Polymorphic effects of *FABP4* and *SCD* genes on intramuscular fatty acid profiles in longissimus muscle from two cattle breeds

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Abstract

The aim of this study was to evaluate the polymorphic effects of two single nucleotide polymorphisms (SNPs) of fatty acid binding protein (FABP4) and stearoyl-CoA desaturase (SCD) genes on intramuscular fatty acid profiles in the longissimus muscle in two cattle breeds. Two previously reported SNPs of bovine *FABP4* (7516G>C) and *SCD* (878C>T) were in turn assessed for their associations with intramuscular fatty acid profiles from the upper sirloin cuts of Aberdeen Angus and Blonde d'Aquitaine cattle. In total, 33 animals were genotyped using PCR-RFLP. Intramuscular fatty acid composition was evaluated using two complementary statistical approaches: a classical univariate regression model and a multivariate approach using a combination of Principal Component Analysis and Random Forests. Significant effect of *FABP4* SNP genotypes was found for several fatty acids including C15:0, C17:0, C18:0, C14:1, C17:1, C18:2n6, C20:4n6, C20:5n3, C22:5n3, total n-3, n-6 and total SFA ($P < 0.05$). These results suggest that *FABP4* is a potential candidate gene affecting fatty acid composition in beef cattle.

Meat, PUFA, MUFA, principal component analysis, random forests

Beef quality can be evaluated on the basis of taste, smell, colour, tenderness or juiciness. It is known and commonly accepted that these factors are influenced by the breed, sex, age and diet of the animal (Homolka and Kudrna 2008). Taniguchi et al. (2004) and Matsuhashi et al. (2011) showed that the fatty acid profile has an impact on beef flavour and tenderness. The resulting nutritional value after culinary preparation of beef (roasting, baking, and grilling) is largely influenced by the content and composition of fatty acids (FA) as they represent the most important component of lipids (Homolka and Kudrna 2008). Fatty acids can be divided into several groups: saturated fatty acids (SFA), unsaturated fatty acids with one double bond (monounsaturated, MUFA), unsaturated fatty acids with two or more double bonds (polyunsaturated, PUFA) (Homolka and Kudrna 2008).

The *FABP4* - fatty acid binding protein - 4 belongs to the FABPs group of small conserved intracellular lipid-binding proteins (Cho et al. 2008). They are involved in the absorption, transport and metabolism of fatty acids (Kulig et al. 2010) and in particular bind long-chain fatty acid with high affinity. *FABP4* mediates the transfer of FA during lipolysis (Hoashi et al. 2008); therefore it has an important role in lipid metabolism and is legitimately considered a candidate gene for obesity (Michal et al. 2006). Together with *FABP5*, *FABP4* gene is located on the bovine chromosome 6 (Cho et al. 2008). It has four exons and three introns with 4.39 kb length and the cDNA encodes a peptide of 132 amino acids (Flicek et al. 2014).

Michal et al. (2006) identified SNP 7516G>C and described the effect of this particular SNP on the carcass trait. Furthermore, the SNP was tested by Lee et al. (2013) and Avilles et al. (2013) with similar observations.

The *SCD* – Stearoyl CoA desaturase - is an enzyme controlled *SCD* gene that catalyses the conversion of SFA to MUFA (Taniguchi et al. 2004; Kühn et al. 2005). The main

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sources of the enzyme *SCD* in cattle are adipose tissues in growing animals and mammary glands in lactating animals (Keating et al. 2006). So the *SCD* gene can be marked as one of the genes responsible for fatty acid composition (Mannen 2011). According to Ohsaki (2009), the *SCD* gene has particular influence on the stearic acid and oleic acid presence in beef. Milanesi (2008) states that the *SCD* enzyme is also involved in the endogenous synthesis of CLA. Bovine *SCD* gene is located on chromosome 26, consists of 6 exons and 5 introns and its size is approximately 17 kb (Alim et al. 2012). Taniguchi et al. (2004) describe 8 SNP sites. In the open reading frame (ORF) substitutions were found at positions 702bp (G>A), 762bp (C>T) and 878bp (T>C) and in the untranslated region (3'UTR) at positions 1905bp (T>C), 3134bp (C>T), 3351bp (A>G), 3537bp (A>G), and 4736bp (A>G). SNP C878T was predicted to cause the substitution of the amino acids valine to alanine at amino acid position 293. This substitution has an effect on the fatty acid composition and melting point of intramuscular fat (IMF). Mannen (2011) confirmed that alanine compared to valine increases the proportion of monounsaturated fatty acids at the expense of saturated fatty acids and also decreases the melting point of IMF. Similar conclusions were reached by other authors (Taniguchi et al. 2004; Matsuhashi et al. 2011; Kaplanová et al. 2013).

