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

S. Dunner*, N. Sevane*, D. Garcia*, H. Levéziel†‡, J. L. Williams§, B. Mangin¶, A. Valentini** and the GeMQual Consortium¹

*Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain. †INRA, UMR 1061, 87000 Limoges, France. ‡Université de Limoges, UMR 1061, 87000 Limoges, France. §Parco Tecnologico Padano, Via Einstein, Polo Universitario, 26900 Lodi, Italy. ¶INRA Chemin de Borde-Rouge-Auzeville, BP 52627, 31326 Castanet-Tolosan Cedex, France. **Department for Innovation in Biological, Agro-Food and Forest Systems, Università della Tuscia, via De Lellis, 01100 Viterbo, Italy.

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, IGF1P3, 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-
date genes involved in adipogenesis, lipid metabolism and energy homeostasis (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 Alberti 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 carcasses were treated under similar conditions (Alberti 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 (FAN) and phospholipid (FAF) 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. 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

\[ Y_{ij} = m_i + a_i G_{ij} + e_{ij}^{(0)} \]

where \( Y_{ij} \) 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 \( G_{ij} \) takes different values according to the genotype of each individual (1 for genotype AA, 0 for Aa and -1 for aa). \( e_{ij}^{(0)} \) are independent and identically distributed normal residuals, also independent of \( G_{ij} \). 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 both 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 \( z \) correction led to very small individual \( z \) 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 (Composite 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 FAMHIAP 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.

<table>
<thead>
<tr>
<th>Locus Symbol</th>
<th>dbSNPs^1</th>
<th>SNP location</th>
<th>Significant Trait Associations^2</th>
<th>Mean^3</th>
<th>SD^4</th>
<th>P-value</th>
<th>Allele^5</th>
<th>Effect^6</th>
<th>Effect/SD^7</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDH2</td>
<td>ss77831990</td>
<td>Intron 8</td>
<td>Flavour group^8 Test panel^9</td>
<td>18:2/18:3</td>
<td>18.774</td>
<td>9.964</td>
<td>0.00005</td>
<td>T</td>
<td>−1.514</td>
</tr>
<tr>
<td>CFL1</td>
<td>ss77831721</td>
<td>Exon 2-NS 47aa lle→Thr Exon 4-5</td>
<td>All fatty acids</td>
<td>N 22:4 n-6</td>
<td>0.677</td>
<td>0.639</td>
<td>0.002</td>
<td>T</td>
<td>0.091</td>
</tr>
<tr>
<td>CHRNE</td>
<td>ss77831830</td>
<td>Exon 4-5</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N</td>
<td>0.004</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRHR2</td>
<td>ss77832125</td>
<td>Intron 4</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N</td>
<td>0.003</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRI1</td>
<td>ss77832128</td>
<td>Exon 1-NS 47aa Ala→Ser</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N</td>
<td>0.005</td>
<td>0.03</td>
<td></td>
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<tr>
<td>DGAT1</td>
<td>ss77831744</td>
<td>3′UTR</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N</td>
<td>0.001</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFL1</td>
<td>ss77831745</td>
<td>Exon 8-NS Dinnucleotide substitution</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N</td>
<td>0.002</td>
<td>0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFL1</td>
<td>ss77832137</td>
<td>232aa Lys→Ala</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N</td>
<td>0.005</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF2R</td>
<td>ss77831728</td>
<td>Intron 25</td>
<td>Test panel</td>
<td>Flavour</td>
<td>N</td>
<td>0.001</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF2R</td>
<td>ss77831883</td>
<td>Intron 55</td>
<td>Test panel</td>
<td>Flavour</td>
<td>N</td>
<td>0.0004</td>
<td>0.0007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF2R</td>
<td>ss77831884</td>
<td>Intron 55</td>
<td>Test panel</td>
<td>Flavour</td>
<td>N</td>
<td>0.0003</td>
<td>C</td>
<td>−0.148</td>
<td>−0.300</td>
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<tr>
<td>IGF2R</td>
<td>ss77831885</td>
<td>Intron 55</td>
<td>Test panel</td>
<td>Flavour</td>
<td>N</td>
<td>0.0002</td>
<td>G</td>
<td>0.160</td>
<td>0.325</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>ss77832346</td>
<td>Intron 2</td>
<td>Test panel</td>
<td>Flavour</td>
<td>N</td>
<td>0.0008</td>
<td>C</td>
<td>0.051</td>
<td>0.030</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>ss77831914</td>
<td>Intron 1</td>
<td>Test panel</td>
<td>Flavour</td>
<td>N 12:0</td>
<td>1.818</td>
<td>1.723</td>
<td>0.0005</td>
<td>C</td>
</tr>
<tr>
<td>MMP1</td>
<td>ss77831916</td>
<td>Intron 1</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N 9c11Tcla</td>
<td>7.561</td>
<td>5.779</td>
<td>0.00007</td>
<td>G</td>
</tr>
<tr>
<td>MYOZ1</td>
<td>ss77831946</td>
<td>Intron 2</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N 16ald</td>
<td>23.277</td>
<td>6.880</td>
<td>0.005</td>
<td>C</td>
</tr>
<tr>
<td>NEB</td>
<td>ss77832090</td>
<td>Intron 149</td>
<td>All fatty acids</td>
<td>Flavour group</td>
<td>N 18:2/18:3</td>
<td>18.774</td>
<td>9.964</td>
<td>0.0002</td>
<td>C</td>
</tr>
<tr>
<td>PLTP</td>
<td>ss77832104</td>
<td>3′UTR</td>
<td>n-6:n-3</td>
<td>Flavour group</td>
<td>N 12:0</td>
<td>1.978</td>
<td>1.815</td>
<td>0.002</td>
<td>C</td>
</tr>
</tbody>
</table>

