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

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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.

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-1α (*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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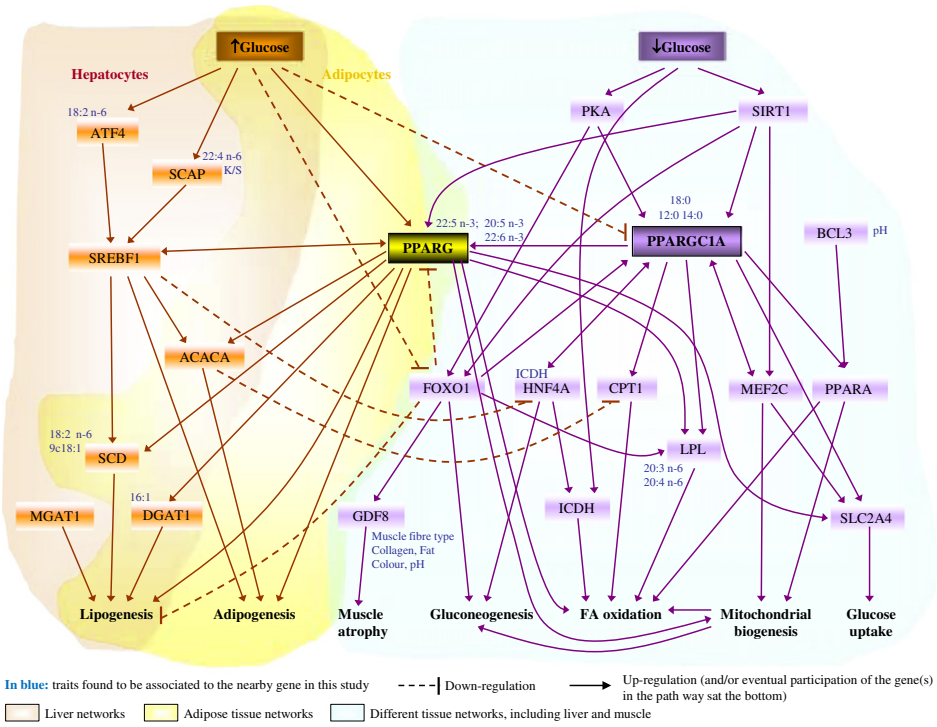

Fig. 1. Associations found in this study incorporated into the *PPARG-PPARGC1A* gene-network and energy metabolism. Apart from the master energy regulator *PPARG*, which function is tissue dependent, two categories of genes are included in the network whether they are up-regulated in the presence of 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 antithrombogenic fatty acids, eicosatrienoic acid (C20:3n−6) and C20:5n−3, and the thrombogenic fatty acid, C20:4n−6 ((C20:3 + C20:5)/C20:4) (Enser, Hallett, Hewitt, Fursey, & 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 typeII (*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 √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 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: 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 & Loor, 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; Soyal, 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 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											
			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)	Overall (314)
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.
ATF4	ss244244311	FA N % 18:2 n−6	3.006	1.533	10.493	G	8.205	0.014	0.005	0.042	0.027
BCL3	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
DGAT1	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
GDF8	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)	MHCIIX	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
HNF4A	ss61961144	ICDH	1.324	0.406	10.294	C	8.865	0.019	0.003	0.003	0.007
LPL	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
PPARGC1A	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
PPARG	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
SCAP	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
SCD	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; MHCIIX: 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 (υmol/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 adrenic 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 dihomo-gamma-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 *ERRα* 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.

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References

Alaynick, W. A. (2008). Nuclear receptors, mitochondria and lipid metabolism. *Mitochondrion*, *8*, 329–337.

Albertí, P., Panea, B., Sañudo, C., Olleta, J. L., Ripoll, G., Ertbjerg, P., et al. (2008). Live weight, body size and carcass characteristics of young bulls of fifteen European breeds. *Livestock Science*, *114*, 19–30.

Allen, D. L., & Unterman, T. G. (2007). Regulation of myostatin expression and myoblast differentiation by FoxO and SMAD transcription factors. *American Journal of Physiology. Cell Physiology*, *292*, C188–C199.

