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REVIEW

Quantitative Trait Loci and Candidate Genes Affecting Fatty Acid Composition in Cattle and Pig

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Abstract

Investigations into fatty acid composition in meats are becoming more important due to consumer demand for high quality healthy food. Marker-assisted selection has been applied to livestock to improve meat quality by directly selecting animals for favorable alleles that affect economic traits. Quantitative trait loci affecting fatty acid composition in cattle and pigs were investigated, and five candidate genes (ACACA, FASN, SCD, FABPs, and SREBP-1) were significantly associated with fatty acid composition. The information presented here should provide valuable guidelines to detect causative mutations affecting fatty acid composition in cattle and pigs.

Key words: quantitative trait locus, candidate genes, fatty acid composition, cattle, pig

Introduction

Dietary fatty acids (FAs) found in meat, dairy products, vegetables, nuts and fish are highly relevant nutrients for human health that influence the risk for several human health problems including heart disease, stroke, diabetes and some cancers (FAO, 2003). High intake of saturated FA (SFA) can result in elevated plasma cholesterol, which leads to cardiovascular disease. SFAs such as lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) most deleteriously influence cardiovascular health (Keys *et al*., 1974). In contrast, monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) decrease the circulating concentration of low density lipoprotein (LDL)-cholesterol by increasing hepatic LDL receptor activity (Woolet *et al*., 1992).

The FA composition of meat is one of the main factors defining meat quality parameters including nutrition, sensory and functional aspects. Meat quality can be improved by an increasing the ratio of UFA to SFA (Yang *et al*., 1999). The variation in FA composition, in particular saturation, affects the firmness and oiliness of adipose tissue (Diana *et al*., 1998; Wood *et al*., 2008) that also influences meat processing. Moreover, FAs are important in controlling cellular metabolism, maintaining an intact cell membrane and improving nutrient use (Kim *et al*., 2006). Oxidative stability of muscle can be affected by FA composition, which in turn affects muscle flavor and muscle color, increasing consumer acceptance. Some studies have been reported the important effects of fatty acid to meat quality. The lower melting point of monounsaturated fatty acid (MUFA) contributes positively to beef flavor and tenderness (Melton *et al*., 1982; Smith *et al*., 2006). For example, one of the major MUFAs in beef fat, oleic acid (C18:1) is not susceptible to oxidation and contribute to favorable tenderness and flavor of beef (Westerling and Hendrick 1979; Melton *et al*., 1982). In contrast, high melting point fat in meat appears whiter than the fats having lower melting point. This indicates fat color was affected by fatty acids (Wood *et al*., 2003). In case of beef texture, the increase of C18:1 in relation to C18:0 and C16:0 effects soft and oily fat. Therefore, producing and selecting animals having desirable high quality meat with healthy FA composition has become an important goal of producers and researchers.

With the aim of producing healthier meat, dietary alterations have been successful in altering the FA composition with respect to the content of UFA in pigs, poultry and fish (Bou *et al*., 2009; Jung *et al*., 2010; Kim *et al*., 2007). However, the FA composition of meat from ruminants is generally more saturated and is difficult to alter

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because UFAs are subject to biohydrogenation in the rumen (Decker and Park, 2010). This is one of the reasons for the temporally steady or slowly decreasing consumption in beef or other animal products from ruminants in many countries.

The selection of animals having important traits including FA composition can be achieved using molecular approaches such as quantitative trait loci (QTL, locations of the genes on the chromosomes affecting quantitative traits) and candidate genes. For this selection, identification of the QTL regions harboring the candidate genes is crucial, and preludes the search for influential gene mutations in these regions. A number of studies have investigated the chromosomal regions and/or genetic variations affecting FA composition by using QTL analyses and candidate gene identification. This article provides a summary overview of the study of QTLs and candidate genes in relation to FA composition in economically important animals including cattle and pigs.

QTL for FA Composition

The locations of the genes on the chromosomes affecting quantitative traits are referred as quantitative trait loci (QTLs). Therefore, the identification of QTL regions is very important because the causative mutation for FA composition can be searched from these regions. Ultimately, the identified causative mutations can be used for the selection of animals having desirable (healthy) FA composition.

Meat quality is multifactorial trait that influenced by genetic and non-genetic factors. Usually, the genetic factors can be improved by conventional breeding methods. However, if the causative mutations were identified for the economic traits (in this case meat quality), then marker assisted selection can be applied for selection of better animals in early stage. Initially, the polymorphic markers were identified across the genome of interest and the associations were tested between markers and traits. If the significant location of chromosome was identified, this region of the genome is called QTL region (Beuzen, *et al.,* 2000).

QTLs affecting FA composition in cattle

Many of the known bovine QTLs are summarized on the National Animal Genome Research Program website (http://www.animalgenome.org/QTLdb). As of December 2010, this database contained 4,281 QTLs collected from 235 study reports. The QTLs represent 359 traits. In particular, the traits include six classes: exterior, health, meat, milk, production and reproduction. Among these, meat-related traits represent only 7.4% of the QTLs thus far identified. None of the top 20 traits based on the number of reported QTLs are related to FA composition, reflecting the paucity of FA-related QTL studies that have been carried out in cattle.

A whole-genome scan using 328 F_2 progenies from a Wagyu and Limousine cross was conducted by Alexander *et al*. (2007). They identified QTLs affecting palatability and FA composition in beef. Seven QTLs affecting lipid metabolisms and tenderness were identified in five chromosomes. Another, more recent study was conducted using the population from crosses between Charolais and Holstein based on the balanced F_2 and backcross breeding designs. A whole-genome scan was carried out to detect QTLs affecting FA composition in beef (Gutierrez-Gil *et al*., 2010). The QTL locations are summarized in Table 1.

The latter study indicates that many loci are responsible for the variation of FA composition (Guitierrez *et al*., 2010). The highest number of QTLs was detected on chromosome 10 near the 133 cM region: in particular, QTLs for the content of gamma-linolenic acid (C20:3n-6) and docosapentaenoic acid (C22:5n-3, DPA) were present and displayed a 5% genome-wide significance level. The second half of this chromosome, at position 91 cM, was also shown to harbor a significant QTL for docosahexaenoic acid (C22:6n-3, DHA).