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, CFL1, CRHR1, 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, CRHR1, DGAT1, IGFB2R, IGF2R, MMP1, NEB, MYOZ1, PLTP, SOCS2, SUSP1, TCF12 (Table 1) and FOXO1 (Table 2). Although some of these effects are considerable (CFL1, CRHR1, IGFB2R, 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.

### Table 1 (continued)

<table>
<thead>
<tr>
<th>Locus Symbol</th>
<th>dbSNPs</th>
<th>SNP location</th>
<th>Significant Trait Associations</th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
<th>Allele</th>
<th>Effect</th>
<th>Effect/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOCS2</td>
<td>ss77832234</td>
<td>Exon 2-S</td>
<td>All fatty acids</td>
<td>8.911</td>
<td>2.452</td>
<td>0.0004 A</td>
<td>−0.071</td>
<td>−0.029</td>
<td></td>
</tr>
<tr>
<td>SUSP1</td>
<td>ss77831761</td>
<td>Exon 25-S</td>
<td>All fatty acids</td>
<td>8.277</td>
<td>1.996</td>
<td>0.00002 A</td>
<td>−0.070</td>
<td>−0.035</td>
<td></td>
</tr>
<tr>
<td>TCF12</td>
<td>ss77831958</td>
<td>Intron 11</td>
<td>All fatty acids</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Locus symbol</th>
<th>dbSNPs (Allele1/Allele2)1</th>
<th>Haplotype ID and alleles</th>
<th>Significant Trait Associations2</th>
<th>P-value</th>
<th>Haplotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGAT1</td>
<td>ss77831744; ss77831745 A/G; ss77832137 A/C</td>
<td>1-TAA; 3-TGC; 2-CAA; 3-GCCCT</td>
<td>18:3 n-3</td>
<td>0.006</td>
<td>0.088</td>
</tr>
<tr>
<td>FOXO1</td>
<td>ss77831726 A/G; ss77831860 A/C; ss77831861 C/T; ss77831862 C/T; ss77831895 C/T</td>
<td>2-GAATAGTCCAC</td>
<td>Texture</td>
<td>0.008</td>
<td>0.945</td>
</tr>
<tr>
<td>IGF2R</td>
<td>ss77831728 G/A; ss77831887 G/A; ss77831883 G/A; ss77831884 T/C; ss77831885 G/A; ss77831877 G/A; ss77831878 T/C; ss77831880 C/T; ss77831881 C/T; ss77831882 G/A; ss77831886 T/C</td>
<td>2-GAATAGTCCAC</td>
<td>Texture</td>
<td>0.008</td>
<td>0.945</td>
</tr>
</tbody>
</table>