Badaoui, B., Serradilla, J. M., Tomás, A., Urrutia, B., Ares, J. L., Carrizosa, J., et al. (2007). Identification of two polymorphisms in the goat lipoprotein gene and their association with milk traits. *Journal of Dairy Science*, *90*, 3012–3017.

Baeza, M. C., Corva, P. M., Soria, L. A., Pavan, E., Rincon, G., & Medrano, J. F. (2012). Genetic variants in a lipid regulatory pathway as potential tools for improving the nutritional quality of grass-fed beef. *Animal Genetics*, *13*. http://dx.doi.org/10.1111/j.1365-2052.2012.02386.x.

Bassel-Duby, R., & Olson, E. N. (2006). Signaling pathways in skeletal muscle remodelling. *Annual Review of Biochemistry*, *75*, 19–37.

Bates, D., & Maechler, M. (2008). The comprehensive R archive network. http://cran.r-project.org

Beer, M., Rosebrough, R. W., Russell, B. A., Poch, S. M., Richards, M. P., & Coon, C. N. (2007). An examination of the role of feeding regimens in regulating metabolism during the broiler breeder grower period. 1. Hepatic lipid metabolism. *Poultry Science*, *86*, 1726–1738.

Berquin, I. M., Min, Y., Wu, R., Wu, J., Perry, D., Cline, J. M., et al. (2007). Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. *Journal of Clinical Investigation*, *117*, 1866–1875.

Bionaz, M., & Loor, J. J. (2008). Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics*, *31*, 366.

Braggins, T. J. (1996). Effect of stress-related changes in sheepmeat ultimate pH on cooked odor and flavour. *Journal of Agricultural and Food Chemistry*, *44*, 2352–2360.

Brennan, K. M., Michal, J. J., Ramsey, J. J., & Johnson, K. A. (2009). Body weight loss in beef cows: I. The effect of increased ^{2}-oxidation on messenger ribonucleic acid levels of uncoupling proteins two and three and peroxisome proliferator-activated receptor in skeletal muscle. *Journal of Animal Science*, *87*, 2860–2866.

Calder, P. C. (2006). Polyunsaturated fatty acids and inflammation. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *75*, 197–202.

Chen, J., Yang, X. J., Xia, D., Chen, J., Wegner, J., Jiang, Z., et al. (2008). Sterol regulatory element binding transcription factor 1 expression and genetic polymorphism significantly affect intramuscular fat deposition in the longissimus muscle of Erhualian and Sutai pigs. *Journal of Animal Science*, *86*, 57–63.

Christensen, M., Ertbjerg, P., Failla, S., Sañudo, C., Richardson, R. I., Nute, G. R., Olleta, J. L., Panea, B., Albertí, P., Juárez, M., Hocquette, J. F., & Williams, J. L. (2011). Relationship between collagen characteristics, lipid content and raw and cooked texture of meat from young bulls of fifteen European breeds. *Meat Science*, *87*, 61–65.

Churchill, G. A., & Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics*, *138*, 963–971.

Corton, J. C., & Brown-Borg, H. M. (2005). Peroxisome proliferator-activated receptor γ coactivator 1 in caloric restriction and other models of longevity. *Journal of Gerontology*, *12*, 1494–1509.

De Caterina, R., Madonna, R., Bertolotto, A., & Schmidt, E. B. (2007). n−3 fatty acids in the treatment of diabetic patients. Biological rationale and clinical data. *Diabetes Care*, *30*, 1012–1026.

De Caterina, R., Madonna, R., Zucchi, R., & La Rovere, M. T. (2003). Antiarrhythmic effects of omega-3 fatty acids: From epidemiology to bedside. *American Heart Journal*, *146*, 420–430.

Dunner, S., Sevane, N., García, D., Levéziel, H., Williams, J. L., Mangin, et al. (in press) Genes involved in muscle lipid composition in 15 European Bos taurus breeds. *Animal Genetics*.

Edwards, I. J., & O'Flaherty, J. T. (2008). Omega-3 fatty acids and PPARγ in cancer. *PPAR Research*, *2008*, 1–14.

