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Candidate gene marker associations with fatty acid profiles in heavy pigs

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ABSTRACT

Heavy pigs are used in Italy to produce fresh meat, dry-cured ham, salami and lard. Fatty acid profile determines both the dietary and organoleptic qualities of these products. The objective was to study the polymorphisms of two genes that code for enzymes of the fatty acid metabolism, namely, Stearoyl-CoA desaturase (*SCD*) and Fatty acid desaturase 2 (*FADS2*). We also investigated the polymorphism of the Sterol regulatory element binding protein gene (*SREBF1*) as it regulates *SCD* and *FADS2* transcription. Significant associations of *SCD* were found with the ratio of oleic to stearic acid; with the concentration (g/100 g of fat) of stearic and oleic acids. The concentrations of arachidonic and linoleic acids and the ratio of dihomo-gamma-linolenic to linoleic acid were significantly associated with *FADS2* polymorphisms. *SREBF1* polymorphism was associated with oleic acid concentrations and the ratio of arachidonic to linoleic acids.
Our findings suggest that the *SCD*, *FADS2* and *SREBF1* polymorphisms are associated with dietary quality of heavy pig meat products.

1. Introduction

In San Daniele (Italy), heavy pigs aged at least 9 months of age and with 160 kg average live weight are used for the production of a typical dry-cured ham (prosciutto). High quality legs (green weight between 13 and 16 kg and fat cover over 20 mm) are required for optimal ham processing. Therefore, upon arrival at the ham maker, fresh legs are submitted to an inspection. One of the main reasons for refusal is a scarce fat cover or improper intramuscular fat content.

The amount and type of fat in the diet have a major impact on human health. High consumption of saturated fatty acids (SFA) raises plasma LDL-cholesterol, which is a major risk factor for atherosclerosis and coronary heart disease (CHD) (Clarke, Frost, Collins, Appleby, & Peto, 1997; Mensink & Katan, 1992; Wolfram, 2003). Although, recent studies suggest that individual SFAs have different physiologic effects. Indeed, SFAs (12:0, 14:0, and 16:0) raise LDL and HDL cholesterol whereas stearic acid is neutral (Hunter, Zhang, & Kris-Etherton, 2010; Astrup et al., 2011) but still some epidemiologic evidence suggests that stearic acid is associated with CHD (Hu et al., 1999). In contrast, cis-monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are beneficial for human health. PUFA have been shown to protect against coronary heart disease (Harris, Poston, & Haddock, 2007). MUFA are also considered to have a hypocholesterolemic effect (Lopez-Huertas, 2010) and, in addition, have been found to have a beneficial effect on insulin sensitivity (Ros, 2003).

High content of unsaturated fatty acids is a predisposing factor for fat with inadequate consistence and for oxidative processes during curing (Isabel et al., 2003; Lo Fiego, Santoro, Macchioni, & De Leonibus, 2005). The San Daniele dry cured ham production procedure requires that iodine number and linoleic acid content to be less than 70 and 15%, respectively (D.O.P. Prosciutto di San Daniele, 1996).

Stearoyl-CoA desaturase (SCD), also called delta-9 desaturase, is an endoplasmic reticulum enzyme not only responsible for the conversion of SFA into MUFA (Ntambi, 1999) but is also involved in the desaturation of *trans*-vaccenic acid into conjugated linoleic acid (Corl et al., 2001; Renaville et al., 2006). SCD is also involved in fat accumulation as *SCD*-1 −/− mice with a targeted disruption or naturally occurring mutations in the *SCD* gene present reduced triglyceride synthesis and levels (Miyazaki, Kim, Gray-Keller, Attie, & Ntambi, 2000; Miyazaki, Kim, & Ntambi, 2001).

