



Association of bovine meat quality traits with genes included in the *PPARG* and *PPARGC1A* networks

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ARTICLE INFO

Article history:

Received 13 July 2012

Received in revised form 8 February 2013

Accepted 19 February 2013

Keywords:

Candidate genes

Meat quality

SNP

Bos

ABSTRACT

Understanding which are the genetic variants underlying the nutritional and sensory properties of beef, enables improvement in meat quality. The aim of this study is to identify new molecular markers for meat quality through an association study using candidate genes included in the *PPARG* and *PPARGC1A* networks given their master role in coordinating metabolic adaptation in fat tissue, muscle and liver. Amongst the novel associations found in this study, selection of the positive marker variants of genes such as *BCL3*, *LPL*, *PPARG*, *SCAP*, and *SCD* will improve meat organoleptic characteristics and health by balancing the n–6 to n–3 fatty acid ratio in meat. Also previous results on *GDF8* and *DGAT1* were validated, and the novel *ATF4*, *HNF4A* and *PPARGC1A* associations, although slightly under the significance threshold, are consistent with their physiological roles. These data contribute insights into the complex gene-networks underlying economically important traits.

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1. Introduction

Many economically important traits in cattle production, such as those related to meat quality, defined by the nutritional and sensory properties of beef, are very complex, involve many genes and are greatly influenced by a variety of environmental factors (Hocquette et al., 2012). Being difficult and expensive to measure (Simm, Lambe, Bünger, Navajas, & Roehe, 2009), they are not usually included in selection programs based on phenotypic performance. However, the identification of molecular markers linked to economically important traits has evolved substantially in the last years and provides an alternative way to evaluate the genetic merit of livestock (Hocquette et al., 2010). Genomic Selection (GS) strategies focus on the incorporation of molecular information in breeding programs in order to directly select the beneficial genetic variants underlying those complex traits (Pimentel & König, 2012). However, GS will not likely be extended in the short term to beef cattle populations due to small population sizes and lack of high accuracy of estimated breeding values, so a candidate gene approach is currently useful to extend the panel of associated SNP and estimate better SNP effects in these breeds.

Apart from meat quality aspects such as tenderness, flavour, juiciness or colour, health concerns are of particular interest given the relationship found between incidence of lifestyle diseases and dietary intake of saturated fatty acids (SFA) and the ratio of n–6 to n–3 fatty acids, currently far from the recommended 1–4:1 (Scollan et al., 2006). Understanding the genetic variation underlying economically important traits will enable us to improve production efficiency and meat quality. For this purpose, we performed an association study between 26 single nucleotide polymorphisms (SNP) located in 20 candidate genes and different production traits measured in 314 muscle samples of individuals belonging to 11 European bovine breeds. Amongst the genes associated so far with production traits, we focussed on those related to energetic metabolism and specifically several genes linked to the peroxisome proliferator activated receptor γ (*PPARG*) and its coactivator the peroxysome proliferator-activated receptor- γ coactivator-1 α (*PPARGC1A*) networks, given their key role in coordinating metabolic adaptation in fat tissue, muscle and liver (Fig. 1).

2. Materials and methods

2.1. Animals

A total of 314 muscle samples from unrelated bulls belonging to 11 European cattle breeds and fed from weaning to adult weight on a similar diet were genotyped (Albertí et al., 2008). The panel of animals consisted of one highly selected dairy breed (n = 26 Holstein); eight

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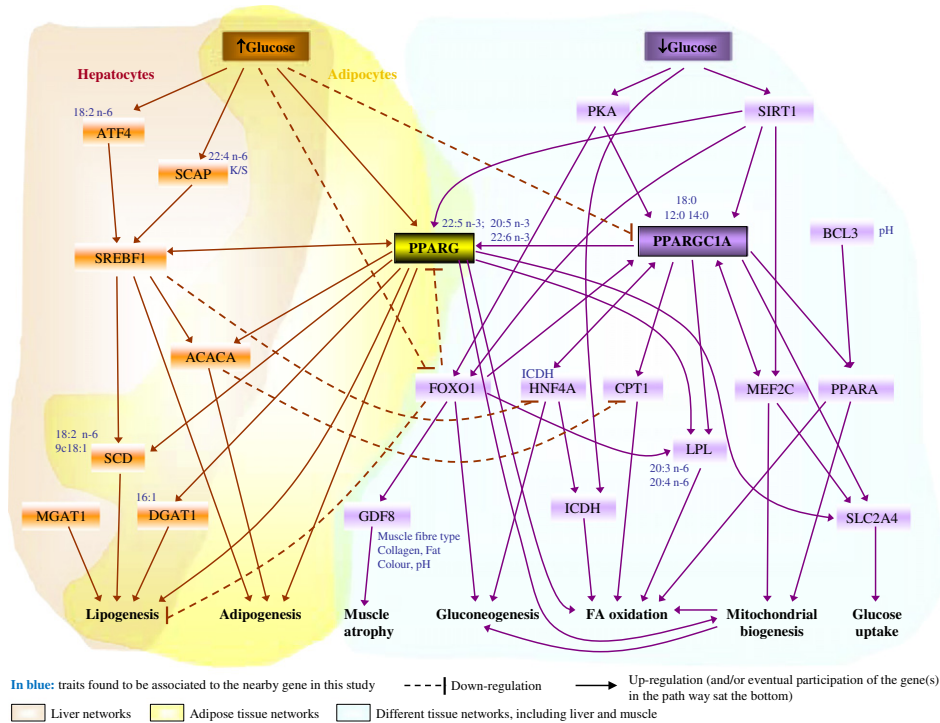