Eivers, S. S., McGivney, B. A., Gu, J., Machugh, D. E., Katz, L. M., & Hill, E. W. (2012). PGC-1α encoded by the PPARGC1A gene regulates oxidative energy metabolism in equine skeletal muscle during exercise. *Animal Genetics*, *43*, 153–162.

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A. J., & Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science*, *42*, 443–456.

Erkens, T., Vandesompele, J., Van Zeveren, A., & Peelman, L. J. (2009). Correlation between porcine PPARGC1A mRNA expression and its downstream target genes in backfat and longissimus dorsi muscle. *Journal of Applied Genetics*, *50*, 361–369.

Esterbauer, H., Oberkofler, H., Linnemayr, V., Iglseder, B., Hedegger, M., Wolfsgruber, et al. (2002). Peroxisome proliferator-activated receptor-gamma coactivator-1 gene locus: Associations with obesity indices in middle-aged women. *Diabetes*, *51*, 1281–1286.

Finley, L. W. S., & Haigis, M. C. (2009). The coordination of nuclear and mitochondrial communication during aging and calorie restriction. *Ageing Research Reviews*, *8*, 173–188.

Gil, M., Serra, X., Gispert, M., Oliver, M. A., Sañudo, C., Panea, B., et al. (2001). The effect of breed-production systems on the myosin heavy Chain 1, the biochemical characteristics and the colour variables of Longissimus thoracis from seven Spanish beef cattle breeds. *Meat Science*, *58*, 181–188.

Glass, D. J. (2005). Skeletal muscle hypertrophy and atrophy signaling pathways. *The International Journal of Biochemistry & Cell Biology*, *37*, 1974–1984.

Graugnard, D. E., Piantoni, P., Bionaz, M., Berger, L. L., Faulkner, D. B., & Loor, J. J. (2009). Adipogenic and energy metabolism gene networks in longissimus lumborum during rapid post-weaning growth in Angus and Angus × Simmental cattle fed high-starch or low-starch diets. *BMC Genomics*, *31*, 10:142.

Grisart, B., Coppieters, W., Farnir, F., Karim, L., Ford, C., Berzi, P., et al. (2002). Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Research*, *12*, 222–231.

Grobet, L., Poncelet, D., Royo, L. J., Brouwers, B., Pirottin, D., Michaux, C., et al. (1998). Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mammalian Genome*, *9*, 210–213.

Grobet, L., Royo Martin, L. J., Poncelet, D., Pirottin, D., Brouwers, B., Riquet, J., et al. (1997). A deletion in the myostatin gene causes doublemuscling in cattle. *Nature Genetics*, *17*, 71–74.

Hara, K., Tobe, K., Okada, T., Kadowaki, H., Akanuma, Y., Ito, C., et al. (2002). A genetic variation in the PGC-1 gene could confer insulin resistance and susceptibility to Type II diabetes. *Diabetologia*, *45*, 740–743.

Hausman, G. J., Dodson, M. V., Ajuwon, K., Azain, M., Barnes, K. M., Guan, L. L., et al. (2009). The biology and regulation of preadipocytes and adipocytes in meat animals. *Journal of Animal Science*, *87*, 1218–1246.

Hoashi, S., Ashida, N., Ohsaki, H., Utsugi, T., Sasazaki, S., Taniguchi, M., et al. (2007). Genotype of bovine sterol regulatory element binding protein-1 (SREBP-1) is associated with fatty acid composition in Japanese Black cattle. *Mammalian Genome*, *18*, 880–886.

Hocquette, J. F., Botreau, R., Picard, B., Jacquet, A., Pethick, D. W., & Scollan, N. D. (2012). Opportunities for predicting and manipulating beef quality. *Meat Science*, *92*, 197–209.

Hocquette, J. F., Gondret, F., Baéza, E., Médale, F., Jurie, C., & Pethick, D. W. (2010). Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal*, *4*, 303–319.

Kamei, Y., Mizukami, J., Miura, S., Suzuki, M., Takahashi, N., Kawada, T., et al. (2003). A forkhead transcription factor FKHR up-regulates lipoprotein lipase expression in skeletal muscle. *FEBS Letters*, *536*, 232–236.

