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Association of single nucleotide polymorphisms in fat metabolism candidate genes with fatty acid profiles of muscle and subcutaneous fat in heavy pigs

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ABSTRACT

Dietary and organoleptic qualities of pork products are largely influenced by the profiles of fatty acids (FAs) of meat and fat. The objective of this work was to investigate the potential associations between FA profile in subcutaneous adipose (back fat and leg fat) and muscular (*longissimus dorsi* and *biceps femoris*) tissues in heavy pigs (n = 129, 9-month-old) with single nucleotide polymorphisms (SNPs) in six candidate genes involved in fat metabolism: Stearoyl-CoA desaturase (*SCD*), Diacylglycerol acyltransferase 1 and 2 (*DGAT1* & *DGAT2*), Microsomal Triglyceride Transfer Protein (*MTTP*), Fatty Acid Synthase (*FASN*) and Heart- fatty acid binding protein (*H*-FABP). Preliminary results suggest a putative association between *MTTP*, *DGAT2* and *FASN* and the FA content in both fat and meat, whereas between *DGAT1*, *SCD* and *H-FABP* the association was found in adipose tissue only. However, the effect of the analyzed genes, needs to be verified in a larger and better characterized pig population to support the hypothesized associations with FA content.

1. Introduction

In the last decades growing consumer interest on the potential impact of diet on health has raised concerns on the fat content and composition of meat products (Vargas-Bello-Peres & Larrain, 2016). The diet-heart (lipid) hypothesis indicates an imbalance of dietary cholesterol and fats as the primary cause of atherosclerosis and cardiovascular disease (Griel & Kris-Etherton, 2006). This hypothesis is mainly based on epidemiological studies that report strong positive correlations between dietary intake of saturated fatty acids (FAs) and increases in peripheral LDL-cholesterol concentrations and the incidence of cardiovascular disease (Clarke, Frost, Collins, Appleby, & Peto, 1997; Hu et al., 1997; Posner et al., 1991). Thus, to meet the consumers' demands, fat content needs to be reduced and FA composition should be controlled in meat products.

By contrast, the processing of the Italian PDO dry-cured ham (prosciutto) requires fresh thighs covered by a suitable layer of fat, in the composition of which linolenic acid content must not exceed 15% of total lipids. These prescriptions are fixed by several consortia, which strictly control through all the productive process the most relevant factors that determine FA deposition, such as commercial hybrid, weight and age at slaughter and diet (De Smet, Raes, & Daniel, 2016). Fat quantity and quality rules, respectively, aim to reduce seasoning loss and to limit the content of polyunsaturated FAs that can affect the consistence of prosciutto fats and increase their oxidability (Bosi & Russo, 2004). As a consequence, the use of genetic markers to modify FA composition would be highly desirable.

Previous works support the potential use of genetic markers in selection schemes to improve eating and dietetic qualities of traditional Italian pork products. Several genomic regions associated with back fat thickness and average daily gain (a target trait included in the selection index) were identified in Italian heavy pig breeds using both candidate gene approach (Fontanesi et al., 2010; Fontanesi et al., 2011; Fontanesi, Galimberti, et al., 2012) and genome-wide association studies (Fontanesi, Schiavo, et al., 2012; Fontanesi, Schiavo, Galimberti, Calò, & Russo, 2014). Also, genomic regions associated with visible intermuscular fat content of ham (Fontanesi, Schiavo, Galimberti, et al., 2017) and ham weight loss at salting (Fontanesi, Schiavo, Gallo, et al., 2017) in Italian Large White pig breed have been recently investigated. Significant associations between single nucleotide polymorphisms (SNPs) in candidate genes and carcass meat quality (Cepica et al., 2013; Davoli et al., 2017; Renaville et al., 2010) and tenderness (Lindholm-

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Table 1
List of candidate gene analyzed and rationale for their selection.