In this study, we investigated the single nucleotide polymorphisms (SNPs) of *FABP4* gene (the 7516G>C SNP) and *SCD* gene (SNP 878T>C), which have been reported to have an effect on the fatty acid composition and content in beef and therefore influence its flavour and juiciness (Taniguchi et al. 2004; Michal et al. 2006; Hoashi et al. 2008; Matsuhashi et al. 2011). The purpose of this work was to evaluate the relationship between these genes and the FA content in Aberdeen Angus and Blonde d'Aquitaine breeds of beef cattle. We presented two complementary analytical approaches for the statistical evaluation of this relationship: i) the conventional method based on general linear models with Tukey's HSD multiple comparisons test and ii) the multivariate indirect method based on a combination of principal components analysis and random forests. To the best of our knowledge, such complementary approach had not been previously attempted. The univariate approach tests for significant associations between genotype and fatty acid profiles by taking into account one FA at a time. The complementary multivariate approach shown in this work takes a more holistic approach of considering all FA and arriving at a minimal set of FA which is different between the genotypes of interest.

The aim of this study was to analyse the relationship between polymorphisms in the two genes (FABP4; SCD) and fatty acid content in the longissimus muscle from two cattle breeds.

Materials and Methods

Animals and samples

Samples of the longissimus muscle (upper sirloin) were obtained on days 7 to 10 after slaughter from Aberdeen Angus (n = 17) and Blonde d'Aquitaine (n = 16) bulls. Each breed was from a different slaughter house. The animals were fed with a traditional mixed ration (TMR) which contained: corn silage, clover grass silage with high dry matter content, meadow hay, barley straw, grain mixes (barley, wheat, soybean extraction meal, rapeseed extraction meal) and mineral supplements according to nutritional value.

Fatty acid extraction and analysis

For analysis, we chose the following fatty acids and their groups: C14:0, C14:1, C15:0, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:1t, C18:2n6, C18:3n3, C18:3n6, C20:0, C20:1, C20:3n3. C20:3n6, C20:4n6, C20:5n3, C22:5n3, C22:6n3, Cla (conjugated linolei acid), general MUFA, PUFA, SFA, n3 and n6 and n6/n3 ratio.

Fatty acids were extracted as follows: to 2 g of homogenized meat, 4 ml of methanol and 2 ml of dichloromethane were added and shaken well. This mixture was filtered through a funnel containing anhydrous sodium sulphate. Two ml of methanol and 2 ml distilled water were added to the filtrate and shaken well. The lower phase was recovered after centrifugation at 115.19 *g* for 5 min at room temperature. This clear solution was concentrated under a gentle stream of N_2. Conversion of extracted fatty acids to methyl esters was done using the methanolic KOH method: To the vial containing the extracted fat, 2 ml heptane were added, followed by 2 ml of

2M methanolic KOH, after which the vial was vortexed for 1 min. One gram of anhydrous sodium sulphate was added, shaken well and allowed to settle. The clear solution was transferred into a GC vial for analysis using a gas chromatograph (Agilent 7890A) fitted with a HP88 column suited for fatty acid analysis. Peak identification was based on matching the retention time with spectra from the Supelco 37 component FAMEs mix as the reference standard. The temperature program was as follows: 60 °C was held for 1 min; followed by a ramp of 15 °/min to 150 °C and then 2.5 °/min to 230 °C for 5 min. The carrier gas was hydrogen (5N purity) at a flow rate of 1.5 ml/min; the solution was injected with a split ratio of 1:20. The injector as well as the flame-ionization detector was set to 250 °C.

DNA genotyping

Total genomic DNA was extracted by JETQUICK Tissue Spin Kit (Genomed, Bad Oeynhausen, Germany) from meat samples according to manufacturer's instructions and stored at -20 °C. Alleles of *FABP4* and *SCD* genes were determined by means of the PCR-RFLP method. The PCR amplification was performed with 50 ng of genomic DNA for *FABP4* gene and 100 ng of genomic DNA for *SCD* gene. PCRs were carried out on a Veriti® 96-Well Thermal Cycler (Applied Biosystems, Foster City, USA) in a volume of 15 µl containing 1 × Combi PPP Master Mix (with 6.25 nkat *Taq* Purple polymerase) (Top-Bio, Prague, Czech Republic), 0.4 µM of each primer of *FABP4* and 0.67 µM of each primer of *SCD*.

Amplification was performed using the following thermo-cycling protocol: initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 20 s (denaturation), 60–64 °C for 20 s (annealing) and 72 °C for 30 s (elongation) with final extension step 72 °C for 7 min for the *SCD* gene. For the *FABP4* gene, each phase (denaturation, annealing and elongation) took 30 s. The primer pairs of *FABP4* and *SCD* were previously reported by Michal et al. (2006) and Taniguchi et al. (2004), respectively. Primer pairs, annealing temperatures, PCR product sizes and restriction nucleases are presented in Table 1.

Table 1. Genes, primer pairs, annealing temperatures, PCR product sizes and restriction nucleases.