Kersten, S. (2001). Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Reports*, *2*, 282–286.

Kgwatalala, P. M., Ibeagha-Awemu, E. M., Mustafa, A. F., & Zhao, X. (2009). Influence of stearoyl-CoA desaturase (SCD) genotype and stage of lactation on desaturase indices and fatty acid composition of Canadian Jersey cows. *Journal of Dairy Science*, *92*, 1220–1228.

Konno, Y., Negishi, M., & Kodama, S. (2008). The roles of nuclear receptors CAR and PXR in hepatic energy metabolism. *Drug Metabolism and Pharmacokinetics*, *23*, 8–13.

Kousteni, S. (2012). FoxO1, the transcriptional chief of staff of energy metabolism. *Bone*, *50*, 437–443.

Kubelka, P., & Mink, K., referred by Swatland, H. J. (1995) On line evaluation of meat. *Technomic editor*, 192.

Lange, P., Moreno, M., Silvestri, E., Lombardi, A., Goglia, F., & Lanni, A. (2007). Fuel economy in food-deprived skeletal muscle: Signaling pathways and regulatory mechanisms. *The FASEB Journal*, *21*, 3431–3441.

Lapsys, N. M., Kriketos, A. D., Lim-Fraser, M., Poynten, A. M., Lowy, A., Furler, S., et al. (2000). Expression of genes involved in lipid metabolism correlate with peroxisome proliferator-activated receptor gamma expression in human skeletal muscle. *Journal of Clinical Endocrinology and Metabolism*, *85*, 4293–4297.

McAinch, A. J., Lee, J. S., Bruce, C. R., Tunstall, R. J., Hawley, J. A., & Cameron-Smith, D. (2003). Dietary regulation of fat oxidative gene expression in different skeletal muscle fiber types. *Obesity Research*, *11*, 1471–1479.

Memisoglu, A., Hu, F. B., Hankinson, S. E., Manson, J. E., De Vivo1, I., Willett, W. C., et al. (2003). Interaction between a peroxisome proliferator-activated receptor γ gene polymorphism and dietary fat intake in relation to body mass. *Human Molecular Genetics*, *12*, 2923–2929.

Merkel, M., Eckel, R. H., & Goldberg, I. J. (2002). Lipoprotein lipase: Genetics, lipid uptake, and regulation. *Journal of Lipid Research*, *43*, 1997–2006.

Muller, Y. L., Bogardus, C., Pedersen, O., & Baier, L. (2003). A Gly482Ser missense mutation in the peroxisome proliferator-activated receptor gamma coactivator-1 is associated with altered lipid oxidation and early insulin secretion in Pima Indians. *Diabetes*, *52*, 895–898.

Ntambi, J. M. (1999). Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *Journal of Lipid Research*, *40*, 1549–1558.

Oh, D., Lee, Y., Lee, C., Chung, E., & Yeo, J. (2011). Association of bovine fatty acid composition with missense nucleotide polymorphism in exon7 of peroxisome proliferator-activated receptor gamma gene. *Animal Genetics*. http://dx.doi.org/10.1111/j.1365-2052.2011.02288.x.

Pimentel, E. C., & König, S. (2012). Genomic selection for the improvement of meat quality in beef. *Journal of Animal Science*, *90*, 3418–3426.

Puigserver, P., & Spiegelman, B. M. (2003). Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): Transcriptional coactivator and metabolic regulator. *Endocrine Reviews*, *24*, 78–90.

Rahmouni, K., & Sigmund, C. D. (2008). Id3, E47, and SREBP-1c: Fat factors controlling adiponectin expression. *Circulation Research*, *103*, 565–567.

Reardon, W., Mullen, A. M., Sweeney, T., & Hamill, R. M. (2010). Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine *M. longissimus* and *M. semimembranosus*. *Meat Science*, *86*, 270–275.

Renaville, B., Piasentier, E., Fan, B., Vitale, M., Prandi, A., & Rothschild, M. F. (2010). Candidate gene markers involved in San Daniele ham quality. *Meat Science*, *85*, 441–445.

Rosen, E. D., & MacDougald, O. A. (2006). Adipocyte differentiation from the inside out. *Nature Reviews Molecular Cell Biology*, *7*, 885–896.

