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Genes involved in muscle lipid composition in 15 European *Bos taurus* breeds

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Summary

Consumers demand healthy and palatable meat, both factors being affected by fat composition. However, red meat has relatively high concentration of saturated fatty acids and low concentration of the beneficial polyunsaturated fatty acids. To select animals prone to produce particular fat types, it is necessary to identify the genes influencing muscle lipid composition. This paper describes an association study in which a large panel of candidate genes involved in adipogenesis, lipid metabolism and energy homoeostasis was tested for effects on fat composition in 15 European cattle breeds. Sixteen genes were found to have significant effects on different lipid traits, and among these, *CFL1* and *MYOZ1* were found to have large effects on the ratio of 18:2/18:3, *CRI1* on the amount of neutral adrenic acid (22:4 n-6), *MMP1* on docosahexaenoic acid (22:6 n-3) and conjugated linoleic acid, *PLTP* on the ratio of n-6:n-3 and *IGF2R* on flavour. Several genes – *ALDH2*, *CHRNE*, *CRHR2*, *DGAT1*, *IGFBP3*, *NEB*, *SOCS2*, *SUSP1*, *TCF12* and *FOXO1* – also were found to be associated with both lipid and organoleptic traits although with smaller effect. The results presented here help in understanding the genetic and biochemical background underlying variations in fatty acid composition and flavour in beef.

Keywords beef cattle, candidate genes, fatty acid profile, genotype-assisted selection.

Introduction

The level of intramuscular fat content and fatty acid (FA) composition is among the main factors determining meat palatability and consumers satisfaction (Lee *et al.* 2007). Muscle lipid characteristics which determine meat flavour, lipid oxidation and contribute to beef colour, can be responsible for abnormal odours and influence the juiciness and tenderness of meat (Bernard *et al.* 2007). Throughout the last decades, consumer concerns for dietary health has increased the desire for meat with a higher ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) (P:S ratio) and in particular with a high n-6:n-3 PUFA content (Simopoulos 1999). Indeed, SFA are implicated in the formation of blood clots leading to heart attacks (Enser *et al.* 2000), while omega-3 fatty acids have been reported to have beneficial effects in the prevention and treatment for a large number of diseases (Simopoulos 1999; De Caterina *et al.* 2007). However, meat from ruminants tends to have high levels of SFA in contrast to non-ruminants. Also in cattle, rumen fat biohydrogenation makes it difficult to alter FA profiles of muscle through changes in the diet (Hausman *et al.* 2009). FA composition has been shown to differ between breeds of cattle (Zembayashi *et al.* 1995), which suggests that, at least to some extent, fat metabolism is under genetic control and that FA profiles could be changed by genetic selection.

The complex and multigenic nature of traits related to meat quality, and the high cost of measuring these traits, most of which can only be evaluated post-mortem, make application of traditional selection methods, as well as the state-of-the-art genomic selection, difficult (Luan *et al.* 2009). An alternative approach is to identify genes with an effect on fat composition and include these in selection objectives.

The aim of this research was to test 389 single nucleotide polymorphisms (SNPs) located in 206 candi-

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date genes involved in adipogenesis, lipid metabolism and energy homoeostasis (Williams *et al.* 2009) for effects on muscle lipid composition in 15 European cattle breeds.

Material and methods

Animals

We used a sample of 436 largely unrelated purebred bulls belonging to 15 breeds: 31 Jersey, 27 South Devon, 30 Aberdeen Angus, 29 Highland, 29 Holstein, 29 Danish Red, 20 Simmental, 30 Asturiana de los Valles, 31 Asturiana de la Montaña, 30 Avileña-Negra Ibérica, 31 Pirenaica, 30 Piedmontese, 28 Marchigiana, 31 Limousin and 30 Charolais. The bulls were fed from weaning to adult weight *ad libitum* on the same diet (barley 82%, soybean 8% and straw 7.5% with appropriate minerals and vitamins and energy of 12.5 kJ/kg dry matter; for details see Albertí *et al.* 2008). A uniform beef management system representative of those used in European Union countries was used for all breeds to homogenise as far as possible the influence of management and rearing systems on meat quality. The animals were slaughtered at 15 months of age, and carcases were treated under similar conditions (Albertí *et al.* 2008).