In chromosome 1, at around 51 cM, three QTLs for linoleic acid (C18:2n-6), linolenic acid (C18:3n-3) and polyunsaturated fatty acids (PUFAs) were detected (Table 1). These three traits displayed different chromosome-wide significance levels. Linoleic acid and PUFA had 1% chromosome-wide significance levels, whereas linolenic acid showed a 5% chromosome-wide significance level. Only linoleic acid had genome-wide significance (0.042, Table 1). Chromosome-wide significance levels of 1% were also detected on chromosome 8 (C20:4n-6), 9 (C20:5n-3), 10 (C20:3n-6 and C22:5n-3) and 22 [C16:0; C16:1; 9cC18:1; conjugated linoleic acid (CLA)] and the weight of total FAs. In addition, fat thickness, another fat-related trait, was detected in chromosome 1 and 7 in the cross population between Brahman and Hereford (Casas *et al*., 2003) and Japanese Black (Wagyu) cattle (Mizoguchi *et al*., 2006). In Wagyu cattle, a significant QTL was detected for arachidonic acid (C20:4n-6) on BTA13 and, in the same location**,** but a QTL for subcutaneous fat depth was not identified (Mizoguchi *et al*., 2006). In a QTL study in Wagyu and Limousine cattle, QTLs for

Table 1. QTL locations affecting fatty acid composition in cattle

Trait[1]	Cattle chromosome	Position (cM)	Pc[2](Pg)[3] value	Reference
C18:2n-6; C18:3n-3; PUFA	1	51;51; 51	0.0025(0.042); 0.0446; 0.0053	Gutierrez *et al.* (2010)
n6:n3	2	91	0.031	Gutierrez *et al.* (2010)
CLA; MUFA; SFA R_2 (ratio of C16:1 to C16:0) R_3 (ratio of C18:1 to C18:0)	2 2 2	46; 5; 10 4 0	(0.013); (0.00012); (0.01) (0.00032) (0.00002)	Alexander *et al.* (2007)
SFA; MUFA; PUFA	2	4; 4; 11	(0.04); (<0.01); (<0.01)	Tshipuliso *et al.* (2008)
CLA	4	92	0.0196	Gutierrez *et al.* (2010)
11t C18:1 (*trans* vaccenic)	5	93	0.037	Gutierrez *et al.* (2010)
C18:0_ald; C22:6n-3; ln-n-6:n-3	6	29; 12; 24	0.0374; 0.0405; 0.046	Gutierrez *et al.* (2010)
CLA; MUFA; SFA;	7	131; 125; 125	(0.092); (0.025); (0.065)	Alexander *et al. (*2007)
C18:2n-6; C20:4n-6; C22:5n-3; PUFA	8	0; 0; 0; 0	0.0359; 0.0058 0.0451; 0.0153	Gutierrez *et al.* (2010)
C20:5n-3; C22:6n-3	9	0; 75	0.0098; 0.0248	Gutierrez *et al.* (2010)
MUFA; PUFA	9	119; 54	(0.09); (<0.01)	Tshipuliso *et al* (2008)
C:16_ald; 11cC18:1; C20:3n-6; C22:5n-3; C22:6n-3; n-3PUFA	10	133; 0; 133(4-133); 133 (1-133); 91; 133	0.0203; 0.0157 0.0013 (0.024); 0.0008(0.015) 0.043; 0.054	Gutierrez *et al.* (2010)
PUFA	10	38	(0.02)	Tshipuliso *et al.* (2008)
C20:5n-3	11	89	0.0184	Gutierrez *et al.* (2010)
MUFA; SFA	12	0; 0	(0.128); (0.052)	Alexander *et al.* (2007)
C20:4n-6	13	5	0.0155	Gutierrez *et al.* (2010)
PUFA	15	14	(<0.01)	Tshipuliso *et al.* (2008)
n6:n3	18	55	0.0114	Gutierrez *et al.* (2010)
11cC18:1	20	9	0.0043	Gutierrez *et al.* (2010)
C14:0; C16:0; C16:1; C18:0; 9cC18:1; CLA; SUMWFA; SFA; P:S	22	29; 31; 33; 29 35; 29; 32 30; 32	0.0142; 0.0053; 0.0041 0.0239; 0.0032; 0.008 0.0092; 0.0112; 0.012	Gutierrez *et al.* (2010)
MUFA	22	47	(0.02)	Tshipuliso *et al.* (2008)

[1]Trait: C14:0 = myristic acid; C16:0 = palmitic acid; C16:0_ald = palmitic aldehyde; C16:1 = palmitoleic acid; C18:0 = stearic acid; C18:0-ald = stearic aldehyde acid; 11tC18:1 = *trans* vaccenic acid; 9cC18:1 = oleic acid; 11cC18:1 = *cis* vaccenic acid; C18:2n-6 = linoleic acid; C18:3n-3 = linolenic acid; CLA = conjugated linoleic acid; C20:3n-6 = gamma-linolenic acid; C20:4n-6 = arachidonic acid; C20:5n-3 = timnodonic acid; C22:5n-3 = docosapentaenoic acid (DPA); C22:6n-3 = docosahexaenoic acid (DHA); SUMWFA = weight of total fatty acids (mg/100 g); SFA = saturated fatty acids; PUFA = poly-unsaturated fatty acids; n-3 PUFA = n-3 poly-unsaturated fatty acids; P:S = poly-unsaturated:saturated fatty acid ratio; n-6:n-3 = n-6:n-3 ratio. MUFA=mono-unsaturated fatty acid

[2]Pc-value = chromosome-wide *p*-value

[3]Pg-value = genome-wide *p*-value (the percentage in side of ( ), it means the level significant in genome-wide)

SFAs, MUFAs and PUFAs were reported on BTA 2 (Tshipuliso *et al*., 2008). QTLs on BTA 10 and 22 were indicative of an association with PUFA (at position 38 cM) and MUFA (at position 47 cM), respectively.