Fig. 1. Associations found in this study incorporated into the *PPARG-PPARGC1A* gene-network and energy metabolism. Apart from the master energy regulator *PPARG*, which function is tissue dependent, two categories of genes are included in the network whether they are up-regulated in the presence of glucose – *ATF4*, *SCAP*, *SREBF1*, *ACACA*, *SCD*, *MGAT1*, and *DGAT1* – enhancing lipogenic and adipogenic metabolic pathways; or up-regulated in the absence of glucose – *PKA*, *SIRT1*, *PPARGC1A*, *BCL3*, *FOXO1*, *HNF4A*, *CPT1*, *LPL*, *MEF2C*, *PPARA*, *SLC2A4*, and *GDF8* – increasing the availability of glucose through different metabolic processes, such as gluconeogenesis, fatty acid oxidation, mitochondrial biogenesis, glucose uptake or muscle atrophy. Interactions between these two main gene categories have also been described as these may drive the cell machinery towards glucose production (e.g., *FOXO1* is up-regulated in absence of glucose and, apart from inhibiting the lipogenic and adipogenic effect of *PPARG* in adipocytes and hepatocytes, also directly down-regulates the lipogenic pathway) or towards expenditure (e.g., *SREBF1* and *ACACA* genes are up-regulated in the presence of glucose and down-regulate *HNF4A* and *CPT1* respectively, diminishing indirectly gluconeogenesis and fatty acid oxidation).

beef breeds, some of them well distributed throughout the world (30 Charolais, 31 Limousin, and 18 Simmenthal and 30 Piedmontese) whilst others more locally used (30 Asturiana de los Valles, 31 Pirenaica, 29 Danish Red, 28 Marchigiana,); and two unimproved local breeds (31 Asturiana de la Montaña, and 30 Avileña-Negra Ibérica).

2.2. Phenotypic data

A comprehensive range of phenotypes were measured which fell into three categories: physical variables, lipid traits and sensory analysis (Table S1). Fat was extracted as described by Christensen et al. (2011). Total lipid content, was taken as the sum of the neutral lipid and phospholipid fractions. Some additional phenotypes were set as are PUFA, n6–n3 ratios, P:S ratios and antithrombotic potential (ATT), which is the ratio between the sum of the antithrombotic fatty acids, eicosatrienoic acid (C20:3n–6) and C20:5n–3, and the thrombotic fatty acid, C20:4n–6 ((C20:3 + C20:5)/C20:4) (Enser, Hallett, Hewitt, Furse, & Wood, 1996). Sensory panel tests assessed meat using a nine-point scale as described in Christensen et al. (2011). Briefly the criteria assessed were: flavour and abnormal flavour intensity, tenderness, and juiciness.

2.3. SNP selection and genotyping

Twenty-six SNPs located in 20 candidate genes known to be involved in beef quality were selected from the literature or the GenBank® database (<http://www.ncbi.nlm.nih.gov>). Whenever possible, non-synonymous polymorphisms or those located in 5' or 3' untranslated

regions (UTR) were chosen to search for causative mutations. Polymorphisms belong to one of the following categories and genes:

Polymorphisms from literature (8): diacylglycerol O-acyltransferase (*DGAT1*) ss77831745 (Grisart et al., 2002); myostatin (*GDF8*) ss77831865, ss77831863, ss77831864 (Grobet et al., 1997, 1998); peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PPARGC1A*) c.1892 + 19T>C, c.5314C>T, c.–920G>A (Weikard, Kühn, Goldammer, Freyer, & Schwerin, 2005); and stearoyl-CoA desaturase (*SCD*) g.10329T<C AY241932 (Taniguchi et al., 2004). Polymorphisms from GenBank® database (17): acetyl coenzyme A carboxylase α (*ACACA*) ss64381883; B-cell CLL/lymphoma 3 (*BCL3*) ss65392310; carnitine palmitoyltransferase-1 (*CPT1*) ss65363345; Dnaj (Hsp40) homologue subfamily A member 1 (*DNAJA1*) ss65351307; forkhead box O1 (*FOXO1*) ss65611802; solute carrier family 2 (facilitated glucose transporter) member 4 (*SLC2A4* or *GLUT4*) ss62538460; hepatocyte nuclear factor 4 α (*HNF4A*) ss61961144; lipoprotein lipase (*LPL*) ss65478732; myocyte enhancer factor 2C (*MEF2C*) ss65449641, ss38329156; protein Kinase cAMP-dependent regulatory tyrosine (*PKA*) ss62837667, ss62837580; peroxisome proliferator activated receptor α (*PPARA*) ss65362714; peroxisome proliferator activated receptor γ (*PPARG*) ss62850198; sirtuin 1 (*SIRT*) ss61550598; SREBP cleavage activating protein (*SCAP*) ss62839002; and sterol regulatory element binding transcription factor 1 (*SREBF1*) ss62543518. Polymorphism inferred from GenBank sequence alignments (1): activating transcription factor 4 or cyclic AMP response element-binding protein 2 (*ATF4* or *CREB2*) ss244244311.

Polymorphisms were genotyped with Multiplex-Capillary Primer Extension as described in Sevane, Crespo, Cañón, and Dunner (2011). Table S2 details 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 for reliability.

2.4. 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 S1). SNPs with minor allele frequency (MAF) less than 0.05 were excluded from the association analysis to avoid bias of the data (Table 1). 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 the linear models and generalized linear mixed models (GLMM) to data (Bates & Maechler, 2008). 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 therefore 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:

$$y = \text{breed} + \text{farm_season} + g\alpha + e$$

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: physical variables and sensory analysis group, 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 (Churchill & Doerge, 1994). For each permutation, SNP genotypes were randomized 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.

2.5. Gene pathways

Gene pathways were built (Fig. 1) using the association results of this study along with previously published gene functions and associations (Alaynick, 2008; Allen & Unterman, 2007; Bassel-Duby & Olson, 2006; Bionaz & Looor, 2008; Brennan, Michal, Ramsey, & Johnson, 2009; Corton & Brown-Borg, 2005; Erkens, Vandesompele, Van Zeveren, & Peelman, 2009; Finley & Haigis, 2009; Glass, 2005; Graugnard et al., 2009; Kamei et al., 2003; Kersten, 2001; Konno, Negishi, & Kodama, 2008; Kousteni, 2012; Lange et al., 2007; McAinch et al., 2003; Scarpulla, 2008; Seo et al., 2009; Soyay, Krempler, Oberkofler, & Patsch, 2006; Wang et al., 2010).

3. Results and discussion

We studied a specific network of genes related to energy metabolism and specifically to *PPARG* and *PPARGC1A* pathways, to find associations between 20 genes and traits influencing meat physical variables, lipid traits and organoleptic characteristics. Fig. 1 shows the network studied, where connections between genes are those

found according to the literature. This summary is not exhaustive, i.e. other genes not analyzed here are not included even if they are known to play a role in this pathway. After eliminating SNPs with MAF under 0.05 (Table 1), 19 polymorphisms belonging to 17 different genes were analysed and 10 SNP located in 10 candidate genes included in the energy metabolism network were found associated with different live, carcass and meat quality traits through linear regression analysis (Table 2). Significant as well as suggestive ($F \text{ Reg} > 8$) associations are shown. Frequencies of the analysed SNP per breed are shown in Table 1, and mean and standard deviation for the traits associated to different genes in Table S3.

There is a clear partition of the whole sample, formed by the different breeds, and this information was taken into account to avoid false positives by including the breed effect in the linear model used. In any case, the 11 different breeds and the relatively few individuals within each population, does possibly miss some positive results and lowers the success of this candidate gene approach, but allows a view on the issues that should be addressed when starting this kind of association studies.

