.001	0.0002	0.004	0.003
	Intron 4	N % SUMFA	93.341	1.644	11.186		9.697	0.001	0.0020	0.004	0.002
		MHCI	16.615	4.115	9.844		9.770	0.079	0.0019	0.061	0.015
		% RFA	6.777	1.410	10.991	A	12.132*	0.011	0.0006	0.038	0.027
		K/S 670 10d	0.715	0.255	10.323		10.048	0.035	0.0017	0.110	0.432
		K/S 740 10d	0.754	0.270	10.410		10.192	0.037	0.0016	0.118	0.438
TG	g.1696 C<T	Stress20 48 h	7.212	3.009	9.836	C	8.748	0.016	0.0038	0.047	0.016
	5′UTR										
TNFA	ss244244313	L 48 h	40.430	3.372	9.817	T	11.038*	0.282	0.0010	0.936	0.278
	Exon 7-S	A 450 48 h	6.871	1.389	10.241		10.583*	0.006	0.0013	0.02	0.014
		A 600 48 h	19.177	3.368	10.249		13.297*	0.007	0.0003	0.025	0.007
		K/S 450 48 h	6.612	1.538	10.466	C	11.091*	0.007	0.0010	0.024	0.016
		K/S 600 48 h	1.787	0.478	10.532		13.227*	0.007	0.0003	0.019	0.039
		n − 6/n − 3	11.202	3.359	10.643		8.896	0.007	0.0031	0.022	0.007
		n − 6 PUFA	220.123	68.209	10.727		8.667	0.008	0.0035	0.023	0.0003
		FA W 18:2 n − 6	165.793	57.650	10.465		9.232	0.009	0.0026	0.027	0.0005
		FA P % 18:2 n − 6	25.332	4.978	10.641		8.886	0.006	0.0031	0.018	0.004
		FA N % 18:2 n − 6	3.008	1.539	10.493		8.062	0.009	0.0048	0.024	0.016

S synonymous SNP

[a] SNP location or GenBank dbSNPs accession number

[b] *pH 3 h* pH at 3 h post mortem; *A* wavelength absorbance; *K/S* ratio of light absorption (K) to light scattering (S); *fatness score* visual fatness cover estimated by UE standard; *pH 24 h*: pH at 24 h post mortem; *9 m pelvis width* pelvis width measured at trochanters at 9 months (cm); *9 m wither height* height measured from the highest point of the wither, between the shoulders, at 9 months (cm); *pH thaw* pH on thawed samples at 10 days post mortem; *LDH* lactate dehydrogenase activity (vmol/min for g of muscle); *cook loss 10d* cook loss percentage at 10 days; *MHCI* myosin heavy chain isoform I (%); *stress20 48 h* compression test, force made by surface area when compression rate is 20 % of total at 48 h (N/cm^2); *L* physical colour measured as lightness at 48 h; *FA* fatty acid; *W* mg/100 g muscles; %: percentage regarding total FA (SUMFA + RFA); P phospholipid; *P* % percentage regarding total phospholipids; *N* neutral FA; *N* % percentage regarding total neutral lipids; *SUMFA* sum of all measured FA; *RFA* residual FA (non measured)

[c] Trait significant thresholds

[d] Allele positively correlated with the trait

[e] F regression statistics

[f] Standard error

[g] *p* value regression statistics

[h] SNP effect on the analyzed trait

[i] SNP effect on the analyzed trait/trait standard deviation

Table 3 Gene symbol and name, main biological functions, previously described trait associations and references of 12 candidate genes associated with different production traits