QTLs located on chromosome 2 showing less than 5% and 1% genome-wide significance effects included CLA at 46 cM (p=0.013), MUFA at 5 cM (p=0.00012), SFA at 10 cM (p=0.01), R_2 at 4 cM (p=0.00032) and R_3 at 0 cM (p=0.00002) (Table 1). These reported QTLs were consistent with those in Wagyu and Limousin populations, which showed increasing lipid content, stearyl-CoA desaturase and desirable flavor. However, Alexander *et al*. (2007) reported that the fat-related genes such as FA synthase (FASN), acetyl-CoA carboxylase alpha (ACACA), solute carrier family 2 member 4 (SLCA4), stearyl-CoA desaturase (SCD) and the gene encoding the subunit of FA elongase were not located in 7 reported QTL regions. Gutierrez-Gil *et al*. (2010) also provided similar evidence that these genes were not good positional candidate genes for the QTLs detected in chromosome 4. Only the leptin gene

(LEP) corresponded with the QTL detected in the flanking interval between DIK26 and IDVG51 on chromosome 4. However, a previous study implicated FASN on chromosome 19 (19q22) as being responsible for FA compositions (Roy *et al*., 2001). Especially, the thioesterase domain of FASN located in exons 39-42 (Bhuiyan *et al*., 2009, Zhang *et al*., 2008) and exon 34 (Abe *et al*., 2009) significantly affected the FA composition. Also, gene ACACA on BTA 19 was implicated in the *de novo* synthesis of FAs (Zhang *et al*., 2009). In addition, the SCD gene located on bovine chromosome 26 has been significantly linked with MUFA, stearic and oleic acids in Japanese Black cattle (Ohsaki *et al*., 2009).

Interestingly, a QTL location can be associated with multiple traits. For example, a QTL on chromosome 11 was highly correlated with C20:5n-3 (Gutierrez-Gil *et al*. 2010) and other traits such as yield force and abnormal odor intensity (Gutierrez-Gil *et al*., 2008). Gutierrez-Gil *et al*. (2010) also reported that no QTL was detected for MUFA. However, another study detected QTLs for MUFA on BTA 2, 7 and 12 (Alexander *et al*., 2007) and BTA 9 and BTA 22 (Tshipuliso *et al*., 2008). MUFA also was detected on chromosome 19 (Taylor *et al*., 1998). Three QTLs on BTA 1, 8 and 10 (Gutierrez *et al*., 2010) and BTA 2, 9 and 15 (Tshipuliso *et al*., 2008) were identified for PUFAs. These results indicate the complexity of the QTLs; especially, some of the related traits have the same QTL locations. This also indicates that many factors influence FA composition. FA modification and synthesis in ruminant animals can be affected by feeding systems (Alfaia *et al*., 2009; Noci *et al*., 2007) and breed (Malau-Aduli *et al*., 1998; Zhang *et al*., 2007)

QTLs affecting fatty acid composition in pigs

The National Animal Genome Research Program database (http://www.animalgenome.org/QTLdb) lists 1705 porcine QTLs. In total, 5986 pig QTLs representing 581 different pig traits have been reported in 268 publications. The number of QTLs in relation to meat quality traits ranked as the most prevalent (69.2%) in comparison with other classes including exterior (6.3%), health (9.8%), production (10.1%) and reproduction (4.6%). Several fat characteristics such as average backfat thickness, backfat at rib, intramuscular fat (IMF) content and subcutaneous fat depth are among the top 20 most numerous of identified the QTLs. Similarly with the cattle QTL database, traits concerning FA composition are not among the 20 most prevalent traits. However, the number of pig QTL studies in relation to FA composition was more than that of cattle.

The significant QTLs affecting palmitoleic acid (C16:1) has been detected in SSC1 (*Sus scrofa* Chromosome 1) and SSC9 (Nii *et al*., 2006). Another studies also detected QTLs for palmitoleic at SSC12 (Munoz *et al*., 2007) and SSC8 (Clop *et al*., 2003). Sanchez *et al*. (2007) also reported two QTLs on SSC10 and SSC12, which had significant associations (5% chromosome-wide level) and one QTL on SSC14 with a 1% genome-wide significance with palmitoleic acid. Palmitoleic acid located in different QTL locations on SSC13 and SSC12 has been reported (Guo *et al*., 2009; Uemoto *et al*., 2009). While QTLs for palmitoleic acid are located in various chromosomal regions, the QTLs for stearic acid (C18:0) and linoleic acid (C18:2) are located in more than 50% chromosomal regions (Table 2). The different populations and breeds used in above three studies may explain the variability of QTL locations for palmitoleic acid.

Only one QTL location was detected both for palmitic acid (C16:0; SSC3) and linoleic acid (C18:2; SSC17). Eighteen QTL locations for 11 FA composition traits were detected in SSC4, whereas 13 QTL locations were detected in SSC7 for 17 FA composition traits. However, two QTL locations in SSC7 at position 62 cM and 59 cM had only a suggestive link and were not significantly associated with C16:1 (Uemoto *et al*., 2009). In the X chromosome, five QTLs regions have been identified in relation to FA composition (Guo *et al*., 2009).

An analysis of 153 QTLs including 63 genome-wide significant QTLs and 90 suggestive QTLs revealed significant effects for FA composition traits on SSC4, 7, 8, 10, 13 and X, with no effects evident on SSC3 and 11 (Guo *et al*., 2009). Interestingly, the QTL observed on SSC 7 for C20:1 displayed an unusually high *F*-value of 118.71 (Table 2). In addition, FA compositions detected in *longissimus* muscle and abdominal fat showed similar QTL locations. However, the different characteristics of two muscle types were reflected in the distinct QTL effect on SSC8 for FA composition in abdominal fat and *longissimus dorsi*. Significant pleiotropic effects in chromosome 4 and 7 were evident for MUFA and PUFA in both *longissimus* muscle and abdominal fat. The same study reported that QTL affecting FA in both tissues (*longissimus* muscle and abdominal fat) were always located at different positions on the same chromosome. This indicates that the genetic factor may influence the FA composition in different tissues.