SNPs in candidate genes and phenotypes

Selection of candidate genes and identification of the SNPs are described by Williams *et al.* (2009). The association analysis was performed using 389 SNPs with minor allele frequencies above 10% in the breeds investigated (Williams *et al.* 2009). These SNPs were genotyped across the 436 bulls.

The phenotypes measured are listed in Table S1. Fat was extracted as described by Christensen *et al.* (2011). Sensory panel tests assessed meat using a nine-point scale as described by Christensen *et al.* (2011). Briefly, the criteria assessed were flavour and abnormal flavour intensity, tenderness and juiciness.

Total lipid content was taken as the sum of the neutral lipid (FA_N) and phospholipid (FA_P) fractions. Some additional phenotypes were set, such as PUFA, n6–n3 ratios, P/S ratio and antithrombotic potential, 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 *et al.* 1996). Other individual traits were grouped as phenotypic groups according to their possible link: all FA (a grouping of all the FA of the lipid profile), the flavour group (all FA, all FA ratios and flavour) and the test panel (all sensory analysis: tenderness, juiciness, beef flavour intensity, abnormal flavour intensity, texture and overall appraisal).

Statistical analysis

A linear model was used to account for the population substructure of the data

$$Y_{i,j} = m_i + a_i G_{i,j} + \varepsilon_{i,j}^{(0)}$$

where $Y_{i,j}$ is the phenotype of individual j in breed i, m_i is the phenotypic mean for breed i, a_i is the additive effect of marker in breed i, and $G_{i,j}$ takes different values according to the genotype of each individual (1 for genotype AA, 0 for Aa and –1 for aa). $\varepsilon_{i,j}^{(0)}$ are independent and identically distributed normal residuals, also independent of $G_{i,j}$. The effects of country, slaughterhouse and day of slaughter are confounded with the breed effect.

Log transformations [Y' = ln(1+Y)] were applied to most of the FA. Multiple testing was accounted for using either a combination of the effective number of markers (Nyholt 2004) and the false discovery rate (see Benjamini & Yekutieli 2005) on the one hand, and on the other, resampling through permutations which were made on the genotypes of the individuals within breeds while keeping their phenotypes fixed, thus generating samples under the null hypothesis. The traditional Bonferroni α correction led to very small individual α values and consequently to a loss of power. For this reason, a multivariate linear analysis was also performed as an alternative to reduce the dimension of the problem, looking for associations between a marker and a phenotypic group, following the method of CPC (Composed Principle Components) (Mangin *et al.* 1998).

Haplotype association analysis was performed on those genes with two or more markers for which a strong association was found with particular traits. Because the pedigree information was insufficient to determine phases, the haplotypes and their frequencies were estimated with FAMHAP v1.6 software (Becker & Knapp 2004) (Table S2).

Gene pathway annotations

SNPPATH was used to analyse the cattle SNPs by enriched KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway terms (Wang *et al.* 2012).

Results

Associations are shown in Tables 1 and 2, and the allele indicated in the table is the one positively correlated with the trait. Allele effects were estimated for the associations detected with the linear univariate model. Some associations only appear when phenotypes are grouped (e.g. test panel which includes all sensory analysis: tenderness, juiciness, beef flavour intensity, abnormal flavour intensity, texture and overall appraisal), highlighting effects which were not seen when used individually. This is the case for most of them and possibly results from their small effect corrected for all SNPs analysed.

Table 1 Significant associations between SNPs and fatty acids, juiciness and flavour.