The genes evaluated in this study are all connected to the energy metabolism and specifically to the *PPARG* and *PPARGC1A* networks. *PPARG* is a critical transcriptional regulator of genes controlling energetic metabolism, adipogenesis and maintenance of the differentiated state (Memisoglu et al., 2003; Rosen & MacDougald, 2006; Xu et al., 1999). Regarding energetic regulation, apparently contradictory functions have been described for this gene depending on the tissue where it is expressed (Fig. 1). Thus, whereas *PPARG* has a lipogenic and adipogenic effect in adipocytes and hepatocytes (Kersten, 2001), it promotes FA oxidation in the muscle, which eventually leads to decreased lipid availability (Lapsys et al., 2000). In the presence of glucose, *PPARG* activates genes such as *SREBF1* in the liver, and *ACACA*, *SCD* and *DGAT1* both in hepatocytes and adipocytes, all of them with a direct impact on lipogenesis/lipolysis balance, adipogenesis and gluconeogenesis. In contrast, when glucose levels are low, activation of *PPARG* in muscle through genes like *SIRT1* and *PPARGC1A* promotes the expression of *LPL* and *SLC2A4* and leads to increased FA oxidation, glucose uptake and mitochondrial biogenesis (Fig. 1). *PPARGC1A*, a coactivator of *PPARG*, has a key function in activating a variety of nuclear hormone receptors and transcription factors regulating energy homeostasis (Puigserver & Spiegelman, 2003). In particular, this gene has been shown to mediate the expression of genes involved in oxidative metabolism, adipogenesis, and gluconeogenesis, such as *HNF4A*, *CPT1*, *LPL*, *PPARA*, *MEF2C* or *PPARG* (Fig. 1). Consistent with these roles, different genotypes of *PPARG* seem to have important effects in physiological responses to dietary fat in humans (Memisoglu et al., 2003), and genetic variation in the human *PPARGC1A* gene were found to be associated with insulin resistance, susceptibility to type II diabetes, indicators for obesity, and altered lipid oxidation (Esterbauer et al., 2002; Hara et al., 2002; Muller, Bogardus, Pedersen, & Baier, 2003).

In the current study, allele A of SNP ss62850198 in the *PPARG* gene was found associated with a considerable increase of several omega-3 PUFA in the muscle: docosapentaenoic acid (DPA, 22:5n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), with increases of 9%, 15% and 18%, respectively for the AA genotype compared to GG homozygous. As omega-3 PUFA and their metabolites are natural ligands for *PPARG* (Edwards & O'Flaherty, 2008), the influence of *PPARG* on omega-3 levels is clearly consistent with its known physiological roles. Consistent with the current results, Oh, Lee, Lee, Chung, and Yeo (2011) found an exonic SNP of *PPARG* associated with both SFA and MUFA in Korean cattle. Many studies have reported the beneficial effects of omega-3 FA in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, cancer, and inflammatory, autoimmune and psychiatric disorders (Berquin et al., 2007; Calder, 2006; De Caterina, Madonna, Bertolotto, & Schmidt, 2007; De

Table 1

Twenty-six polymorphisms genotyped, dbSNP accession number or location, and allele frequencies per breed.

Locus symbol	GenBank dbSNP/Location ^a	Allele1/Allele2	Frequency of allele 1											Overall (314)
			HOL ^b (n = 26)	DR ^b (n = 29)	SM ^b (n = 18)	LIM ^b (n = 31)	CHA ^b (n = 30)	PIE ^b (n = 30)	MAR ^b (n = 28)	AST ^b (n = 30)	CAS ^b (n = 31)	AVI ^b (n = 30)	PI ^b (n = 31)	
ACACA	ss64381883	G/A	0.827	0.759	1	1	1	0.900	0.982	0.983	1	0.967	0.984	0.946
ATF4	ss244244311	G/T	0.167	0	0	0.139	0.023	0.420	0	0	0	0	0.750	0.161
BCL3	ss65392310	T/C	0.154	0.035	0.278	0.016	0.067	0.067	0	0.017	0	0	0.194	0.067
CPT1 ^{*c}	ss65363345	G/C	1	0.983	1	0.983	0.983	1	0.981	1	1	0.914	0.977	0.983
DGAT1	ss77831745	A/G	0.442	0.121	0.094	0.097	0.100	0.017	0.463	0.328	0.250	0.267	0.333	0.228
FOXO1 [*]	ss65611802	T/C	0	0	0	0	0	0	0	0.020	0.113	0.093	0.048	0.026
GDF8 _{del11}	ss77831865	G/del	1	1	1	1	1	1	1	0.317	0.984	1	0.887	0.921
GDF8 _{F94L}	ss77831863	C/A	1	1	1	0.016	0.983	1	1	1	1	1	0.694	0.869
GDF8 _{Q204X[*]}	ss77831864	C/T	1	1	1	0.984	0.833	1	1	1	1	1	1	0.973
SLC2A4	ss62538460	G/A	0.827	0.793	1	1	1	0.900	0.982	0.983	1	0.967	0.984	0.949
HNF4A	ss61961144	T/C	0.039	0.017	0.083	0	0	0.267	0.071	0.138	0.210	0.035	0.161	0.095
LPL	ss65478732	T/C	0.096	0.052	0.056	0	0.100	0.050	0	0	0.083	0.086	0.016	0.048
MEF2C [*]	ss65449641	G/T	1	1	1	1	1	1	1	1	1	1	1	1
MEF2C	ss38329156	G/T	0.423	0.397	1	0.710	0.650	0.600	0.500	0.648	0.677	0.638	0.694	0.621
MGAT1 [*]	ss65425229	T/C	1	1	1	1	1	1	0.929	0.983	0.968	1	1	0.989
PPARGC1A	c.1892 + 19T>C	A/G	0.173	0.121	0.028	0.032	0.167	0.183	0.304	0.276	0.371	0.383	0.016	0.192
PPARGC1A	c.5314C>T	T/C	0.154	0.224	0.667	0.194	0.083	0.300	0.054	0.120	0.016	0.069	0.113	0.164
PPARGC1A	c.—920G>A	G/A	0.077	0	0.028	0.050	0.017	0.183	0.071	0.021	0.167	0.250	0.117	0.092
PKA	ss62837667	T/C	0.135	0.138	0.083	0.387	0.190	0.517	0.196	0.304	0.516	0.357	0.355	0.301
PKA	ss62837580	T/C	0.962	0.839	0.917	0.694	0.828	0.483	0.804	0.625	0.500	0.828	0.661	0.730
PPARA [*]	ss65362714	C/T	1	1	1	1	1	1	1	1	1	1	1	1
PPARG	ss62850198	G/A	0.885	0.810	0.861	0.823	0.750	0.883	0.893	0.900	0.897	0.944	0.839	0.861
SCAP	ss62839002	G/A	0.846	1	0.971	1	0.983	0.917	0.911	0.850	0.887	0.850	0.968	0.925
SCD	g.10329T<C	T/C	0.385	0.173	0.306	0.419	0.467	0.350	0.429	0.333	0.742	0.667	0.436	0.437
SIRT [*]	ss61550598	G/A	1	1	1	1	0.967	1	1	1	1	1	1	0.997
SREBF1	ss62543518	T/C	0.500	0.293	0.708	0.161	0.233	0.350	0.232	0.233	0.400	0.345	0.258	0.314