Clop *et al*. (2003) detected FA composition QTLs using F2 crosses between Iberian and Landrace pigs. The QTLs

Table 2. QTL locations affecting fatty acid composition in pig

Trait[1]	pig chromosome	Position (cM)	Pc[2](Pg)[3] value	Reference
C16:1; C18:0 (inner layer backfat)	1	20.1; 16.1	Significant	Nii *et al.* (2006)
C16:1; C18:0 (outer layer backfat)	1	22.8; 23.8	Significant	Nii *et al.* (2006)
C18:2	1	60.7	Significant	Lee *et al.* (2003)
C18:1; C18:2n-6; MUFA; P:S	1	124; 29; 122; 29	5%; 1%; 5%; 5%	Sanchez *et al.* (2007)
C18:1(perirenal fat)	2	63.2	Significant	Nii *et al.* (2006)
C22:5n-6	2	122	5%	Sanchez *et al.* (2007)
C16:0 (perirenal fat)	3	87.8	Significant	Nii *et al.* (2006)
C18:2	4	68	(P<0.05)	Kim *et al.* (2006)
C18:2	4	79; 59	(1%); (1%)	Guo *et al.* (2009)
C18:3	4	81; 59	(1%); (1%)	Guo *et al.* (2009)
C18:1	4	53	(1%)	Guo *et al.* (2009)
C20:2; C20:2; C20:3; C20:4	4	76; 60; 61; 64	(5%); (1%); (1%); (1%)	Guo *et al.* (2009)
MUFA	4	79; 55	(1%); (1%)	Guo *et al.* (2009)
PUFA	4	79; 58	(1%); (1%)	Guo *et al.* (2009)
C18:2 ; DBI; PI	4	75; 73; 75	(p<0.01; p=0.02; p<0.01)	Clop *et al.* (2003)
C18:2 (inner layer back fat	4	69.7	Significant	Nii *et al.* (2006)
C18:2 (perirenal fat)	4	65.5	Significant	Nii *et al.* (2006)
C18:1	4	71.6	Significant	Nii *et al.* (2006)
C18:2 and C18:1	4	81	(p<0.01)	Perez-Enrico *et al.* (2000)
C22:5n-6	4	117	5%	Sanchez *et al.* (2007)
C18:2	5	49	Significant	Uemoto *et al.* (2009)
PUFA (inner layer back fat)	5	48.0	Significant	Nii *et al.* (2006)
PUFA (perirenal fat)	5	49.9	Significant	Nii *et al.* (2006)
MUFA	5	118	5%	Sanchez *et al.* (2007)
C16:0 (perirenal fat)	6	73.2	Significant	Nii *et al.* (2006)
DBI;UI	6	105; 34	(p=0.04; p=0.05)	Clop *et al.* (2003)
C18:1	7	26	(p<0.05)	Kim *et al.* (2006)
C18:3	7	50; 57	(1%); (1%)	Guo *et al.* (2009)
C18:1	7	57; 55	(1%); (1%)	Guo *et al.* (2009)
MUFA	7	57; 55	(1%); (1%)	Guo *et al.* (2009)
C18:2	7	58; 59	(1%)	Guo *et al.* (2009)
C20:3; C20:1; C20:4	7	59; 71; 71	(1%); (1%); (1%)	Guo *et al.* (2009)
C16:0; SFA; P/S	7	92; 92; 55;	(1%); (5%); (1%)	Guo *et al.* (2009)
UI; DBI	7	57; 60	(1%); (1%)	Guo *et al.* (2009)
C20:1	7	118.71	(1%)	Guo *et al.* (2009)
C20:2	7	73; 55	(1%); (1%)	Guo *et al.* (2009)
C16:1 (not corrected for BF thickness)	7	62	suggestive linkage	Uemoto *et al.* (2009)
C16:1 (corrected for BF thickness)	7	59	not significant	Uemoto *et al.* (2009)
C18:1; C18:2n-6	7	66; 60	5%; 5%	Sanchez *et al.* (2007)
C22:4n-6; C22:5n-6	7	48; 48	1%; (1%)	Sanchez *et al.* (2007)

[1]Trait: C14:0 = myristic acid; C16:0 = palmitic acid; C16:1 = palmitoleic acid; C16:1(n-7) = palmitelaidic acid; C16:1(n-9) = palmitoleic acid; C17:1= *cis*-10-heptadecenoic Acid; C18:0 = stearic acid; C18:1= oleic acid; C18:1(n-7) = vaccenic acid; C18: 1n-9 = oleic acid; C18:2n-7 = vaccenic acid; C18:2 = linoleic acid; C18:2n-6 = linoleic acid; C18:3 = linolenic acid; C18:3(n-3) = linolenic acid; C20:1 = gadoleic acid; C20:2 = eicosadienoic acid; C20:2(n-6) = eicosadienoic acid; C20:3 = eicosatrienoic acid; C20:3n-3 = eicosatrienoic acid; C20:4 = arachidonic acid; C22:6n-3 = docosahexaenoic acid; C22:4n-6 = adrenic acid; C22:5n-6= docosapentaenoic acid; SFA = saturated fatty acids; PUFA = poly-unsaturated fatty acids; MUFA = mono-unsaturated fatty acid; P/S = poly-unsaturated:saturated fatty acid ratio; DBI = double bond index

[2]Pc-value = chromosome-wide *p*-value

[3](Pg)-value = genome-wide *p*-value (the value in side of ( ) means the level significant in genome-wide)