Locus Symbol	dbSNPs[1]	SNP location	Significant Trait Associations[2]	Mean[3]	SD[4]	*P*-value	Allele[5]	Effect[6]	Effect/SD[7]
ALDH2	ss77831990	Intron 8	Flavour group[8]			0.04			
			Test panel[9]			0.01			
CFL1	ss77831721	Exon 2-NS 47aa Ile→Thr	18:2/18:3	18.774	9.964	0.00005	T	−1.514	−0.152
CHRNE	ss77831830	Exon 4-S	All fatty acids			0.04			
CRHR2	ss77832125	Intron 4	All fatty acids			0.04			
CRI1	ss77832128	Exon 1-NS 47aa Ala→Ser	N 22:4 n-6	0.677	0.639	0.002	T	0.091	0.142
			All fatty acids			0.004			
			Flavour group			0.01			
DGAT1	ss77831744	3′UTR	All fatty acids			0.03			
			Flavour group			0.01			
	ss77831745	Exon 8-NS	12:0	1.978	1.815	0.009	G	0.072	0.040
		Dinucleotide substitution	16:1	85.998	68.882	0.006	G	0.131	0.002
			All fatty acids			0.0002			
			Flavour group			0.0003			
	ss77832137	232aa Lys→Ala	All fatty acids			0.005			
			Flavour group			0.001			
IGF2R	ss77831728	Intron 25	Test panel			0.01			
	ss77831887	Intron 55	Test panel			0.01			
			Flavour	3.608	0.493	0.0006	A	0.142	0.288
	ss77831883	Intron 55	Flavour	3.608	0.493	0.0009	A	−0.131	−0.266
			Test panel			0.004			
	ss77831884	Intron 55	Test panel			0.0007			
			Flavour	3.608	0.493	0.0003	C	−0.148	−0.300
	ss77831885	Intron 55	Test panel			0.001			
			Flavour	3.608	0.493	0.0002	G	0.160	0.325
IGFBP3	ss77832346	Intron 2	Test panel			0.0006			
MMP1	ss77831914	Intron 1	N 12:0	1.818	1.723	0.0008	C	0.051	0.030
			14:0	68.858	56.393	0 .001	C	0.226	0.004
			N 14:0	64.505	54.323	0.0009	C	0.098	0.002
			9c11Tcla	7.561	5.779	0 .0002	C	0.210	0.036
			N 9c11Tcla	6.560	5.430	0.0002	C	0.096	0.018
			N 20:1	3.047	2.786	0.002	C	0.061	0.022
			N t18:1	63.534	65.720	0.0002	C	0.109	0.002
			N 18:2 n-6	52.412	43.051	0.0004	C	0.082	0.002
			N 18:3 n-3	7.879	8.336	0.0008	C	0.072	0.009
	ss77831916	Intron 1	All fatty acids			0.01			
			Flavour group			0.02			
			9c11tCLA	7.561	5.779	0.00007	G	−0.250	−0.043
			12:0	1.978	1.815	0.001	G	−0.133	−0.073
			14:0	68.858	56.393	0.0005	G	−0.269	−0.005
	ss77831919	Intron 1	All fatty acids			0.03			
	ss77831921	Intron 1	All fatty acids			0.05			
	ss77831923	Intron 3	All fatty acids			0.04			
	ss77831924	3′UTR	16ald	23.277	6.880	0.005	C	−0.155	−0.023
			18ald	16.005	5.060	0.001	C	−0.171	−0.034
			22:6 n-3	0.892	0.567	0.0008	C	0.126	0.222
			All fatty acids			0.003			
			Flavour group			0.01			
MYOZ1	ss77831945	Intron 2	18:2/18:3	18.774	9.964	0.0002	C	−1.470	−0.148
NEB	ss77832090	Intron 149	All fatty acids			0.01			
			12:0	1.978	1.815	0.002	C	0.072	0.040
			16:0	611.473	451.855	0.007	C	0.104	0.00023
			16:1	85.998	68.882	0.003	C	0.126	0.002
			20:1	3.707	2.957	0.007	C	0.073	0.025
			9c11tCLA	7.561	5.779	0.0003	C	0.139	0.024
			9c18:1	797.417	611.702	0.003	C	0.126	0.00021
			18:2/18:3	18.774	9.964	0.001	C	−0.050	−0.005
PLTP	ss77832104	3′UTR	n-6:n-3	9.587	3.974	0.0001	A	−0.756	−0.190

Table 1 (continued)

Locus Symbol	dbSNPs[1]	SNP location	Significant Trait Associations[2]	Mean[3]	SD[4]	*P*-value	Allele[5]	Effect[6]	Effect/SD[7]
SOCS2	ss77832234	Exon 2-S	20:3n-6	8.911	2.452	0.0004	A	−0.071	−0.029
			P 20:3 n-6	8.277	1.996	0.00002	A	−0.070	−0.035
SUSP1	ss77831761	Exon 25-S	All fatty acids			0.01			
TCF12	ss77831958	Intron 11	All fatty acids			0.04			