Gene	Forward primer Reverse primer	Annealing temperature	PCR product sizes (bp)	Restriction nuclease
	FABP4 F 5′ ATATAGTCCATAGGGTGGCAAAGA 3′			
FABP4	FABP4 R 5′ AACCTCTCTTTGAATTCTCCATTCT 3′	60 °C	452	MspA1I (166.7 nkat/µl)
	SCD F 5′ ATGTATGGATACCGCCCTTATGAC 3′			
SCD	SCD R 5′ CTGTCCCTTAGTTTTATAGTGGAATG 3′	64 °C	209	Fnu4HI (83.35 nkat/µl)

Successful gene amplifications were verified by electrophoresis on 3–4% agarose gel containing TBE and ethidium bromide for visualization under ultraviolet light. PCR products were digested with restriction nucleases as shown in Table 1 that were obtained from New England Biolabs, Inc., USA. Digestion was conducted at 37 °C for 3–4 h using a 15 µl reaction solution contained 10 µl of PCR products, 10 × NEBuffer 4 (New England BioLabs), and 66.68–83.35 nkat of restriction nucleases. The DNA fragments after digestion were separated by electrophoresis on 3–4% agarose gel. To determine the size of the fragments we used the DNA length standard (O'gene ruler low range DNA ladder, manufacturer Thermo Scientific). The variation at the position *FABP4* and *SCD* was resulting in the presence/absence of the specific restriction site.

Statistical analysis of data

The PowerMarker program (Liu and Muse 2005) was used for the calculation indicators of genetic diversity. Both the genetic equilibrium and significance were estimated using the $\chi 2$ test and LRT value (Hardy-Weiberg equilibrium). Calculations of i) genetic diversity (defined as the probability that two randomly chosen alleles from the population are different), ii) heterozygosity (the proportion of heterozygous individuals in the population) and iii) PIC values (polymorphism information content) for the breeds were carried out. Polymorphism information content is a modification of the heterozygosity measure that subtracts from the H-value an additional probability that an individual in a linkage analysis does not contribute information to the study (Speer 1999; Nagy et al. 2012).

Relevant variables were estimated as follows:

Polymorphism information content: $$PIC_l = 1 - \sum_{u=1}^{k} \overline{p}_{lu}^{2} - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\overline{p}_{lu}^{2}\,\overline{p}_{lv}^{2}$$

Heterozygosity: $$H_l = 1 - \sum_{u=1}^{k} \overline{P}_l$$

Gene diversity: $D = (1 - \sum_{u=1}^{k} p_{lu}^{-2})$

The area under each peak of the chromatogram was converted to percentage data. The effect of genes (*FABP4* and *SCD*) on individual fatty acids was checked using a linear model, where the genotype was the only (fixed) effect. A few fatty acids violated assumptions of homogeneity of variances so they were analysed after log-transformation. Homogeneity of variances was tested using Flinger-Killeen test. Results show percent estimates from the model. Analysis was performed separately for each breed because we did not want a possible effect of breed to add variability to our dataset.

To complement our results we also employed a multivariate approach. Here, we used a combination of principal component analysis (PCA) and random forests (RF). We used random forests version 4.6–10 (Breiman 2001; Liaw and Wiener 2002) to arrive at a minimum set of variables (fatty acids) to differentiate groups of interest. These fatty acids are used to compare the PCA plot with all the variables before the variable selection to a PCA plot with only the selected fatty acids.

We used the varSelRF package version 0.7–3 (Uriarte and deAndrés 2006) for the random forests algorithm to find a minimum set of predictor variables to distinguish genotypes, i.e., to classify CC from CG from GG. We employed 100 bootstrapping iterations of the random forests algorithm to arrive at a minimal set of FA which could differentiate fatty acid profiles of CC, CG and GG for FABP4. All statistical analyses were performed using R software version 3.0.2 (R Core Team 2013).

Results

Evaluation of genetic diversity, heterozygosity and PIC is an important part of the genetic characteristics of populations. Basic characteristic of the gene polymorphism in meat samples (allele and genotype frequencies, genetic diversity, heterozygosity and PIC) found in this study are presented in Table 2. For both genes (*FABP4* and *SCD*) we obtained values of genetic diversity (0.3967, 0.4927), heterozygosity (0.3636, 0.4545) and PIC value (0.3180, 0.3713). Both genes (*FABP4* and *SCD*) were shown to be polymorphic (Oh et al. 2012; Kaplanová et al. 2013) and our study confirms this observation. Both genes were found to be in the genetic Hardy-Weinberg equilibrium.

Table 2. Genotype and allele frequencies, genetic diversity, heterozygosity and PIC of SCD and FABP4 genes.

Gene	Genotype	n	Frequency	Allele	Frequency
FABP4	CC	18	0.5455	C	0.7273
	CG	12	0.3636		
	GG	3	0.0909	G	0.2727
SCD	CC	11	0.3333	G	0.5606
	CT	15	0.4545		
	TT	7	0.2121	T	0.4394

Gene	Genetic diversity	Heterozygosity	PIC	HWE Disequilibrium	
				Chi Square value	Exact *P*-value
FABP4	0.3967	0.3636	0.318	0.2292	0.690
SCD	0.4927	0.4545	0.3713	0.1975	0.747

n = number of animals with a relevant genotype, HWE = Hardy-Weinberg equilibrium,
PIC = polymorphism information content

For Aberdeen Angus, SNP 7516G>C of the *FABP4* gene was polymorphic. Frequency of the major allele (C) was 0.6471, and of the rare genotype (GG) 0.1176. This polymorphism showed a significant difference ($P = 0.022$) between CC and CG genotypes for the myristoleic acid content. We also observed a significant difference

($P = 0.048$, $P = 0.031$ and $P = 0.03$) between CC and CG genotypes for C17:0, C18:2n6, and total n-6, respectively.