Gene symbol	Gene name	Biological process	Previous trait association	References
AANAT	Arylalkylamine N-acetyltransferase	Encodes the penultimate enzyme in the production of melatonin and reduces lipid peroxidation, hence protecting long chain PUFAs	Total fatty acid, omega-3 PUFA, fatty aldehyde, and collagen muscle content, flavour, and pH on thawed samples at 10 days post mortem in cattle	[29, 43, 44]
CAPN1	Calpain − 1	Responsible for protein breakdown in meat post-mortem. Implicated in the regulation of adipocyte differentiation and associated with high free fatty acid levels	Meat tenderness in cattle	[9, 10, 30, 31]
CAST	Calpastatin	Inhibitor of calpains	Meat tenderness, juiciness, water-holding capacity, colour, and fatty acid profile in cattle muscle	[11, 29, 45, 46]
CAV3	Caveolin 3	Implicated in the development of the T-tubule system in muscle	Unknown	[47]
CGGBP1	CGG triplet repeat-binding protein 1	Involved in cell cycle regulation and progression	Several carcass traits	[41, 42, 48]
CRH	Corticotrophin-releasing hormone	Iindirectly causes the release of glucocorticoids, which are purported to be growth inhibitors	Growth and carcass yield in beef cattle	[12, 49]
CSN3	Kappa-casein	Encodes a lactoprotein	Milk traits (fat and protein composition, coagulation properties, cheese performance), carcass traits (growth)	[13, 50]
HSPB1	Heat shock 27 kDa protein 1	Involved in stress resistance and actin organization	Tenderness in beef cattle	[23]
POMC	Pro-opiomelancortin	Its expression leads to increased synthesis of alpha melanocyte stimulating hormone (αMSH), which reduces appetite by bound to the melanocortin-4 receptor (MC4R)	Growth and carcass yield	[11, 12, 51]
RORA	RAR-related orphan receptor alpha	Plays an important role in the regulation of lipid homeostasis and the promotion of myogenesis in skeletal muscle	Its disruption has been associated with severe obesity in humans	[52, 53]
TG	Thyroglobulin	Glycoprotein hormone precursor of both triiodothyronine (T3) and tyroxin (T4), which play important roles in regulating lipid metabolism	Marbling in cattle	[18, 39]
TNFA	Tumor necrosis factor alpha	Involved in the regulation of immune cells, skeletal muscle atrophy signalling pathways and lipid metabolism, decreasing lipoprotein lipase (LPL) activity and increasing de novo fatty acid synthesis in liver	Unknown	[54, 55]

compared to TT homozygous. On the other hand and in agreement with previous data [23], this same SNP was found to be associated to pH on thawed samples at 10 days post mortem, which is related to microfibrillar breakdown and reflects the conservation properties of meat [24]. This effect can be explained by a lower stress resistance that diminishes glycogen stores and gives rise to an elevated pH [25].

The C allele of the synonymous *TNFA* SNP ss244244313 was found to increase the amount of LA (18:2 n − 6), the n − 6/n − 3 ratio and the total amount of n − 6 PUFA in muscle. This SNP was also associated with variations in meat colour traits: the T allele increases L*—the TT genotype accounting for 4.6 % of the observed variation compared to CC homozygous–, and absorbance at wavelengths between 450 and 600 nm both at 48 h, both of them related to paler meat [26]; on the other hand, the C allele increases the ratio of light absorption (K) to light scattering (S) (K/S) at the same wavelengths and time. The trait S is known to be influenced by pH (when pH falls, S increases) and is related to protein denaturation among other processes [27, 28]. Thus, an increase in the K/S ratio implies low protein denaturation and elevated pH, generating tougher meat. Therefore, the choice of the C allele results in more tender meat but also in the increase of n − 6 PUFA.

The *CRH* polymorphism analysed here is located in exon 2, alters residue 4 of the signal sequence (proline to arginine), and its G allele has been positively related to growth and carcass yield in beef cattle [12]. In the current study, a significant association was found between its C allele and the increase of arachidonic acid (AA 20:4 n − 6) in muscle. In the case of CSN3 locus, SNP g.13100 C<A results in an aspartic acid to alanine substitution at aa 148 [13] and was found associated with the increase of the percentage of neutral myristic acid (14:0) in muscle.

Also in this study previous results on *AANAT* and *CAST* were validated. The T allele of the *AANAT* SNP ss62584155 increases the percentage of phospholipid myristic acid 14:0 in muscle by 4.3 % for the TT genotype compared to CC homozygous, and pH at 3 h post-mortem. These results are in agreement with those of Dunner et al. [29], who found associations between another polymorphism in *AANAT* and muscle pH and overall fatty acid content. Regarding *CAST*, the A allele of polymorphism g.2959 G<A in 3′UTR, associated so far with tenderness [11], also seems to influence fatness score with an increase of 10 % for the AA genotype compared to GG homozygous. As discussed in Dunner et al. [29], calpains have been implicated in the regulation of adipocyte differentiation and associated with high free fatty acids levels [30, 31]. Thus *CAST*, which is a calpain inhibitor, may influence the amount of fatty acids through the regulation of calpain activity.