Table 2. Continued

Trait[1]	Pig chromosome	Position (cM)	Pc[2](Pg)[3] value	Reference
C16:0; C16:1 (n-9)	8	86; 86	(p=0.05; p<0.01)	Clop *et al.* (2003)
C18:2; C20:2; C18:0	8	38; 40; 75	(1%); (1%); (5%)	Guo *et al.* (2009)
DBI; P/S; UI; SFA	8	38; 39; 54; 71	(1%); (1%); (1%)	Guo *et al.* (2009)
C16:0	8	66	5%	Sanchez *et al.* (2007)
C16:1; SFA	9	64.6; 78.6	Significant	Nii *et al.* (2006)
C18:0	9	67.6 & 67.6	Significant	Nii *et al.* (2006)
C14:0; C18:0; C20:3	9	27; 14; 88	1%; 1%; (1%)	Sanchez *et al.* (2007)
C14:0	10	82	(p=0.05)	Clop *et al.* (2003)
C14:0	10	66	(1%)	Guo *et al.* (2009)
C16:1; C18:1; C18:2n-6	10	106; 103; 108	5%; 5%; 5%	Sanchez *et al.* (2007)
C20:5; C22:5n-6; MUFA	10	27; 42; 41	(5%); 1%; (5%)	Sanchez *et al.* (2007)
C17:1; C22:6n-3	11	92; 51	5%; 1%	Sanchez *et al.* (2007)
PUFA; P:S	11	89; 63	5%; 5%	Sanchez *et al.* (2007)
C14:0 (not corrected for BF thickness)	12	1	Significant	Uemoto *et al.* (2009)
C16:1 (not corrected for BF thickness)	12	62	Suggestive linkage	Uemoto *et al.* (2009)
C14:0 (corrected for BF thickness)	12	1; 2	Suggestive linkage; significant	Uemoto *et al.* (2009)
C16:1 (corrected for BF thickness)	12	59	Non significant	Uemoto *et al.* (2009)
C14:0; C16:0; C16:1(n-7); C18:0	12	11; 20; 68; 75	1%; 1%; 1%; 1%	Munoz *et al.* (2007)
C18:1(n-7); C20:1(n-); C20:2(n-6)	12	76; 34; 1	1%; 1%; 1%	Munoz *et al.* (2007)
C18:3(n-3)	12	31	(p=0.04)	Clop *et al.* (2003)
C16:1	12	40	5%	Sanchez *et al.* (2007)
C16:1	13	89	(1%)	Guo *et al.* (2009)
C18:1; C22:5n-6; MUFA	13	45; 102; 45	5%; 5%; 5%	Sanchez *et al.* (2007)
C16:0 (perirenal fat)	14	17.2	Significant	Nii *et al.* (2006)
C16:0; C16:1; C18:0	14	22; 67; 67	5%; (1%); (0.1%)	Sanchez *et al.* (2007)
C18:2n-6; C20:3n-3; C22:5n-6	14	22; 21; 32	5%; 1%; 5%	Sanchez *et al.* (2007)
SFA; MUFA; PUFA; P:S	14	45; 67; 23; 23	(1%); 5%; 5%; 1%	Sanchez *et al.* (2007)
C16:0; C18:1; SFA	15	35.9; 57; 45.3	Significant	Nii *et al.* (2006)
C14:0	15	44	(5%)	Sanchez *et al.* (2007)
C16:0 (perirenal fat)	16	0.0	Significant	Nii *et al.* (2006)
C16:0	16	33	5%	Sanchez *et al.* (2007)
C18:2	17	62.3	Significant	Nii *et al.* (2006)
C14:0	18	42.8	Significant	Lee *et al.* (2003)
C20:3; PUFA; C20:3; P/S	X	50; 50; 51; 51	(5%); (5%); (5%); (5%)	Guo *et al.* (2009)
DBI; UI; C16:0; SFA	X	52; 52; 53;55	(1%); (1%); (1%); (1%)	Guo *et al.* (2009)

on SSC4, 6, 8, 10 and 12 both displayed 1% and 5% genome-wide significant effects for FA composition (Table 2). The QTL for C18:2 have been identified in pig chromosome 4. However, a QTL for backfat thickness was identified in the same region from the same pig breed (Varona *et al*., 2002). The discrepancy might be due to the effect of pleiotropic QTL effects between FA and backfat thickness. Also, significant QTLs were detected for the double bond index (DBI), equals the sum of the products of the mole fraction and the number of double bonds for each fatty acid, and unsaturated index (UI), the sum of the percentage unsaturated fatty acids multiplied by their number of double bonds, in SSC6. However, Ovilo *et al*. (2000) also reported that this location also has been associated with intramuscular fat and loin eye area. Also, de Koning *et al*. (1999) described the influence of this region on intramuscular fat content.

Three studies reported QTLs on SSC12 associated with eight FA composition traits, as shown in Table 2 (Clop *et al*., 2003; Munoz *et al*., 2007; Uemoto *et al*., 2009). Munoz *et al*. (2007) suggested that the QTLs closely located at the QTL region affecting C18:0 and C18:1 is in the vicinity of the ACACA gene, which was mapped at SSC12p13-p12 (Calvo *et al*., 2000). Moreover, Wakil *et*

Table 3. List of candidate genes affecting fatty acid composition in cattle and pig