For Blonde d'Aquitaine, SNP 7516G>C of the *FABP4* gene was polymorphic. Frequencies of the major allele (C) was 0.8125 and of the rare genotype (GG) was 0.0625. Among our samples of Blonde d'Aquitaine only one was a GG homozygote; therefore we excluded this sample from the statistical analyses. However, we observed significant differences between genotypes CC and CG and contents of generally SFA ($P = 0.026$), C15:0 ($P = 0.012$) and C18:0 ($P = 0.042$), where the homozygote CC had significantly lower contents compared to heterozygote. Similarly, homozygote CC had a significantly lower content of monounsaturated FA C17:1 ($P = 0.046$). On the other hand, animals with the genotype CC showed significantly higher percentage content of C20:5n3 ($P = 0.050$), C22:5n3 ($P = 0.011$) and generally n-3 ($P = 0.044$) compared with the CG ones. The content of C20:4n6 ($P = 0.044$) was significantly higher in the CC genotype than CG.

Table 3. Association between *FABP4* genotypes and the fatty acid composition in Aberdeen Angus.

Fatty acid	CC (n = 7)	CG (n = 8)	GG (n = 2)	*P*-value	Pairwise differences
C14.0	1.98 ± 0.16	1.61 ± 0.15	1.65 ± 0.31	0.278	
C14.1	0.11 ± 0.05	0.23 ± 0.04	0.28 ± 0.09	0.022	a b ab
C15.0	0.60 ± 0.08	0.42 ± 0.08	0.41 ± 0.16	0.273	
C16.0	22.51 ± 0.83	21.54 ± 0.78	23.27 ± 1.56	0.530	
C16.1	2.95 ± 0.20	2.40 ± 0.19	2.40 ± 0.38	0.149	
C17.0	2.07 ± 0.20	1.36 ± 0.19	1.33 ± 0.37	0.048	a b ab
C17.1*	0.06 ± 0.37	0.19 ± 0.34	0.25 ± 0.68	0.054	
C18.0	9.83 ± 0.70	10.98 ± 0.66	7.07 ± 1.32	0.055	
C18.1	33.74 ± 2.09	29.07 ± 1.96	28.47 ± 3.92	0.248	
C18.1t	2.76 ± 0.77	2.00 ± 0.72	2.70 ± 1.43	0.753	
C18.2n6	12.20 ± 1.51	17.91 ± 1.42	18.88 ± 2.83	0.031	a b ab
C18.3n3	1.11 ± 0.54	1.66 ± 0.51	1.47 ± 1.01	0.758	
C18.3n6	0.52 ± 0.17	0.51 ± 0.16	0.14 ± 0.33	0.584	
C20.0	0.11 ± 0.09	0.25 ± 0.09	0.29 ± 0.18	0.506	
C20.1	1.65 ± 0.36	0.98 ± 0.34	0.22 ± 0.68	0.172	
C20.3n3	0.07 ± 0.03	0.09 ± 0.02	0.03 ± 0.05	0.615	
C20.3n6	0.67 ± 0.13	1.08 ± 0.12	1.18 ± 0.25	0.074	
C20.4n6	3.63 ± 0.53	5.09 ± 0.49	5.15 ± 0.99	0.137	
C20.5n3	0.68 ± 0.16	0.65 ± 0.15	0.83 ± 0.30	0.867	
C22.5n3	1.25 ± 0.16	1.19 ± 0.15	1.27 ± 0.31	0.954	
C22.6n3	0.18 ± 0.04	0.09 ± 0.04	0.07 ± 0.07	0.245	
cla	0.39 ± 0.05	0.28 ± 0.05	0.26 ± 0.09	0.216	
SMUFA	36.39 ± 2.23	31.47 ± 2.09	30.88 ± 4.18	0.220	
S (n-3)	3.21 ± 0.78	3.60 ± 0.73	3.65 ± 1.47	0.928	
S (n-6)	17.61 ± 2.12	25.47 ± 1.99	26.69 ± 3.97	0.030	a b ab
(n-6)/(n-3)	6.30 ± 1.57	9.79 ± 1.47	7.31 ± 2.94	0.291	
SPUFA	20.30 ± 2.38	28.27 ± 2.23	29.04 ± 4.46	0.061	
SSFA*	28.50 ± 1.04	28.50 ± 1.03	31.82 ± 1.07	0.659	

*Fatty acid what was not homogenous and was analyzed after log transformation; a–b Different letters within a row indicate significant differences between genotypes; ± value is standard error;
CLA = conjugated linolei acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid

Table 4. Association between FABP4 genotypes and the fatty acid composition in Blonde d'Aquitaine.