The fatty acid desaturase 2 (FADS2), also called delta-6 desaturase, is a membrane-bound protein with amino-terminal cytochrome b5 domains carrying heme-binding motifs, two-membrane-spanning domains, and three His-box motifs (Cho, Nakamura, & Clarke, 1999). FADS2 converts linoleic acid (LA, 18:2 n−6) to gamma-linolenic acid (GLA, 18:3n−6). After an elongation step (resulting in dihomo-gamma-linolenic acid (DGLA, 20:3n−6)), delta-5 desaturase (FADS1) catalyzes the formation of arachidonic acid (20:4n−6) (Lattka, Illig, Heinrich, & Koletzko, 2010). Although three enzymes are involved in

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the production of arachidonic acid from linoleic acid, FADS2 is the rate-limiting step in this biosynthetic pathway (Baylin, Ruiz-Narvaez, Kraft, & Campos, 2007).

Sterol regulatory element binding proteins (SREBPs) belong to the original basic helix loop–helix leucine zipper family of transcription factors (Eberlé, Hegarty, Bossard, Ferré, & Foufelle, 2004). Three SREBPs have been described: SREBP-1a, -1c, and -2, which stimulate transcription of more than 30 genes involved in the uptake and synthesis of cholesterol, fatty acids, triglycerides, and phospholipids (Horton et al., 2003). In particular, SREBP-1c mainly activates lipogenic genes, SREBP-2 acts more specifically on cholesterol biosynthesis genes and SREBP-1a regulates both metabolic pathways (Amemiya-Kudo et al., 2002). The same gene (*SREBF1*) encodes for SREBP-1c and SREBP-1a (Shimano, 2001) but this last protein is a more potent transcriptional activator than SREBP-1c, due to its longer NH2-terminal transactivation domain (Eberlé et al., 2004). Of particular interest for this study, SREBP-1c regulates the transcription of the *SCD* and the *FADS2* gene (Nakamura & Nara, 2002).

The diet of pigs is strictly regulated by the consortium of Prosciutto di San Daniele which controls all the steps of San Daniele ham production, indeed, only a list of permitted feed is allowed in the diet in certain quantities and the amount of linoleic acid cannot be over 2% of the feed dry weight.

Increasing the ratio of MUFA to SFA in meat products may increase its dietary properties, on the contrary PUFA are undesirable for dry-cured ham production as they are subject to oxidation, therefore, the aim of this study was to investigate the effect of polymorphism in the *SCD*, *SREBF1* and *FADS2* genes on the fatty acid profile of meat and fat in heavy pigs.

2. Materials and methods

2.1. Animals

White heavy pigs (n = 129) from four different commercial hybrids used for the production of San Daniele hams were tracked through the harvesting process and through the cutting of the carcass into primal cuts including hams. The hybrids were: two traditional hybrids obtained from the Italian selection by using Italian Duroc (ID) or Large White (LW) boars and Landrace × Large White (L × LW) sows, and two industrial lines, GOLAND and DANBRED as described in Renaville, Piasentier, Bacciu, and Prandi (2012). Animals were slaughtered at 9 months of age by electrical stunning before exsanguination. Sex was recorded at slaughter. No information was available on the pedigree of the animals. All animals were fed according to the recommendations of the consortium of Prosciutto di San Daniele consisting of two third of cereals with a maximum 2% of linoleic acid of the dry weight of feed.

2.2. Fatty acid profiles

Fatty acid profiles were analyzed on 129 samples of *Longissimus dorsi* (LD), *Biceps femoris* (BF), backfat and leg fat cover. Samples of 150 to 200 g of tissue were collected right after slaughter and minced and two aliquots of 2.5 g were used for lipid extraction.

Lipids were extracted according to the methanol–chloroform method of Folch, Lees, and Stanley (1957). After conversion to fatty acid methyl esters with acid catalysis in acetyl chloride/methanol (1:10, vol/vol) as described in Christie (1993), at 70 °C for 2 h they were separated by gas chromatography (HRGC 5300 mega-series) with an Omegawax 320 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm, Supelco Inc., Bellafonte, PA). FAME were identified using external standards (37 components Fame mix and PUFA no. 2, Supelco, Bellefonte, PA), quantified using C19:0 as internal standard and expressed in g/100 g of fatty acids.