Gene name	Species	GenBank Acc.No.	SNP name	Favorable trait[1]	Favorable genotype or haplotype	Reference
ACACA	Cattle	AJ276223	g.2203 G>T	Triacylglycerol (TAG), total lipid, total SFA and total MUFA	GG	Zhang *et al.* (2009)
			g.2350T>C	C14:0;C14:1; C16:1; C17:1	TC	Zhang *et al.* (2009)
		EU168399	linked SNP (c.4899G>A & c.5196T>C) c.5634T>C	SFA (C14:0; C16:0; C18:0), PUFA (C18:2) & MUFA	Haplotype $A_{4899}C_{5196}$	Gallardo *et al.* (2009)
	Pig	EF618729	c.6681G>T	C16:1(n-9); C18:1(n-7)	C allele	Munoz *et al.* (2007)
				C18:0	G allele	
FASN	Cattle	AF285607	g.17924A>G	C18:1; total MUFA C20:3 & PUFA	GG AA	Zhang *et al.* (2008)
		AF285607	g.17924A>G	C18:1; C16:0	GG	Bhuiyan *et al.* (2009)
		AF285607	g.16024A>G & g.16039T>C	C18:0; C18:1; Ratio MUFA: SFA	TW haplotype	Abe *et al.* (2009)
		AF285607	32 SNPs in chromosome 19 (identified using Illumina BovineSNP50 BeadChip) g.16024A>G	C18:1	No information	Uemoto *et al.* (2010)
		AF285607		C18:1	Haplotype AR>TW	Matsuhashi *et al.* (2010)
LXR-alpha	Cattle	NC_007313	397bpG>A (V1331)	C18:2	Heterozygote	Hoashi *et al.* 2008
SCD	Cattle	NC_007327	878bpT>C	MUFA & low in melting point	Type A A	Taniguchi *et al.* (2004)
		NC_007327	Genotype effect (no SNP information)	C14:1; C18:1; MUFA	Type AA	Ohsaki *et al.* (2009)
		NC_007327	A293V	C14:0; C14:1; C18:0; C18:1 & MUFA	AV	Matsuhashi *et al.* (2010)
		NC_007327	32 SNPs in chromosome 19 (identified using Illumina SNP Chip)	No effect in C18:1	No information	Uemoto *et al.* (2010)
FABP4	Cattle	NC_007312	328 bpG/A (174V)	C16:1	I/I homozygote	Hoashi *et al.* (2008)
FABP3	Pig	X98558	Genotype effect using restriction enzyme	SFA, MUFA & ratio of PUFA to SFA	hh genotype	Lee *et al.* (2010)
MTTP	Pig	NM_214185	c.2573T>C	C16:0; C16:1 (n-9); C18:1(n-2)	C allele	Estelle *et al.* (2009)
ACSL4	Pig	DQ144454	c. 2645G>A	C18:2	No information	Mercade *et al.* (2006)
SREBP-1	Cattle	NC_007317	84-bp indel of SREBP-1	MUFA & low melting point	SS type	Hoashi *et al.* (2007)
		NC_007317	84-bp indel of SREBP-1	C18:0 C18:2; PUFA	LL type SS type	Bhuiyan *et al.* (2009)

[1]Trait: C14:0 = myristic acid; C16:0 = palmitic acid; C16:1 = palmitoleic acid; C16:1(n-9) = palmitoleic acid; C17:1= *cis*-10-heptadecenoic acid; C18:0 = stearic acid; C18:1= oleic acid; C18:2= linoleic acid; SFA = saturated fatty acids; PUFA = poly-unsaturated fatty acids; MUFA=mono-unsaturated fatty acid

al. (1989) suggested that FASN gene is involved in the synthesis of long-chain FAs. The gene was subsequently mapped to HSA17q25 (Jayakumar *et al*., 1994).

In SSC13, Guo *et al*. (2009) reported significant QTLs associated with C16:1 in both *longissimus dorsi* and abdominal fat. A previous study indicated the same chromosome locations harboring QTLs for C18:1, C22:5 and MUFA (Sanchez *et al*., 2007). From the total of 153 QTLs for FA composition in a whole genome reported by Guo *et al*. (2009), no significant QTL was detected on SSC14 for the FA composition traits. On the other hand, Nii *et al*. (2006) reported QTLs in relation to C16:0 in perirenal fat on SSC14. In addition, in SSC14 at 59.3–60.0 cM, the SCD gene was assigned and shown to be highly responsible for conversion of SFAs in MUFA in mammalian adipocytes (Ren *et al*., 2003).

For SSC 15, a QTL has been associated with C16:0, C18:1 and SFA (Nii *et al*., 2006). The possible candidate

gene for this region is FA coenzyme A ligase, long chain 2 (FACL2) (Vidal and Amills, 2004). The formation of fatty acyl-CoAs plays an essential role in many cellular biochemical processes such as lipid metabolism, enzyme activation and protein transport. They are used as substrate of phospholipids (Weimar *et al*., 2002).

Candidate genes affecting FA composition

Candidate genes are the possible causative genes that strongly affect the variations in the target traits. If the candidate genes are located in the QTL region, then they are termed positional candidate genes. The candidate genes in relation to meat quality traits including FA composition have been extensively investigated in the last few decades. In this section, we describe the candidate genes with known associations to FA composition in cattle and pig.

Acetyl-CoA carboxylase alpha (ACACA)

The ACACA enzyme is rate-limiting for the *de novo* synthesis of long-chain FAs (Mao *et al*., 2001). This enzyme plays an important role in the conversion of acetyl-CoA to malonyl-CoA (Abu-Elheiga *et al*., 1997) and acts as a precursor for palmitate (Gallardo *et al*., 2009). Malonyl-CoA inhibits the importation of FAs into the mitochondria for β-oxidation (Barber *et al*., 2005). This enzyme is present in cells as diverse as adipose cells, liver cells and lactating mammary gland cells. Three promoters, promoter I (PI), II (PII) and III (PIII) appear to be involved in the initiation of transcription of the ACACA gene in cattle (Mao *et al*., 2001). PI is the main ACACA promoter in liver cells and is very active in adipose tissue. However, PI is repressed in adipose tissue during lactation. PII is a housekeeping promoter that is expressed constituently (Luo and Kim, 1990) and PIII plays an important role in lipogenesis during lactation (Barber and Travers, 1998; Mao *et al*., 2001). Malonyl-CoA as the product of ACACA is the intermediate substrate for FASN (John *et al*., 1991) in which SFA is synthesized by FASN and undergoes elongation and/or denaturation in bovine adipose tissue to produce SFA and MUFA.

Eight single nucleotide polymorphisms (SNPs) have been identified in PI of the ACACA gene in cattle (Zhang *et al*., 2009). Two of these SNPs are associated with FA composition. Cattle having GG genotype of the g.2203G>T SNP are significantly associated with greater triacylglycerol (TAG), total lipid, total SFA and total MUFA, compared with the animals having GT genotype (Zhang *et al*., 2009). This result indicates that ACACA expression could catalyze the conversion of acetyl-CoA to malonyl-CoA in the cytoplasm, serving as precursors for fat as food storage (Mao *et al*., 2001). Moreover, concerning the g.2350T>C SNP, TC heterozygous animals were reported to possess more myristoleic acid (C14:1), palmitoleic acid (16:1) and cis-10-heptadecenoic acid (C17:1) in the *longissimus dorsi* than CC homozygous animals (Zhang *et al*., 2009).