Fatty acid	CC (n = 11)	CG (n = 4)	GG (n = 1)	*P*-value	Pairwise differences
C14.0	1.24 ± 0.16	1.70 ± 0.26		0.163	
C14.1	0.20 ± 0.07	0.33 ± 0.12		0.366	
C15.0	0.43 ± 0.04	0.64 ± 0.06		0.012	a b
C16.0	20.37 ± 0.60	21.79 ± 1.00		0.244	
C16.1	2.11 ± 0.21	2.26 ± 0.35		0.718	
C17.0	1.55 ± 0.09	1.73 ± 0.15		0.311	
C17.1	0.10 ± 0.02	0.20 ± 0.04		0.046	a b
C18.0	10.19 ± 0.48	12.27 ± 0.79		0.042	a b
C18.1	27.51 ± 1.43	26.52 ± 2.37		0.726	
C18.1t	1.11 ± 0.19	1.79 ± 0.32		0.088	
C18.2n6	22.29 ± 1.19	20.76 ± 1.98		0.520	
C18.3n3	1.97 ± 0.35	1.17 ± 0.59		0.260	
C18.3n6	0.44 ± 0.15	0.84 ± 0.25		0.196	
C20.0	0.08 ± 0.01	0.12 ± 0.02		0.112	
C20.1	0.90 ± 0.27	1.16 ± 0.45		0.633	
C20.3n3	0.05 ± 0.02	0.06 ± 0.03		0.813	
C20.3n6	1.22 ± 0.10	0.9 ± 0.16		0.104	
C20.4n6	5.25 ± 0.34	3.80 ± 0.56		0.044	a b
C20.5n3	0.90 ± 0.08	0.56 ± 0.13		0.050	a b
C22.5n3	1.72 ± 0.12	1.02 ± 0.20		0.011	a b
C22.6n3	0.11 ± 0.01	0.07 ± 0.02		0.082	
cla	0.24 ± 0.03	0.32 ± 0.06		0.288	
SMUFA	29.62 ± 1.53	29.62 ± 2.54		0.781	
S (n-3)	4.70 ± 0.44	2.28 ± 0.72		0.044	a b
S (n-6)	30.73 ± 1.59	26.62 ± 2.64		0.205	
(n-6)/(n-3)*	6.72 ± 1.11	11.06 ± 1.19		0.031	a b
SPUFA	33.95 ± 1.75	29.18 ± 2.90		0.182	
SSFA*	31.76 ± 1.02	35.55 ± 1.04		0.026	a b

*Fatty acid what was not homogenous and was analyzed after log transformation
a–b Different letters within row indicate significant differences between genotypes ± value is standard error; CLA = conjugated linolei acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid

Principal component analysis

For Aberdeen Angus meat samples with a model frequency of 84%, C14:1 + (n-6) represented the minimum set of fatty acids needed to distinguish the three genotypes CC, CG and GG. In other words, the combination of C14:1 and (n-6) appeared in 84% of the iterations to clearly distinguish the three genotypes. A PCA with all the fatty acids was able to explain only 52.59% of the variation in the first two principal components (Plate III, Fig. 1). But a PCA with only these two variables was able to explain 100% of the variation present in the data (Plate III, Fig. 2).

For Blonde d'Aquitaine meat samples with a model frequency of 98%, C20:1 + C18:1t represented the minimum set of fatty acids needed to distinguish the two genotypes CC and CG. In other words, the combination of C20:1 and C18:1t appeared in 98% of the iterations to clearly distinguish the two genotypes. A PCA with all the fatty acids was able to explain only 61.04% of the variation in the first two principal components (Plate IV, Fig. 3). But a PCA with only these two variables was able to explain 100% of the variation present in the data (Plate IV, Fig. 4), as was the case of Aberdeen Angus meat samples.

The SNP 878T>C was polymorphic too. Frequencies of the minor allele (T) was 0.43 and of the rare genotype (TT) was 0.2121. Results for the *SCD* gene did not show any significant results (as shown in Tables 5 and 6) either within a separate analysis according to the breed, or when combined together. Therefore, we did not carry out further PCA analyses with the SCD gene, only for the FABP4 gene.

Table 5. Association between SCD genotypes and the fatty acid composition in Aberdeen Angus.