^a GenBank dbSNP accession numbers or location for the interrogated SNP.^b Complete breed names: Holstein (HOL), Danish Red (DR), Simmental (SM), Limousin (LIM), Charolais (CHA), Piedmontese (PIE), Marchigiana (MAR), Asturiana de los Valles (AST), Asturiana de la Montaña (CAS), Avileña-Negra Ibérica (AVI), Pirenaica (PI).^c Superscript (*): SNP with minor allele frequency (MAF) less than 0.05 excluded from the association analysis.

Table 2
Significant and suggestive associations between SNP and different traits.

Locus symbol	GenBank dbSNP ^a	Trait associations ^b	Mean	Stand. Dev.	F Th ^c	Allele ^d	F Reg ^e	SE	p-value	Effect	Effect/s.d.
<i>ATF4</i>	ss244244311	FA N % 18:2 n-6	3.006	1.533	10.493	G	8.205	0.014	0.005	0.042	0.027
<i>BCL3</i>	ss65392310	pH thaw	5.576	0.089	9.886	C	12.554*	0.001	0.0005	0.002	0.023
	Exon 3-S	pH 3 h	6.422	0.319	10.046		11.729*	0.002	0.0007	0.008	0.025
<i>DGAT1</i>	ss77831745	FA % 16:1	3.053	0.725	10.741	G	13.333*	0.005	0.0003	0.018	0.025
		FA N % 16:1	3.666	0.588	10.757		10.624	0.004	0.001	0.014	0.024
<i>GDF8</i>	ss77831865	L 10d	42.235	3.668	10.113	del 11	13.785*	0.721	0.0002	2.676	0.730
	3'UTR	L 48 h	40.430	3.372	9.817		12.533*	0.671	0.0005	2.377	0.705
	nt821(del11)	MHCIIIX	42.107	12.837	9.956		9.351	0.202	0.002	0.383	0.030
		A ₆₁₀ 48 h	23.590	3.571	10.305		10.321*	0.737	0.0015	2.368	0.663
		A ₆₇₀ 48 h	30.590	4.252	10.481		11.175*	0.809	0.0009	2.705	0.636
		A ₆₇₀ 10d	33.272	5.520	10.103		10.685*	1.029	0.0012	3.363	0.609
		Collagen total	3.398	0.711	10.034	G	19.778*	0.012	0.00001	0.055	0.077
		MHCI	16.615	4.115	9.844		13.666*	0.108	0.0003	0.161	0.039
		pH thaw	5.576	0.089	9.886		9.608	0.001	0.002	0.003	0.034
		K/S ₆₁₀ 10d	1.132	0.349	10.445		13.974*	0.068	0.0002	0.255	0.732
		K/S ₆₇₀ 10d	0.715	0.255	10.323		11.820*	0.048	0.0007	0.166	0.652
<i>HNF4A</i>	ss61961144	ICDH	1.324	0.406	10.294	C	8.865	0.019	0.003	0.003	0.007
<i>LPL</i>	ss65478732	FA N W 20:3n-6	0.812	0.880	10.682	T	16.704*	0.018	0.00006	0.078	0.089
	Exon 2-S	FA N W 20:4n-6	0.898	0.712	10.518		9.371	0.023	0.006	0.073	0.103
<i>PPARGC1A</i>	c.5314C>T	FA % 18:0	15.095	1.937	10.674	T	9.861	0.006	0.002	0.018	0.009
	3'UTR	FA N % 12:0	0.066	0.018	10.640	C	9.332	0.001	0.006	0.003	0.167
		FA N % 14:0	2.837	0.463	10.653		8.113	0.006	0.005	0.016	0.035
<i>PPARG</i>	ss62850198	FA % 22:5n-3	0.544	0.337	10.641	A	13.499*	0.007	0.0003	0.025	0.074
	5'UTR	FA % 20:5n-3	0.233	0.188	10.815		11.013*	0.005	0.001	0.017	0.091
		FA % 22:6n-3	0.055	0.040	10.860		10.920*	0.001	0.001	0.005	0.124
<i>SCAP</i>	ss62839002	FA P % 22:4n-6	1.030	0.265	10.677	A	8.220	0.007	0.004	0.020	0.075
		K/S ₆₀₀ 10d	1.574	0.471	10.365		13.664*	0.051	0.0003	0.173	0.368
<i>SCD</i>	g.10329 T < C	FA P % 18:2n-6	25.332	4.978	10.641	T	11.516*	0.005	0.0008	0.018	0.004
	Exon 5-NS 293aa	FA % 18:2 n-6	10.142	6.129	10.733		9.575	0.012	0.002	0.036	0.006
	Ala → Val	FA N % 18:2 n-6	3.006	1.533	10.493		8.011	0.008	0.005	0.022	0.014
		FA % 9c18:1	29.076	6.039	10.684	C	8.023	0.006	0.005	0.018	0.003