Ross, B. M., Seguin, J., & Sieswerda, L. E. (2007). Omega-3 fatty acids as treatments for mental illness: Which disorder and which fatty acid? *Lipids in Health and Disease*, *18*, 6–21.

Sagoo, G. S., Tatt, I., Salanti, G., Butterworth, A. S., Sarwar, N., Maarle, et al. (2008). Seven lipoprotein lipase gene polymorphisms, lipid fractions, and coronary disease: A HuGE association review and meta-analysis. *American Journal of Epidemiology*, *168*, 1233–1246.

Scarpulla, R. C. (2008). Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiological Reviews*, *88*, 611–638.

Scollan, N., Hocquette, J., Nuernberg, K., Dannenberger, D., Richardson, I., & Moloney, A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, *74*, 17–33.

Seo, J., Fortuno, E. S., III, Suh, J. M., Stenesen, D., Tang, W., Parks, E. J., et al. (2009). Atf4 regulates obesity, glucose homeostasis, and energy expenditure. *Diabetes*, *58*, 2565–2573.

Sevane, N., Crespo, I., Cañón, J., & Dunner, S. (2011). A primer extension assay for simultaneous use in cattle genotype assisted selection, parentage and traceability analysis. *Livestock Science*, *137*, 141–150.

Simm, G., Lambe, N., Bünger, L., Navajas, E., & Roehe, R. (2009). Use of meat quality information in breeding programmes. In J. P. Kerry, & D. Ledward (Eds.), *Improving the sensory and nutritional quality of fresh meat* (pp. 680). UK: Woodhead Publishing Ltd.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *American Journal of Clinical Nutrition*, *70*, 560S–569S.

Sladek, F. M., Zhong, W. M., Lai, E., & Darnell, J. E., Jr. (1990). Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes & Development*, *4*, 2353–2365.

Soyal, S., Krempler, F., Oberkofler, H., & Patsch, W. (2006). PGC-1α: a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes. *Diabetologia*, *49*, 1477–1488.

Swatland, H. J. (2004). Progress in understanding the paleness of meat with a low pH. *South African Journal of Animal Science*, *34*, S1–S7.

Taniguchi, M., Utsugi, T., Oyama, K., Mannen, H., Kobayashi, M., Tanabe, et al. (2004). Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. *Mammalian Genome*, *14*, 142–148.

Thaller, G., Kühn, C., Winter, A., Ewald, G., Bellmann, O., Wegner, J., et al. (2003). DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Animal Genetics*, *34*, 354–357.

Von Schacky, C. (2000). n − 3 fatty acids and the prevention of coronary atherosclerosis. *American Journal of Clinical Nutrition*, *71*, 224S–227S.

Wang, C., Huang, Z., Du, Y., Cheng, Y., Chen, S., & Guo, F. (2010). ATF4 regulates lipid metabolism and thermogenesis. *Cell Research*, *20*, 174–184.

Weikard, R., Kühn, C., Goldammer, T., Freyer, G., & Schwerin, M. (2005). The bovine PPARGC1A gene: Molecular characterization and association of an SNP with variation of milk fat synthesis. *Physiology Genomics*, *21*, 1–13.

Xu, H. E., Lambert, M. H., Montana, V. G., Parks, D. J., Blanchard, S. G., Brown, P. J., et al. (1999). Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Molecular Cell*, *3*, 397–403.

Yamamoto, T., Shimano, H., Nakagawa, Y., Ide, T., Yahagi, N., Matsuzaka, T., et al. (2004). SREBP-1 interacts with hepatocyte nuclear factor-4 alpha and interferes with PGC-1 recruitment to suppress hepatic gluconeogenic genes. *Journal of Biological Chemistry*, *279*, 12027–12035.

Yang, J., Williams, R. S., & Kelly, D. P. (2009). Bcl3 interacts cooperatively with peroxisome proliferator-activated receptor gamma (PPARgamma) coactivator 1alpha to coactivate nuclear receptors estrogen-related receptor alpha and PPARalpha. *Molecular and Cellular Biology*, *29*, 4091–4102.