Fatty acid	CC (n = 9)	CT (n = 4)	TT (n = 4)	*P*-value
C14.0	1.68 ± 0.15	2.02 ± 0.22	1.71 ± 0.22	0.435
C14.1	0.34 ± 0.05	0.36 ± 0.08	0.23 ± 0.08	0.425
C15.0	0.47 ± 0.08	0.64 ± 0.11	0.40 ± 0.11	0.334
C16.0	21.68 ± 0.72	23.49 ± 1.08	21.83 ± 1.08	0.382
C16.1	2.48 ± 0.19	2.99 ± 0.28	2.60 ± 0.28	0.342
C17.0	1.53 ± 0.21	2.03 ± 0.31	1.53 ± 0.31	0.390
C17.1	0.16 ± 0.07	0.13 ± 0.11	0.34 ± 0.11	0.337
C18.0	10.73 ± 0.70	9.81 ± 1.05	8.74 ± 1.05	0.313
C18.1	29.66 ± 1.97	33.25 ± 2.95	31.44 ± 2.95	0.599
C18.1t	2.82 ± 0.67	2.14 ± 1.00	1.69 ± 1.00	0.626
C18.2n6	15.76 ± 1.62	13.35 ± 2.43	17.81 ± 2.43	0.449
C18.3n3	1.84 ± 0.46	0.88 ± 0.69	0.97 ± 0.69	0.412
C18.3n6	0.48 ± 0.15	0.62 ± 0.23	0.29 ± 0.23	0.604
C20.0	0.22 ± 0.09	0.11 ± 0.13	0.22 ± 0.13	0.760
C20.1	1.08 ± 0.35	1.63 ± 0.53	0.90 ± 0.53	0.593
C20.3n3	0.10 ± 0.02	0.07 ± 0.03	0.02 ± 0.03	0.199
C20.3n6	0.90 ± 0.13	0.74 ± 0.19	1.17 ± 0.19	0.316
C20.4n6	4.70 ± 0.51	3.7 ± 0.77	4.82 ± 0.77	0.512
C20.5n3	0.80 ± 0.13	0.42 ± 0.20	0.70 ± 0.20	0.329
C22.5n3	1.31 ± 0.14	1.01 ± 0.21	1.25 ± 0.21	0.514
C22.6n3	0.14 ± 0.03	0.16 ± 0.05	0.06 ± 0.05	0.369
cla	0.32 ± 0.04	0.44 ± 0.06	0.22 ± 0.06	0.064
SMUFA	32.14 ± 2.10	36.24 ± 3.16	34.04 ± 3.16	0.562
S (n-3)	4.08 ± 0.65	2.48 ± 0.97	2.98 ± 0.97	0.364
S (n-6)	23.19 ± 2.26	18.68 ± 3.40	24.77 ± 3.40	0.428
(n-6)/(n-3)*	6.36 ± 1.19	8.17 ± 1.28	8.33 ± 1.28	0.567
SPUFA	26.01 ± 2.42	20.97 ± 3.36	27.10 ± 3.63	0.440
SSFA	34.09 ± 1.06	35.32 ± 1.58	32.28 ± 1.58	0.414

*Fatty acid what was not homogenous and was analyzed after log transformation
± value is standard error; CLA = conjugated linolei acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid

Discussion

Our results presented in Table 2 are in accordance with results of heterozygosity of polymorphism 7516G>C of the *FABP4* gene obtained by others. Lee et al. (2013) tested 141 bulls of the breeds Korean brindle cattle and Black cattle, and calculated the heterozygosity of H = 0.3964; Avilles et al. (2013) calculated the heterozygosity at 0.35 for 98 Charolais bulls and Michal et al. (2006) calculated H = 0.3103 for 232 crossbred Wagyu × Limousine bulls.

Table 6. Association between SCD genotypes and the fatty acid composition in Blonde d'Aquitaine.

Fatty acid	CC (n = 2)	CT (n = 10)	TT (n = 3)	*P*-value
C14.0	1.43 ± 0.43	1.32 ± 0.18	1.18 ± 0.35	0.834
C14.1	0.47 ± 0.16	0.21 ± 0.07	0.10 ± 0.13	0.230
C15.0	0.38 ± 0.11	0.51 ± 0.05	0.42 ± 0.09	0.388
C16.0	22.22 ± 1.54	20.33 ± 0.66	20.18 ± 1.26	0.551
C16.1	2.85 ± 0.49	2.02 ± 0.21	1.75 ± 0.40	0.212
C17.0	1.48 ± 0.23	1.57 ± 0.10	1.61 ± 0.19	0.873
C17.1	0.10 ± 0.05	0.15 ± 0.02	0.06 ± 0.04	0.267
C18.0	10.34 ± 1.23	11.24 ± 0.52	9.57 ± 1.00	0.404
C18.1	30.01 ± 3.43	25.66 ± 1.46	28.66 ± 2.80	0.516
C18.1t	0.76 ± 0.47	1.42 ± 0.20	1.00 ± 0.39	0.307
C18.2n6	20.08 ± 3.51	23.17 ± 1.49	22.22 ± 2.86	0.805
C18.3n3	1.69 ± 0.85	1.64 ± 0.36	2.73 ± 0.70	0.294
C18.3n6	0.13 ± 0.37	0.61 ± 0.16	0.50 ± 0.30	0.474
C20.0	0.09 ± 0.03	0.09 ± 0.01	0.08 ± 0.02	0.669
C20.1	0.16 ± 0.59	1.17 ± 0.25	0.53 ± 0.48	0.158
C20.3n3	0.04 ± 0.05	0.06 ± 0.02	0.04 ± 0.04	0.877
C20.3n6	1.18 ± 0.25	1.09 ± 0.11	1.23 ± 0.20	0.844
C20.4n6	4.31 ± 0.94	4.99 ± 0.40	5.13 ± 0.77	0.798
C20.5n3	0.57 ± 0.21	0.82 ± 0.09	0.99 ± 0.17	0.327
C22.5n3	1.38 ± 0.37	1.56 ± 0.16	1.65 ± 0.30	0.862
C22.6n3	0.08 ± 0.03	0.10 ± 0.01	0.11 ± 0.03	0.797
cla	0.16 ± 0.08	0.28 ± 0.03	0.23 ± 0.07	0.278
SMUFA	32.86 ± 3.71	27.67 ± 1.58	30.42 ± 3.03	0.496
S (n-3)	3.74 ± 1.19	4.12 ± 0.51	5.49 ± 0.97	0.333
S (n-6)	27.27 ± 4.70	30.89 ± 2.00	31.32 ± 3.84	0.745
(n-6)/(n-3)	7.45 ± 2.94	9.17 ± 1.26	5.72 ± 2.40	0.430
SPUFA	29.49 ± 5.03	34.04 ± 2.15	34.62 ± 4.11	0.678
SSFA	34.00 ± 2.23	32.89 ± 0.95	30.93 ± 1.82	0.490