1 In bold, SNPs also found to be associated with some trait individually.
2 See Table S1.
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, CR1, 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-3) (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%. CR1, which reduces PPARα 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 adrenal 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 transe 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 SNP 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). IGFR2 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
<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Biological process</th>
<th>Previous trait association</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDH2</td>
<td>Aldehyde dehydrogenase 2, family (mitochondrial)</td>
<td>Catalyses the detoxification of aldehydes to carboxylates, key components of beef flavour</td>
<td>Unknown</td>
<td>Conklin et al. (2007) and Resconi et al. (2010)</td>
</tr>
<tr>
<td>CHRNE</td>
<td>Cholinergic receptor nicotinic, epsilon (muscle)</td>
<td>Activation of this ligand-gated ion channel results in membrane depolarisation</td>
<td>Congenital myasthenic syndrome (CMS): carriers have body weight advantage at different stages</td>
<td>Kraner et al. (2002) and Thompson et al. (2007)</td>
</tr>
<tr>
<td>CRHR2</td>
<td>Corticotropin releasing hormone receptor 2</td>
<td>Appetite and gastrointestinal motor regulation, regulation of energy homeostasis and mediation of the anorexic effect of CRH at the adipose level</td>
<td>Unknown</td>
<td>Bale et al. (2003) and Doyon et al. (2004)</td>
</tr>
<tr>
<td>CRI1 (BD1)</td>
<td>EP300 interacting inhibitor of differentiation 1</td>
<td>Reduces fat accumulation in adipose cells and induces expression of brown fat genes in white pre-adipocytes</td>
<td>Unknown</td>
<td>Lizcano &amp; Vargas (2010)</td>
</tr>
<tr>
<td>DGAT1</td>
<td>Diacylglycerol acyltransferase 1</td>
<td>Catalyses the final stage of triacylglycerol synthesis</td>
<td>Milk fat content, marbling</td>
<td>Grisart et al. (2002) and Thaller et al. (2003)</td>
</tr>
<tr>
<td>FOXO1 (FKHR)</td>
<td>Forkhead box O1</td>
<td>Influences a variety of cellular functions, including lipid metabolism through the regulation of master adipogenic transcription factors</td>
<td>Unknown</td>
<td>Kostner (2012)</td>
</tr>
<tr>
<td>IGF2R</td>
<td>Insulin-like growth factor 2 receptor</td>
<td>IGF2 can signal via this receptor and affect cellular functions such as differentiation, migration and apoptosis in a variety of cell types</td>
<td>Reduced lipid metabolism and fat deposition in GDF8 hypertrophic muscles</td>
<td>Nezer et al. (1999), Sherman et al. (2008), Pérez et al. (2010)</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>Insulin-like growth factor binding protein-3</td>
<td>Transporter and regulator of IGFs Affects cellular functions independent of IGFs</td>
<td>Slaughter and carcass traits, including back-fat thickness and beef fat content (cattle, pigs)</td>
<td>Wang et al. (2009) and Sun et al. (2003)</td>
</tr>
<tr>
<td>MMP1</td>
<td>Matrix metalloproteinase 1</td>
<td>Plays a key role in adipogenesis, stimulating tissue remodelling during adipose tissue expansion in obesity</td>
<td>Unknown</td>
<td>Meissburger et al. (2011)</td>
</tr>
<tr>
<td>MYOZ1</td>
<td>Myozenin 1</td>
<td>Regulation of muscle fibre type composition</td>
<td>Unknown</td>
<td>Frey et al. (2008)</td>
</tr>
<tr>
<td>NEB</td>
<td>Nebulin</td>
<td>Encodes a giant protein component of the cytoskeletal matrix</td>
<td>Marbling</td>
<td>Stedman et al. (1988) and Lee et al. (2008)</td>
</tr>
<tr>
<td>PLTP</td>
<td>Phospholipid transfer protein</td>
<td>Transports a large number of different amphipathic molecules, playing an important role in lipid and lipoprotein metabolism</td>
<td>Unknown</td>
<td>Albers et al. (1995)</td>
</tr>
<tr>
<td>SOCS2</td>
<td>Suppressor of cytokine signalling 2</td>
<td>Involved in the negative regulation of cytokine signal transduction and body growth</td>
<td>Growth</td>
<td>Starr et al. (1997) and Horvat &amp; Medrano (2001)</td>
</tr>
<tr>
<td>SUSP1 (SENPa)</td>
<td>SUMO1/Sentrin-specific peptidase 6</td>
<td>Belongs to the small ubiquitin-like modifier (SUMO) protein family, which contributes to the regulation of many cellular processes</td>
<td>Unknown</td>
<td>Herskow &amp; Ciechanover (1998)</td>
</tr>
<tr>
<td>TCF12 (NEB)</td>
<td>Transcription factor 12 (NEB)</td>
<td>Orchestrates the regulation of myogenic factor activity through myogenic differentiation</td>
<td>Unknown</td>
<td>Massari &amp; Murre (2000) and Parker et al. (2006)</td>
</tr>
</tbody>
</table>
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.

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References


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.