[1]dbSNPs accession number.
[2]See Table S1.
[3]Trait mean.
[4]Trait standard deviations.
[5]Allele positively correlated with the trait.
[6]Allele effect.
[7]Effect measured in standard deviations.
[8]Flavour group includes all fatty acids, all fatty acid ratios and flavour.
[9]Test panel includes all sensory analysis: tenderness, juiciness, beef flavour intensity, abnormal flavour intensity, texture and overall appraisal.

Table 2 Significant associations between haplotypes and fatty acids and texture.

Locus symbol	dbSNPs (Allele1/Allele2)[1]	Haplotype ID and alleles	Significant Trait Associations[2]	*P*-value	Haplotype frequency
DGAT1	**ss77831744 T/C; ss77831745 A/G; ss77832137 A/C**	1-TAA	18ald	0.006	0.088
		2-CAA	18ald	0.001	0.140
		3-TGC	18:3 n-3	0.006	0.765
FOXO1	ss77831726 A/G; ss77831860 A/C; ss77831861 C/T; ss77831862 C/T; ss77831859 C/T	3-GCCCT	t18:1	0.0003	0.033
			18:2 n-6	0.02	
IGF2R	**ss77831728 G/A; ss77831887 G/A; ss77831883 G/A; ss77831884 T/C; ss77831885 A/G;** ss77831877 G/A; ss77831878 T/C; ss77831880 C/T; ss77831881 C/T; ss77831882 G/A; ss77831886 T/C	2-GAATAGTCCAC	Texture	0.008	0.495

[1]In bold, SNPs also found to be associated with some trait individually.
[2]See Table S1.

The association analysis performed on 15 European breeds revealed a total of 73 associations influencing muscle lipid composition and, hence, physical and sensory meat attributes among 389 tested SNP in 206 candidate genes. These associations involved 37 SNPs, either individually considered (Table 1) or included in one haplotype (Table 2), in 16 different genes. Among these, *CFL1* and *MYOZ1* were found to have large effects on the ratio of 18:2/18:3, *MMP1* on docosahexaenoic acid (22:6 n-3) and conjugated linoleic acid (CLA), *CRI1* on the amount of neutral adrenic acid (22:4 n-6), *PLTP* on the ratio of n-6:n-3 and *IGF2R* on flavour. *ALDH2*, *CHRNE*, *CRHR2*, *DGAT1*, *IGFBP3*, *NEB*, *SOCS2*, *SUSP1*, *TCF12* and *FOXO1* were newly found to be associated with both lipid and organoleptic traits. Mean and standard deviations for all the traits associated with different genes in the 15 breeds are given in Table S3-A. Table S4 shows the allele frequencies per breed of the 26 polymorphisms found to be individually associated with different traits, and Table S2 the haplotype frequencies of those genes with two or more markers for which a strong association was found. Table S5 shows the gene pathway annotations currently available for some of the associated genes. Only *MMP1* and *PLTP* shared a common biological pathway, whereas other genes are included in the PPAR signalling, insulin signalling, Type II diabetes mellitus, fatty acid metabolism or glycerolipid pathways. Differences in lipid profiles and allele frequencies among breeds are summarised in Table S3-B.

Discussion

A total of 16 genes were found to have significant effects on several traits: *ALDH2*, *CFL1*, *CHRNE*, *CRHR2*, *CRI1*, *DGAT1*, *IGF2R*, *IGFBP3*, *MMP1*, *NEB*, *MYOZ1*, *PLTP*, *SOCS2*, *SUSP1*, *TCF12* (Table 1) and *FOXO1* (Table 2). Although some of these effects are considerable (*CFL1*, *CRI1*, *IGF2R*, *MMP1*, *MYOZ1*, *PLTP*), most of the 73 significant associations reported here had an overall low effect. This may be because either, the SNPs examined were not causative but in linkage disequilibrium or, more likely, that the traits are polygenic and the genes detected account for only a small amount of the total effect.