CLA = conjugated linolei acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid

Avilles et al. (2013) calculated the heterozygosity of H = 0.58 for 99 Limousine bulls; Kaplanová et al. (2013) tested 143 bulls of the Czech Fleckvieh breed and calculated H = 0.5944, and Taniguchi et al. (2004) calculated heterozygosity of H = 0.6331 for the 878G>C SNP of the *SCD* gene in 1,003 Japanese black bulls.

Previous works examining the effect of polymorphisms of the *FABP4* gene were either focused on the influence of the gene on the carcass trait or on the associations between the *FABP4* gene and the FA content and composition in beef. Latter studies were focused on different polymorphisms than we studied. Oh et al. (2012) reached similar results, when the genotype of the SNPs 280A>G, 408G>C and 456A>G showed an effect on the content of C18: 2n6 and the ratio of total saturated and monounsaturated FA. Hoashi et al. (2008) and Mannen (2012) agreed on the influence of the most appearing SNP 220A>G polymorphism, (sometimes referred as I74V) on the content of palmitoleic acid. Similar results for the effect of *FABP4* genotype on saturated fatty acid and also on monounsaturated fatty acid were reported by Oh et al. (2012) for the Hanwoo cattle in Korea. Cho et al. (2008) described the effect of this SNP on back fat thickness.

Based on our results we can assume that the SNP 7516G>C of the *FABP4* gene affects not only the quality of carcass traits, as previously reported (Michal et al. 2006; Avilles et al. 2013; Lee at al. 2013), but also the fatty acid composition. Considering all these findings and facts we can claim the gene *FABP4* generally as a candidate gene for the FA content and composition in beef.

A similar result of the SNP 878T>C was achieved by Li et al. (2012), wherein frequencies for the minor allele (T) and minor genotype (TT) were 0.36 and 0.1166, respectively. Mannen (2012) described frequencies of the minor allele and the minor genotype as 0.41 and 0.0897, respectively. Aviles et al. (2013) described frequencies for the minor allele (T) and TT as rare genotype as 0.39 and 0.1735, respectively.

Previous studies suggested that polymorphism of the *SCD* gene should affect the FA composition of fatty acids in beef (Matsuhashi et al. 2011; Li et al. 2012; Mannen et al. 2012; Ishii et al. 2013). Our results, however, did not confirm this claim.

To conclude, we have demonstrated in this work that the FABP4 gene (with SNP 7516G>C), can be considered as the candidate gene affecting the FA content in the beef cattle. We also demonstrated that in addition to classical association data analysis, other approaches such as principal component analysis can also be used. As we have shown using the example of FA content in beef, its advantage is that it provides a more holistic representation of the similarities or differences compared to classical association analyses.

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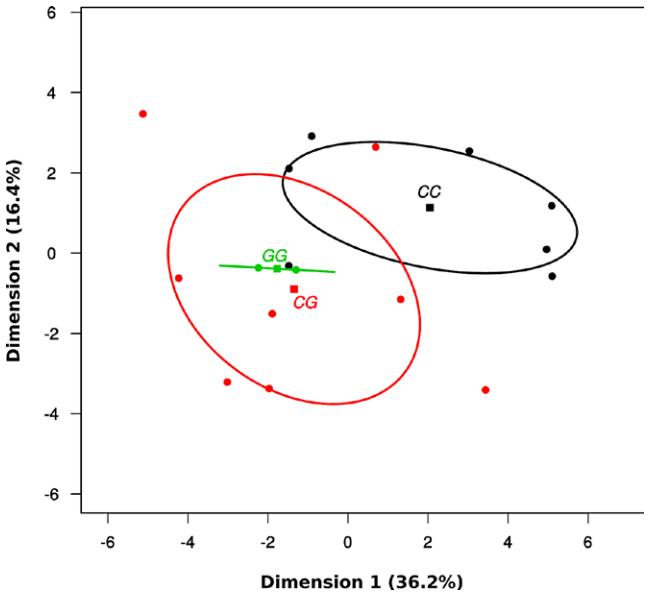

Fig. 1. PCA for FABP4 with all the fatty acids together explains 52.59% of variation for Aberdeen Angus meat samples.