In pigs, the ACACA gene was mapped on SSC12 in the QTL region at position 75.6 cM in the Duroc commercial line (Gallardo *et al*., 2009; Munoz *et al*., 2007). Of the 21 SNPs, 10 were segregated for lipid metabolism. Two linked SNPs (c.4899G>A and c.5196T>C) were considered to associate with percentage of carcass lean, intramuscular fat (IMF), MUFA, SFA (myristic, palmitic and stearic) and PUFA (linoleic) in the *longissimus thorachis et lumborum* muscle. In addition, the same study indicated that fatter pigs had higher SFA and MUFA, but a lower percentage of PUFA. For cattle, this situation reflects dietary intake, since cattle can not synthesize PUFA precursors such as linoleic acid and linolenic acid (Zhang *et al*., 2009). Therefore, the percentage of PUFA in cattle remains lower than SFA and MUFA. Another study reported that the ACACA gene was a candidate gene for fatty acid composition. The ACACA c.5634C allele had a positive effect on C16:1n-9 and C18:1n-7, whereas c.6681G allele had a positive effect on C18:0 (Munoz *et al*., 2007).

Fatty acid synthase (FASN)

The FASN gene encodes a multifunctional enzyme complex that catalyses the synthesis of long chain SFA (Zhang *et al*., 2008). In the FASN complex, the thioesterase (TE) domain is responsible for termination of FA synthesis. Moreover, this domain has an important role in the determination of the chain length for the FASN protein. Therefore, TE domain variations are implicated as influential for differences in FA composition. TE domains are important regions in the FASN gene because they are potential substrates for elongation and denaturation that are responsible for length determination of the FASN gene. The polymorphisms detected in this TE domain may influence the structure of substrate-binding site and affect the specific activity in TE towards C14-acyl ACP (Zhang *et al*., 2008)

The TE domain is located near the C terminus of the FASN multienzyme complex (Joshi and Smith, 1993) and at the 3'-end of the FASN encoding between exons 39–42 (Zhang *et al*., 2008). This gene has seven active sites that help to catalyze all the reaction steps in the conversion of

acetyl-CoA and malonyl-CoA to palmitate (Roy *et al*., 2005). The QTL for the FASN gene has been detected in chromosome 19 (Morris *et al*., 2007; Ordovas *et al*., 2008; Roy *et al*., 2006). The FASN gene is widely used to identify associations with meat quality traits including FA composition. Mutations in bovine FASN and their associations with FA composition in cattle have been documented (Abe *et al*., 2009, Bhuiyan *et al*., 2009, Matsuhashi *et al*., 2010, Uemoto *et al*., 2010, Zhang *et al*., 2008).

Three SNPs located in the FASN TE domain were identified in Angus bulls (Zhang *et al*., 2008). Two of these SNPs were revealed to be significantly associated with FA composition. The g.17924GG genotypes had significantly greater health index, C18:1 and total MUFA compared to the AA genotypes, whereas the g.17924AA genotypes were more favorable in C20:3 and PUFA. These studies differ from a study with Japanese Black cattle that detected a lower concentration of C14:0, C16:0, C14:1 and C16:1 and higher concentration of C18:0, C18:1, MUFA and SFA (Abe *et al*., 2009). In addition, Moris *et al*. (2007) identified a QTL on bovine chromosome 19 and detected that the FASN gene in the QTL region, which was associated with C14:0 in subcutaneous adipose tissue and milk fat. Another study reported five SNPs in the FASN TE domain that were identified in Korean Hanwoo cattle. Of these SNPs, significant associations were identified with C16:0 and oleic C18:1 (Bhuiyan *et al*., 2009). In case of Japanese Black and Limousin cattle, the TW FASN gene haplotype had significant effects with increasing C18:0 and C18:1 and decreasing C14:0, C14:1, C16:0 and C16:1 (Abe *et al*., 2009). Using haplotype analysis, Matsuhashi *et al*. (2010) reported that the AR>TW haplotype of the FASN gene was decreasing the proportion of C14:0, C14:1, C16:0 and C161, but increasing C18:1. Very recently, FASN was associated with C18:1 within Japanese Black cattle using Illumina Bovine SNP50 BeadChip (Uemoto *et al*., 2010).

The porcine FASN gene has been mapped on chromosome 12 (Jayakumar *et al*., 1994; Wakil, 1989) and the FASN map location was consistent with the comparative mapping information between human and pig (Munoz *et al*., 2003). Previously, Clop *et al*. (2003) identified a significant QTL for backfat FA composition and related metabolic traits. However, no study has reported an association of the FASN gene with FA composition in pig.

Stearoyl-CoA desaturase (SCD)

The SCD gene has been extensively studied in cattle, compared with pig and chicken. SCD is an enzyme that catalyzes the conversion of SFA into MUFA in mammalian adipocytes (Taniguchi *et al*., 2004) and catalyzes the delta (Δ) 9 desaturation of SFA and MUFA (Ohsaki *et al*., 2009). SCD also contributes the synthesis of UFAs by insertion of a cis-double bond in the delta 9 position of FA substrate (Kim and Ntambi, 1999). The preferable substrates are palmitate and stearate, which are converted to palmitoleate and oleate, respectively. These substrates are the major constituent membrane phospholipids and triacylglycerol that is stored in adipose tissue. The ratio of stearic acid to oleic acid is one of the important factors influencing membrane fluidity, and this ratio plays an important role in various human diseases such as cancer, diabetes, obesity, hypertantion and neurological disease (Ntambi, 1995).

The type A SCD gene has been linked to high a MUFA percentage and low melting point in the intramuscular fat of Japanese Black cattle (Taniguchi *et al*., 2004). The lower melting point contributes positively to favorable beef flavor and tenderness. Similarly, Taniguchi *et al*. (2004) analyzed the level of SCD mRNA expression in muscle and fat tissue in different breeds, and reported that a transcriptional regulator of SREBP1 (sterol responsible-element binding protein 1c) might affect MUFA variation.