Genes affecting growth traits and physical variables

CAPN1, *CAV3*, *POMC*, *RORA*, and *TG* genes seem to exclusively influence physical traits, including light parameters, pH, the activity of the enzyme lactate dehydrogenase (LDH), cook loss or myofribrilar resistance at 20 % of compression rate, whereas CGGBP1 shows a considerable effect on growth traits.

SNP ss77832259 in *CAPN1* results in a glycine to alanine substitution at amino acid position 316 and its G allele has been associated with tougher meat in cattle [9, 10]. In accordance with these data, here the G allele was found associated with an increase in the ratio K/S at wavelength 440 nm. As explained in the previous section (see *TNFA*), higher scattering coefficients imply low protein denaturation and have been related to higher Warner–Bratzler shear force [32] and elevated pH, giving rise to tougher meat [33]. On the other hand, the C allele increases the absorbance at wavelength 420 nm, which results in the purplish-red or purplish-pink colour typically associated with vacuum packaged product and muscle immediately after cutting, and can be assigned to deoxymyoglobin absorption [33, 34]. In agreement with these data, Ribeca et al. [35] recently described the effect of another SNP in *CAPN1* (V530I) [9] on meat colour, although in this case the variation is on the yellow range.

The C allele of *CAV3* SNP ss62797050 was found to affect pH at 24 h post mortem and the K/S ratio at 390 nm, whereas the T allele increases the absorbance at wavelength 390 nm. All these traits are related to post-mortem muscle biochemical changes, specifically post-mortem pH, which is a key factor controlling meat quality [25, 33, 36].

The synonymous *POMC* SNP ss77832219, previously related to growth and carcass yield -being T the favourable allele—[11, 12], was associated with the activity of the enzyme LDH, which reflected the glycolitic potential of muscle fibres to catabolize glucose and to produce lactate, and with cook loss at 10 days. Given that glucose metabolism affects post-mortem maturation processes [37], these results are in agreement with Gill et al. [38] work, where associations between two polymorphisms in *POMC* and mechanical and taste panel assessed tenderness were reported.

RORA gene was found to influence muscle fatty acid profile -mainly through modifications in the neutral fraction-, the levels of myosin heavy chain isoform I (MHCI), which is related to the ability of the muscle to contract in a slow fashion and is more abundant in red muscles, and the K/S ratio at wavelengths between 670 and 740 nm at 10 days, such that the individuals with the AA genotype had greater scattering coefficients by 30 and 32 % respectively, compared to GG homozygous. These effects are consistent with the functions previously described for RORA (Table 3).

The T allele of the *TG* SNP g.1696 C<T was previously associated with marbling in cattle [18, 39]. In the present study, the C allele increases meat toughness at 48 h of maturation, related to myofribrilar resistance at 20 % of compression rate and explained by the fact that the reduction of marbling results also in the reduction of meat tenderness [40].

Finally, the non-synonymous *CGGBP1* SNP ss65141555, which caused the substitution of lysine to arginine at aa 69, was found associated with a considerable increase in pelvis width and wither height both at 9 months –the AA genotype accounting for 5.4 and 3 % of the observed variation, respectively, compared to GG homozygous. Singh et al. [41, 42] reported functions of *CGGBP1* in cell cycle regulation and progression which can explain its association with growth traits.

Conclusions

The candidate gene approach performed has revealed novel association for the genes *AANAT*, *CAPN1*, *CAST*, *CAV3*, *CGGBP1*, *CRH*, *CSN3*, *HSPB1*, *POMC*, *RORA*, *TG*, and *TNFA*, among which stand out the considerable effect (between 3 and 32 %) of *CAST* on fatness score, *CGGBP1*

on growth traits, *HSPB1* on the percentage of 12:0 and phospholipid 22:6 n − 3, *RORA* on K/S, and *TNFA* on L*. In particular, genes associated with meat colour and pH may contribute to the improvement of the appearance and other sensory traits that are key issues in consumers' decisions. Genes related to the complex architecture of muscle fatty acid profile may also be an aid in the design of a healthier product. All these data offer scientific community a starting point from which to study some complex gene-networks underlying economically important traits.

Acknowledgments This work was supported in part by an EC grant QLK5 – CT2000-0147.

Appendix

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