^a GenBank dbSNPs accession number and SNP location. S: synonymous SNP; NS: non-synonymous SNP.

^b pH thaw: pH on thawed samples at 10 days post mortem; pH 3 h: pH at 3 h post mortem; L: physical colour measured as lightness at 10 days or 48 h; MHCIIIX: myosin heavy chain isoform IIX (%); A: wavelength absorbance; collagen total: total amount of collagen (mg/g meat); MHCI: myosin heavy chain isoform I (%); K/S: ratio of light absorption (K) to light scattering (S); ICDH: isocitrate dehydrogenase activity (μmol/min for g of muscle); FA: fatty acid; W: mg/100 g muscles; %: percentage regarding total FA; P: phospholipid; P %: percentage regarding total phospholipids; N: neutral FA; N %: percentage regarding total neutral lipids.

^c Trait significant thresholds.

^d Allele positively correlated with the trait.

^e F regression statistics. *: significant associations.

Caterina, Madonna, Zucchi, & La Rovere, 2003; Ross, Seguin, & Sieswerda, 2007; Simopoulos, 1999; Von Schacky, 2000), so selection of animals carrying AA or AG genotypes in the *PPARG* SNP ss62850198 may help to balance the n-6 to n-3 ratio and improve meat healthfulness. The *PPARGC1A* gene has been found to be responsible for variation in milk fat synthesis in cattle (Weikard et al., 2005), and oxidative energy metabolism in equine skeletal muscle during exercise (Eivers et al., 2012). In the present study, three polymorphisms previously published by Weikard et al. (2005) were analyzed (c.1892 + 19T>C, c.5314C>T, c.-920G>A). The intronic c.1892 + 19T>C polymorphism linked previously with a QTL for fat in milk was not associated with any trait included in this study. However, another *PPARGC1A* SNP, c.5314C>T, was associated with the amount of stearic acid (18:0), neutral lipid lauric acid (12:0), and neutral lipid myristic acid (14:0) in muscle.

Apart from the master energy regulators *PPARG* and *PPARGC1A*, two categories of genes are included in the network depending on their up-regulation in the presence – *ATF4*, *SCAP*, *SREBF1*, *ACACA*, *SCD*, *MGAT1*, and *DGAT1* – or the absence – *PKA*, *SIRT1*, *BCL3*, *FOXO1*, *HNF4A*, *CPT1*, *LPL*, *MEF2C*, *PPARA*, *SLC2A4*, and *GDF8* – of glucose (Fig. 1). Amongst them, *ATF4*, *SCAP*, *SCD*, *DGAT1*, *HNF4A*, *LPL*, *BCL3* and *GDF8* were found associated with different production traits (Table 2).