Several metabolic pathways are implicated (Table S5): PPAR signalling pathway, fatty acid metabolism, glycerolipid metabolism, pyruvate metabolism, limonene and pinene degradation, fat digestion and absorption, insulin signalling and diabetes mellitus II pathways. This is in accordance with the literature, which describes some of the genes as having biological functions related to lipid metabolism (Table 3 summarises published associations for these genes).

Genes affecting muscle fatty acid profile

If we group the associations by traits, some genes, such as *CFL1, MYOZ* and *NEB*, affect the 18:2/18:3 ratio, whereas *PLTP* is associated with n6/n3 ratio. Also, *CRI1, DGAT1, FOXO1, MMP1* and *SOCS2* influence the amount of specific n-6 and n-3 FA in muscle (18:3 n-3, 18:2 n-6, 22:4 n-6, 22:6 n-3, 20:3 n-6) (Tables 1 and 2). Specifically, an association between the SNP ss77831721 of *CFL1* and the ratio of linoleic/linolenic acids (18:2 n-6/18:3 n-3) was found. The dietary n-6 to n-3 ratio, and in particular n-3 FA, has been proven to be beneficial for human health (MacLean *et al.* 2006; Gissi-Hf Investigators *et al.* 2008). This SNP is non-synonymous, causing a *CFL1:* p.Ile47Thr substitution. The effect of the favourable allele *T* (Table 1) is considerable, decreasing the ratio of 18:2/18:3 by 8%. Also, an appreciable effect of the intronic SNP in *MYOZ1* is found on the FA ratio of 18:2/18:3, decreasing the trait by 8%, possibly explained by the different FA profile which depends on muscle fibre types. Janovská *et al.* (2010) already determined that, compared with glycolytic muscles, oxidative fibres preferentially accumulated C18 over C16 FA and n-6 over n-3 PUFA.

Variations in the *NEB* gene (specifically SNP ss77832090) were strongly associated with the amount of different FA in muscle (12:0, 16:0, 16:1, 20:1, 9c11tCLA, 9c18:1, 18:2/18:3) and with the overall amount of FA, which may be explained by the fact that an increase in the cytoskeletal matrix can cause a decrease in adipose tissue (Anderson & Kunkel 1992). In fact, this gene has been found to be highly expressed in individuals that produce meat with low marbling (Lee *et al.* 2008). *PLTP* was found to affect the amount of FA in muscle, and the *A* allele of SNP ss77832104 (located in the 3′-UTR) decreases the ratio n-6:n-3 by 8%. *CRI1,* which reduces PPARG transactivation and pRB levels, leading to increased expression of *UCP1* and *PGC-1A,* all of these genes involved in lipid metabolism, seems to influence the total amount of lipids in muscle and the 'flavour group' (which includes, among other traits, total FA). The *T* allele of SNP ss77832128 increases the amount of neutral adrenic acid (22:4 n-6) by 13.4%. The associated polymorphism, located in exon 1, results in a SNP ss77832128*:* p.Ala47Ser substitution and therefore may play a functional role in expression or function of the gene product. Three SNPs of *DGAT1* were shown to be associated with 18:3 n-3, n-octadecanal (18ald) fatty aldehyde (Table 2), palmitoleic acid (16:1), lauric acid (12:0), muscle FA content and the phenotypic flavour group (Table 1), which may be a consequence of the variation caused by this gene on fatty aldehyde profiles, described as a key component of beef flavour (Resconi *et al.* 2010). One of the SNPs is located in the 3′-UTR, whereas the other two polymorphisms are the ApA to GpC dinucleotide substitution in exon 8 described by Grisart *et al.* (2002), causing a *DGAT1:* p.Lys232Ala substitution.