2.3. Molecular genetic marker evaluation

The DNA was extracted from the muscle samples using a standard DNA extraction method. The mutation of Stearoyl-coA desaturase (*SCD*) was the T/C mutation in the promoter described by Ren et al. (2004) at position −233 and deposited in Genbank, access number AY487830. Primers were designed to amplify a fragment containing the mutation (Table 1). The *SREBF1* polymorphism was described in Renaville, Glenn, Mote, Fan, Stalder, and Rothschild (2010) and Renaville, Piasentier, Fan, Vitale, Prandi, and Rothschild (2010). For *FADS2*, a primer set (Fwd: ACCGCGACCTTGATTTAGTG; Rvs: TGAGAG GTAGCAA GGATGAAGG) was designed in order to amplify a fragment of FADS2 (Genbank AY692366) gene. PCR products were sequenced using an ABI automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). A new primer set (Table 1) was designed in order to create a PCR-RFLP test for the polymorphism found in exon 3. Amplifications were performed using 12.5 ng of porcine DNA, 1× PCR buffer, 2.5 mM of MgCl2, 0.125 mM each dNTP, 0.3 mM of each primer and 0.35 U Taq polymerase (Promega, Madison, WI, USA). Digestions were performed using the restriction enzyme for each PCR-RFLP test (Table 1) following the recommendations of the manufacturer.

2.4. Statistical analyses

Single locus associations were analyzed using the General Linear Mixed Model of SPSS version 17 (SPSS Inc., Illinois, USA) with a model that included sex, hybrid and marker genotypes (*SREBF1*, *SCD*, *FADS2*) as fixed effects. Significant differences were declared when the marker genotype effect was a significant source of variation in the analysis of variance and the *P*-value for the difference between the least squares means for each marker genotype was less than 0.05. Levene's test was used to test the equality of error variance. LSD was used to compare means unless the variances were not equal, and in these cases the *Tamhane's T2* post-hoc test was used.

3. Results

3.1. SCD polymorphism effects on fatty acid profiles

The genotypic frequencies of the *SCD* polymorphism were: 10% homozygous TT, 62% homozygous CC and 28% heterozygous CT. The *SCD* polymorphism is associated with the concentration of one substrate (stearic acid) and both products (palmitoleic and oleic acids) of the

Table 1
Genes analyzed, primers, PCR annealing temperature, restriction enzymes used, PCR-RFLP fragment sizes and allele frequencies in a study of the association between genetic markers and fresh and dry-cured ham processing characteristics.

Gene[a]	Primer	Primer sequence (5′-3′)	Fragment size (bp)	Annealing temp. (°C)	Restriction enzyme	Fragment sizes (bp) and allele freq. (%)
SCD	SCD-F	CTCTGTCTCCTCCCCTCTCC	322	59	PflF1	322 bp (allele T) 24(%)
	SCD-R	GATCACTTTCCCAGGGATGA				212, 110 bp (allele C) (76%)
FADS2	FADS2-F	CTGAACTGGCTGTGGACAAA	374	63	BsiE1	374 bp (allele C) (12%)
	FADS2-R	TGAGAGGTAGCAAGGATGAAGG				218, 156 bp (allele G) (88%)
SREBF1	SREBF-F	ATG CCT GCC TGC CCT AAC	503	60	NlaIII	503 bp (allele G) (26%)
	SREBF-R	GCC ATC TGT CCT CTT TGC TG				373 + 130 (allele A) (74%)

[a] The genes analyzed were: stearoyl-CoA desaturase (*SCD*), fatty acid desaturase 2 (*FADS2*), and sterol regulatory element binding transcription factor 1 (*SREBF1*).