Activating transcription factor 4 (*ATF4*), also known as *CREB2*, is a bZIP class transcription factor and, amongst its large number of regulatory roles, a link between this gene and lipid metabolism has been reported (Seo et al., 2009; Wang et al., 2010). *ATF4*-deficient mice were used in both studies, revealing increases in lipolysis and decreases in expression of lipogenic genes, thus pointing at a role of *ATF4* in the

up-regulation of lipogenic genes such as *SREBF1*. In the current study, a SNP in the 5'UTR of the *ATF4* gene was found associated with the increase of neutral linoleic acid (LA, 18:2n-6) in the muscle. In parallel, *SREBP* cleavage activating protein (*SCAP*) neutralizes *SREBP* precursors, which controls the nutritional activation of lipogenic genes and suppresses expression of gluconeogenic genes through the competitive inhibition of *PPARGC1A* recruitment, a requirement for *HNF4A* activation (Fig. 1) (Yamamoto et al., 2004). Consistent with these functions, several polymorphisms in *SREBF1* were previously associated with meat FA composition in cattle (Hoashi et al., 2007) and intramuscular fat (Chen et al., 2008) and leg weight (Renaville et al., 2010) in pigs, and SNPs in *SCAP* were correlated with lean percentage, back-fat thickness, fat colour and salting losses in pigs (Renaville et al., 2010). Although one SNP in *SREBF1* was included in the current analysis, no association was detected with any trait. However, the SNP located in *SCAP* was associated with the amount of the phospholipid fraction of adipic acid (22:4n-6) and the ratio of light absorption (K) to light scattering (S) (K/S) at 600 nm at 10 days post mortem, such that the individuals with the AA genotype had greater trait values by 4% and 22% respectively, compared to GG homozygous. The trait S is known to be influenced by pH (when pH falls, S increases) and is related to protein denaturation amongst other processes (Kubelka & Mink, 1995; Swatland, 2004). Thus, an increase in the ratio K/S implies low protein denaturation and elevated pH, giving rise to tougher meat. pH ultimately depends on ATP availability, and connects *SCAP*'s role in the regulation of *SREBF1* and energetic metabolism with meat pH.

Further downstream in the pathway, *PPARG* over-expression has a direct influence on the activity of *SCD* and *DGAT1* when glucose intake

is increased (Fig. 1). Stearoyl-CoA desaturase (SCD) is the enzyme responsible for conversion of SFA into MUFA in mammalian adipocytes, either synthesized *de novo* or derived from diet (Ntambi, 1999). Moreover, SCD activity seems to be essential for lipogenic capacity and development of subcutaneous adipose tissue (Hausman et al., 2009), and it is regulated by *SREBF1* (Rahmouni & Sigmund, 2008). In this study, the SNP g.10329T<C described by Taniguchi et al. (2004), which causes a valine to alanine substitution in the fifth exon, was analysed. The C allele has previously been positively correlated with MUFA content and lower melting point in beef cattle (Taniguchi et al., 2004), with higher intramuscular fat content in bovine *M. longissimus* and *M. semimembranosus* (Reardon, Mullen, Sweeney, & Hamill, 2010), and with MUFA profile in milk (Kgwatalala, Ibeagha-Awemu, Mustafa, & Zhao, 2009). The results obtained here show the association of T allele with the increase of the amount of LA (18:2n–6) in muscle, related both to phospholipid and neutral lipids, whereas the C allele is associated with an increase in oleic acid (9c18:1n–9). The influence of this gene on the index C18:1/(C18:0 + C18:1), as well as on C14:1/(C14:0 + C14:1), has been recently reported by Baeza et al. (2012). Acyl-CoA:diacylglycerol acyltransferase 1 (*DGAT1*) utilizes diacylglycerol and fatty acyl-CoA as substrates in order to catalyze the final stage of triacylglycerol synthesis, and is known to affect fat content in milk (Grisart et al., 2002). The SNP ss77831745 analyzed here is the A → G polymorphism of the ApA to GpC dinucleotide substitution in exon 8 described by Grisart et al. (2002), causing a lysine to alanine substitution at aa 232 with documented effects on milk fat content and marbling (Grisart et al., 2002; Thaller et al., 2003). Also, Dunner, Sevane, García, Levéziel, and Williams (in press) recently described an effect of this SNP on beef flavour, and 16:1 and 12:0 muscle content in cattle. In agreement with these data, the G allele was found to be associated with the amount of palmitoleic acid (16:1) in the muscle in the current study.

The second category of genes of the *PPARG-PPARGC1A* network includes genes activated when glucose is decreased. Hepatocyte nuclear factor 4 α (*HNF4A*) is a highly conserved member of the nuclear receptor superfamily (Sladek, Zhong, Lai, & Darnell, 1990). Specifically, the *HNF4A/PPARGC1A* pathway plays a crucial role in the transcriptional regulation of hepatic gluconeogenic genes that are activated at fasting and inhibited by *SREBP1* in a fed state (Yamamoto et al., 2004). In the present study, the association analysis suggests that one SNP near *HNF4A* (ss61961144) influences the activity of the enzyme isocitrate dehydrogenase (ICDH), which is related to the oxidative potential of muscle fibres to catabolize FA (Beer et al., 2007).