The haplotype analysis performed in this study associated haplotype 3 (GCCCT) of *FOXO1*, a regulator of master adipogenic transcription factors such as PPARα and C/EBPα (Kousteni 2012), with the amount of 18:2 n-6 and trans 18:1 FA in muscle (Table 2), although no associations were detected for individual SNPs. This may suggest that the real effect is another gene in linkage with *FOXO1*. Also, nine SNPs in *MMP1* were examined, six of which were associated with the amount of FA in muscle, either taken individually (9c11tCLA, 12:0, 14:0, 16ald, 18ald and 22:6n-3) or grouped (all fatty acids group and flavour group traits), although with small effects except for SNPs ss77831914 and ss77831916 that influence the amount of CLA, and SNP ss77831924 in 3′UTR that affects docosahexaenoic acid (22:6 n-3), for which the *C* allele is associated with an increase in the amount of this beneficial n-3 in muscle by 14%. This allele is also associated with a slight decrease in the amount of 16ald and 18ald fatty aldehydes. Finally, an association was detected between *SOCS2* and the amount of DGLA 20:3 n-6 in muscle. $SOCS2^{-/-}$ knockout mice show a twofold increase in PGC-1α (Rico-Bautista *et al.* 2005), which is a key regulator of energy homoeostasis in skeletal muscle, including lipid metabolism (Puigserver & Spiegelman 2003).

Genes affecting organoleptic characteristics

The main effect of another group of genes (*ALDH2, IGFR2* and *IGFBP3*) is on different sensory appraisals, such as flavour, texture or test panel, which includes all sensory analysis. *ALDH2* influences the flavour group and test panel, probably through its effect on the key components of beef flavour aldehydes and carboxylates (Resconi *et al.* 2010). *IGF2R* was found to have a strong effect on different lipid traits, with five of the 11 SNPs (Table 1) and one of the 10 haplotypes (Table 2) associated with the test panel and flavour groups and texture measurements. *IGFBP3* affects the test panel group, most likely by affecting the muscle fat content (Sun *et al.* 2003; Wang *et al.* 2009).

Genes affecting total fatty acid content in muscle

Finally, *CRHR2, CHRNE, SUSP1* and *TCF12* seem to exclusively influence the amount of FA in muscle. Concerning *CRHR2,* although Jiang *et al.* (2006) did not find

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Table 3 Gene symbol and name, main biological functions, previously described trait associations and references of 16 candidate genes associated with different lipid traits.

Gene symbol	Gene name	Biological process	Previous trait association	References
ALDH2	*Aldehyde dehydrogenase 2 family (mitochondrial)*	Catalyses the detoxification of aldehydes to carboxylates, key components of beef flavour	Unknown	Conklin *et al.* (2007) and Resconi *et al.* (2010)
CFL1	*Cofilin 1 (non-muscle)*	Organisation of actin filament assembly/disassembly. Lipid metabolism, gene regulation, apoptosis	Unknown	Bamburg & Bernstein (2010)
CHRNE	*Cholinergic receptor nicotinic, epsilon (muscle)*	Activation of this ligand-gated ion channel results in membrane depolarisation	Congenital myasthenic syndrome (CMS): carriers have body weight advantage at different stages	Kraner *et al.* (2002) and Thompson *et al.* (2007)
CRHR2	*Corticotropin releasing hormone receptor 2*	Appetite and gastrointestinal motor regulation, regulation of energy homoeostasis and mediation of the anorexic effect of CRH at the adipose level	Unknown	Bale *et al.* (2003) and Doyon *et al.* (2004)
CRI1 (EID1)	*EP300 interacting inhibitor of differentiation 1*	Reduces fat accumulation in adipose cells and induces expression of brown fat genes in white pre-adipocytes	Unknown	Lizcano & Vargas (2010)
DGAT1	*Diacylglycerol acyltransferase 1*	Catalyses the final stage of triacylglycerol synthesis	Milk fat content, marbling	Grisart *et al.* (2002) and Thaller *et al.* (2003)
FOXO1 (FKHR)	*Forkhead box O1*	Influences a variety of cellular functions, including lipid metabolism through the regulation of master adipogenic transcription factors	Unknown	Kousteni (2012)
IGF2R	*Insulin-like growth factor 2 receptor*	IGF2 clearance receptor IGF2 can signal via this receptor and affect cellular functions such as differentiation, migration and apoptosis in a variety of cell types	Reduced lipid metabolism and fat deposition in *GDF8* hypertrophic muscles	Nezer *et al.* (1999), Sherman *et al.* (2008), Pérez *et al.* (2010)
IGFBP3	*Insulin-like growth factor binding protein-3*	Transporter and regulator of IGFs Affects cellular functions independent of IGFs	Slaughter and carcass traits, including back-fat thickness and beef fat content (cattle, pigs)	Wang *et al.* (2009) and Sun *et al.* (2003)
MMP1	*Matrix metalloproteinase 1*	Plays a key role in adipogenesis, stimulating tissue remodelling during adipose tissue expansion in obesity	Unknown	Meissburger *et al.* (2011)
MYOZ1	*Myozenin 1*	Regulation of muscle fibre type composition	Unknown	Frey *et al.* (2008)
NEB	*Nebulin*	Encodes a giant protein component of the cytoskeletal matrix	Marbling	Stedman *et al.* (1988) and Lee *et al.* (2008)
PLTP	*Phospholipid transfer protein*	Transports a large number of different amphipathic molecules, playing an important role in lipid and lipoprotein metabolism	Unknown	Albers *et al.* (1995)
SOCS2	*Suppressor of cytokine signalling 2*	Involved in the negative regulation of cytokine signal transduction and body growth	Growth	Starr *et al.* (1997) and Horvat & Medrano (2001)
SUSP1 (SENP6)	*SUMO1/Sentrin-specific peptidase 6*	Belongs to the small ubiquitin-like modifier (SUMO) protein family, which contributes to the regulation of many cellular processes	Unknown	Hershko & Ciechanover (1998)
TCF12 (HEB)	*Transcription factor 12 loop-helix transcription factors 4*	Orchestrates the regulation of myogenic factor activity through myogenic differentiation	Unknown	Massari & Murre (2000) and Parker *et al.* (2006)