Ohsaki *et al*. (2009) reported the effect of SCD genotypes in the sire of Japanese Black cattle; there were highly significant associations with C14:1, C18:0, C18:1, MUFA, SFA, MUFA/SFA, C14:1/C14:0+C14:1 and C18:1 /C18:0+C18:1. However, based on the Tukey's HSD (Honestly Significant Differences) test, the SCD gene showed significant effects or has favorable traits in C14:1, C18:1 and MUFA. A previous study in the same cattle reported that animals having AA genotype of the SCD gene have a higher MUFA and a lower melting point (Taniguchi *et al*. 2004). However, the authors suggested that SCD was not a major factor for FA composition in Japanese Black cattle because SCD genotypes contributed to only 4% of MUFA variation. Another study reported that the AV genotype in bovine SCD gene displays a statistically higher level of C14:0, C14:1, C18:0, C18:1 and MUFA (Matsushasi *et al*., 2010). However, a very recent study using Illumina BovineSNP50 did not reveal an association between the SCD gene and C18:1 (Uemoto *et al*., 2010).

Fatty acid binding proteins (FABPs)

FABPs belong to the member of a superfamily of lipid-binding protein (Chmurzynska, 2006). The FABP genes

are divided into two main groups based on the cellular level: FABPs located in plasma membrane and those that are intracellular or cytoplasmic (Glatz and van der Vusse 1996). Based on the tissue-specific distribution, Chmurzynska (2006) reviewed FABPs in nine different tissues and designated them as: L (liver), I (intestinal), H (muscle and hearth), A (adipocyte), E (epidermal), Il (ileal), B (brain), M (myelin) and T (testis).

FABPs act mainly as FA transporters in the metabolic pathway (Estelle *et al*., 2009), where the peroxisome proliferators-activated receptor (PPAR) family members cooperatively participate as transcription factors. Therefore, the PPAR family plays an important role in regulating the transcription of many genes involved in lipid metabolisms (Desvergne and Wahli, 1999).

Many studies have been carried out for the association with FABP polymorphisms and fat-related traits in animals. In cattle, the association between FABP4 polymorphisms and fatty acid composition (C16:1) has been identified (Hoashi *et al*., 2008). This FABP4 gene also affects the marbling and subcutaneous fat in Wagyu and Limousin F2 crosses (Michal *et al*., 2006) and IMF in Australian cattle (Barendse *et al*., 2009). In Korean cattle (Hanwoo), the FABP4 gene was associated with backfat thickness (Cho *et al*., 2008), marbling and carcass weight (Lee *et al*., 2010). However, no association was evident between FABP4 and IMF in crossbred *Bos taurus* cattle (Pannier *et al*., 2010).

In pig, some FABP family members are involved in fat-related traits. Heart-FABP (H-FABP; FABP3) is most widely investigated gene in relation to fat. Significant associations between the H-FABP gene and IMF contents have been reported in pig (Arnyasi *et al*., 2006; Gerbens *et al*., 2001; Li *et al*., 2006; Urban *et al*., 2002). H-FABP was also significantly associated with backfat thickness in purebred Yorkshire pigs (Cho *et al*., 2010) and Polish Large White and Polish Landrace (Chmurzynska *et al*., 2007). In addition, the positional candidate gene, FABP2, has been investigated for the QTL on porcine chromosome 8. The association between FABP2 and FA composition was not be significant (Estelle *et al*., 2009). However, pigs having hh genotypes had higher UFA content, and a higher ratio of PUFA to SFA in Berkshire breed (Lee *et al*., 2010).

Other candidate genes

The microsomal triglyceride transfer protein (MTTP) gene provides instructions for the manufacture of microsomal triglyceride transfer protein. This gene plays an essential role in lipid transfer during the assembly of lipoproteins in the liver and intestine (Hussain *et al*., 2003). A MTTP gene SNP was highly associated with palmitoleic acid and oleic acid in a F2 cross pig population (Estelle *et al*., 2009). The most likely explanation is that the FA composition of monogastric meat and fat depends mainly on dietary fat (Mourot and Hermier 2001). However, weight and slaughter age also contributes to the differences in FA profile.

Long-chain-CoA synthase 4 (ACSL4) is a member of the ACSL family that is involved in the two main metabolic pathways: the pathway for anabolic conversion of FAs to cellular lipids and the pathway for catabolism of FAs through β-oxidation (Van Horn *et al*., 2005). Mercade *et al*. (2006) reported that the ACSL4 polymorphisms were associated with percentage of oleic FA in pigs.

Sterol regulatory binding proteins (SREBPs) are membrane bound transcription factors that function mainly in energy homeostasis by promoting glycolysis, lipogenesis and adipogenesis (Hoashi *et al*., 2007). The SREBP1 gene regulates the enzyme responsible for synthesis of cholesterol, FA and the low density lipoprotein receptor (Horton *et al*., 1998). This gene is closely related with the SCD gene. The expression level of the SREBP1 gene may affect the expression level of SCD gene, leading to differences in FA composition. Polymorphisms in an 84 bp insertion/deletion (indel) in intron 5 of the bovine SREBP-a gene have been associated with higher MUFA and lower melting point in the SS genotype of Japanase Black cattle (Hoashi *et al*., 2007). A similar study in Korean cattle (Hanwoo) reported higher C18:0 and C18:2 with the LL and SS genotype, respectively (Bhuiyan *et al*., 2009).

Conclusion

QTLs and candidate genes, which were associated with FA composition, have been amply identified in cattle and pig. The present review can give a useful guideline for the current researches for FA composition in cattle and pig. Appropriate markers, that will be developed soon, can be applied to select animals that produce healthy and good quality beef and pork. Therefore, more molecular markers are developed, animal and meat industry will have benefits not only for decreasing of the selection intervals, but also producing meat products that consumers need.

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