Lipoprotein lipase (*LPL*) plays a key role in lipid metabolism by hydrolyzing triglyceride-rich particles, thereby generating free FA and glycerol for energy utilization and storage (Merkel, Eckel, & Goldberg, 2002). Several studies have reported associations of this gene with plasma lipid levels (Sagoo et al., 2008), and with milk fat content and dry weight in goat (Badaoui et al., 2007). Here, the T allele of the exonic SNP ss65478732 was found associated with an increase of both neutral dihomogamma-linolenic acid (DGLA, 20:3n–6) and arachidonic acid (AA, 20:4n–6) in muscle. The large effects of this SNP, for which the TT genotype increased the amount of DGLA in muscle by 16% and of arachidonic acid by 19% compared to CC homozygous, are consistent with its documented physiological role and previous associations.

B-cell CLL/lymphoma 3 (*BCL3*) is a transcriptional regulator of genes controlling energetic metabolism through the activation of diverse pathways, such as the coactivation of the nuclear receptors *ERR α* and *PPARA* synergistically with *PPARGC1A* (Yang, Williams, & Kelly, 2009). This energy-regulatory role of *BCL3* can explain the novel associations found in this study between the C allele of the synonymous SNP ss65392310 and the increase of pH at 3 h *post mortem*, as well as on thawed samples at 10 days (pH thaw). Both traits are ultimately related to *post mortem* ATP availability and influence juiciness (Braggins, 1996).

Forkhead box O1 (*FOXO1*) belongs to a protein subfamily that influences a variety of cellular functions, including energy metabolism through the regulation of master transcription factors such as *PPARG*

and *PPARGC1A* (Corton & Brown-Borg, 2005; Kousteni, 2012). *FOXO1* transcription factors also regulate the expression of myostatin (*GDF8*) and contribute to the control of muscle cell growth and differentiation (Allen & Unterman, 2007). Although no association was detected between the polymorphism in *FOXO1* and any trait in the current study, the 11-bp deletion in *GDF8* (nt821-del11) was found associated with several carcass and meat quality traits. Three polymorphisms in *GDF8* gene were genotyped, all of them previously associated with increased muscularity: an 11-bp deletion (nt821-del11) resulting in the truncation of the bioactive carboxyterminal domain of the protein (ss77831865); a transition C → T at bp 610 that yields a premature stop codon at amino acid position 204 (Q204X); and a conservative phenylalanine to leucine substitution at amino acid position 94 (F94L, C → A) (Grobet et al., 1998). Only nt821(del11) and F94L mutations had a MAF exceeding 0.05 (Table 1) and were included in the association analysis, and only the first one was found associated with different traits. In agreement with previous results (Gil et al., 2001), the nt821-del11 allele responsible for the double muscle phenotype was associated with colour parameters related to paler meat, increasing lightness (L^*), and absorbance at several wavelengths (Fig. S1), but especially between 670 and 700 nm – red spectrum –, all of them at 48 h and 10 days. Apart from these, nt821-del11 was associated with the increase in myosin heavy chain isoform IIX (MHCIIX), and the wild-type allele with an increase in collagen content, MHCI, pH on thawed samples at 10 days post mortem (pH thaw), and the ratio K/S at several wavelengths, and specially between 670 and 700 nm at 10 days (Fig. S1). The influence of this polymorphism on the muscle fibre profile is consistent with previous data (Gil et al., 2001) showing that the hypertrophic allele increases MHCIIX fibres.

4. Conclusions

The candidate gene approach performed has revealed a total of 42 associations involving 10 different genes, some of them suggesting new relationships between genes and meat quality traits. Most of these associations have an overall low effect probably due to the fact that the traits measured are influenced by multiple genes and the genes detected only account for a small amount of the total effect, and in addition, the SNP screened may not be causative mutations but in linkage disequilibrium with them. However, amongst the novel associations found in this study, it is worth highlighting the considerable effect of *PPARG* on the beneficial omega-3 PUFA DPA, EPA and DHA, and *LPL* on DGLA and AA. Also, the associations found here between the genes *ATF4*, *BCL3*, *HNF4A*, *PPARGC1A*, *SCAP*, and *SCD*, and meat organoleptic characteristics and lipid profile, despite having small effects, are described here for the first time and may bring insights into the complex gene-networks underlying economically important traits. Regarding *GDF8* and *DGAT1*, the results obtained confirm previously described associations. All these data offer scientific community a starting point from which to study some complex gene-networks underlying economically important traits.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.meatsci.2013.02.014>.

Acknowledgements

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

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