any association between several polymorphisms in this gene and intramyocellular lipid accumulation or subcutaneous fat depth in cattle, the data presented here show a significant link between this gene and the total amount of FA in muscle. Unfortunately, our experimental design did not provide records of individual feed intake, and therefore, it was not possible to assess whether the effects of variations in *CRHR2* on fat accumulation were mediated through differences in appetite and feed intake. On the other hand, the association of *CHRNE*, *SUSP1* and *TCF12* with lipid traits have no clear biochemical link (see Table 3), and further research is needed to validate these associations.

Conclusions

We can conclude that, although most of the 73 significant associations reported here had an overall low effect, some of the genes show considerable and novel effects such as *CFL1* and *MYOZ1* on the 18:2/18:3 ratio, as well as *CRI1* on the amount of 22:4 n-6, *MMP1* on 22:6 n-3 and CLA, *PLTP* on n-6:n-3 ratio and *IGF2R* on flavour. In addition, the effects of *DGAT1*, *IGF2R* and *IGFBP3* on muscle FA content, and consequently on meat palatability, confirm the associations previously described for these genes, and *ALDH2*, *CHRNE*, *CRHR2*, *NEB*, *SOCS2*, *SUSP1*, *TCF12* and *FOXO1* were newly found to be associated with both lipid and organoleptic traits. The results presented here provide valuable information to help dissect the complex gene networks underlying muscle FA composition in cattle and understand the factors influencing meat sensory aspects. The development and implementation of low-density SNP panels with predictive value for economically important traits, such as those reported here, may be used to improve production efficiency and meat quality in the beef industry as a molecular signature of TGGAGTGCCAA for *CFL1* (ss77831721), *CRI1* (ss77832128), *DGAT1* (ss77831745), *IGF2R* (ss77831887, ss77831883, ss77831884, ss7783 1885), *MMP1* (ss77831924), *MYOZ1* (ss77831945), *PLTP* (ss77832104) and *SOCS2B* (ss77832234).

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Phenotypes measured on 436 purebred bulls belonging to 15 cattle breeds.

Table S2 Haplotype frequencies of those genes with two or more markers for which a strong association was found with particular traits.

Table S3 A) Mean and standard deviation for the traits associated to different genes in 15 cattle breeds; B) Main differences (in bold) in lipid profiles among breeds. Values are expressed as means ± SE.

Table S4 Allele frequencies per breed of 26 polymorphisms found to be individually associated to different lipid and organoleptic traits.

Table S5 Gene pathway annotations.