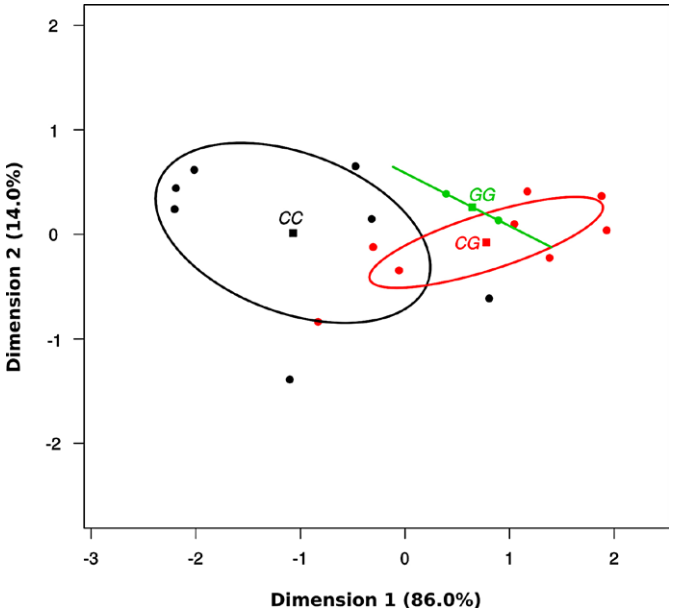

Fig. 2. PCA for FABP4 with only the two fatty acids (C14:1 and n-6) explains 100% of variation for Aberdeen Angus meat samples.

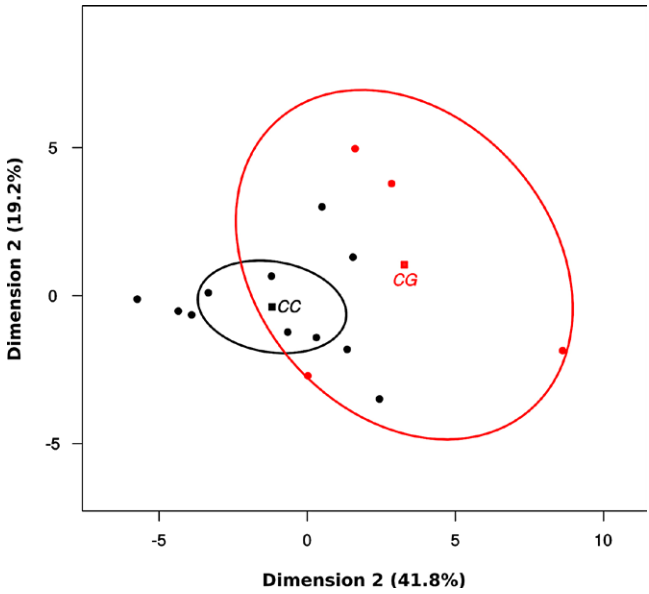

Fig. 3. PCA for FABP4 with all the fatty acids together explains 61.04% of variation for Blonde d'Aquitaine meat samples

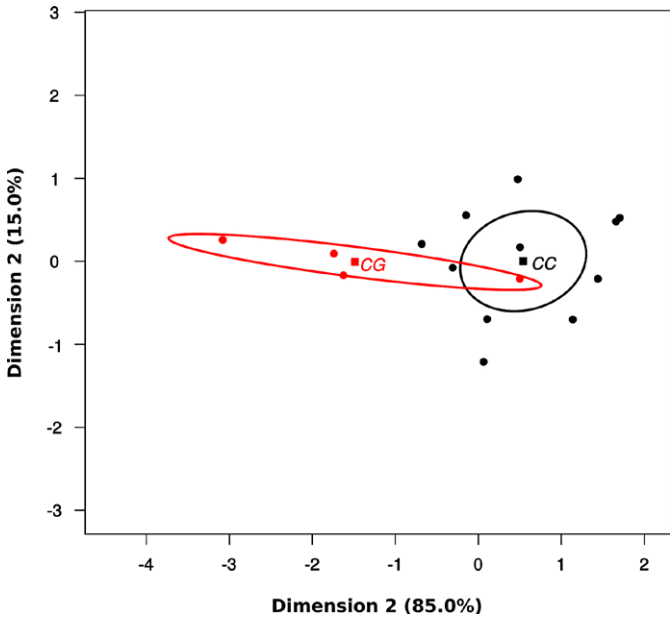

Fig. 4. PCA for FABP4 with only the two fatty acids (C15:0 and C22:5n3) explains 100% of variation for Blonde d'Aquitaine meat samples.