Gene name and symbol	Rationale for selection	References
Stearoyl-CoA desaturase (*SCD*)	The enzyme Stearoyl-CoA desaturase is involved in the conversion of stearic into oleic acid	(Ntambi, 1999)
Diacylglycerol acyltransferase 1 (*DGAT1*) *and* Diacylglycerol acyltransferase 2 (*DGAT2*)	The enzyme Diacylglycerol acyltransferase catalyzes the final step in the sn-glycerol-3-phosphate pathway leading to triglycerides	(Liu, Siloto, Lehner, Stone, & Weselake, 2012, Oelkers, Behari, Cromley, Billheimer, & Sturley, 1998)
Microsomal triglyceride transfer protein (*MTTP*)	The MTTP protein is essential for lipid transfer during the assembly of nascent lipoproteins by the liver and the small intestine	(Wetterau, Lin, & Jamil, 1997, Wetterau et al., 1992)
Fatty acid synthase (*FASN*)	The fatty acid synthase enzyme catalyzes the de novo synthesis of saturated fatty acids	(Jensen-Urstad & Semenkovich, 2012)
Heart fatty acid-binding proteins (*H-FABP*)	The heart type FABP (H-FABP) protein participates in the uptake, intracellular metabolism and/or transport of long-chain fatty acids	(Spener et al., 1990)

Table 2
Genes analyzed, primers, PCR annealing temperature, restriction enzymes and PCR-restriction fragment length polymorphism (PCR-RFLP) fragment sizes used to investigate the association between candidate genes and fatty acid concentrations in adipose (subcutaneous leg and back fat) and muscular (*longissimus dorsi* and *biceps femoris*) tissues in 9-month-old heavy pigs (n = 129).

Gene name and symbol	Primer sequence (5′–3′)	Fragment size (bp)	Annealing temperature (°C)	Restriction enzyme	PCR-RFLP Fragment size (bp)
Stearoyl-CoA desaturase (*SCD*)	F: ctctctcccagctctgcact R: agcccaccacaacacctaag	462	60	*HypCH4IV*	TT: 462 CC: 130 + 332
Diacylglycerol acyltransferase 1 (*DGAT1*)	F: cctgctcaccagggtgcctg R: gtacatggtcccacagtgtcc	1236	64	*AvaII*	GG:10 + 287 + 412 + 527 AA:10 + 62 + 225 + 412 + 527
Diacylglycerol acyltransferase 2 (*DGAT2*)	F: agcaggacattgacctctac R: ccaatgtgaagaaagagtgac	338	58	*BcnI*	AA: 338 GG:237 + 101
Microsomal triglyceride transfer protein (*MTTP*)	F: cagcgcggaaacagaagccg R: acggtgcatcgtacccctttcca	1370	64	*SmlI*	TT:1370 CC:226 + 1144
Fatty acid synthase (*FASN*)	F: atcaaccctgcttcccttcgtg R: cgcgctggcagcctatcat	130	55.1	*Fnu4HI*	TT:10 + 120 CC:10 + 54 + 66
Heart fatty acid-binding proteins (*H-FABP*)	F: attgcttcggtgtgtttgag R: tcaggaatgggagttattgg	850	57	*MspI*	AA: 750 + 100 AA: 850

Table 3
Genes, number of pigs in which each gene was detected, alleles and genotypic frequencies (%) of the polymorphisms of each gene analyzed to investigate the association between candidate genes and fatty acid concentrations in adipose (subcutaneous leg and back fat) and muscular (*longissimus dorsi* and *biceps femoris*) tissues in 9-month-old heavy pigs (total no. = 129).

Gene	No. of pigs	Genotypic frequencies of alleles (%)		
MTTP	126	TT	TC	CC
		27	71	28
		21%	55%	22%
DGAT2	127	AA	AG	GG
		8	53	66
		6%	41%	51%
FASN	127	TT	TC	CC
		7	65	55
		5%	50%	43%
DGAT1	123	GG	GA	AA
		3	31	89
		2%	24%	69%
SCD	127	TT	TC	CC
		0	24	103
			19%	80%
H-FABP	126	AA	Aa	aa
		78	47	1
		60%	36%	1%

Perry et al., 2009; Nonneman et al., 2011) have been reported in different pig populations. Further, we provided evidence for the association between polymorphisms of estrogen receptors and thickness of subcutaneous leg fat in heavy pigs (Renaville, Piasentier, Bacciu, & Prandi, 2012) and we identified associations between polymorphisms of genes that code for enzymes involved in FA metabolism and back fat thickness, weight loss during salting and shear force (Renaville, Bacciu, Lanzoni, Corazzin, & Piasentier, 2015) and FA composition of meat and fat (Renaville et al., 2013).

Here, we hypothesize that SNPs in fat metabolism candidate genes may be associated with variations of the FA composition of meat and fat in heavy pigs. To test this hypothesis, we selected SNPs in six candidate genes that code for enzymes involved in FA metabolism. The rationale for the selection of each gene is summarized in Table 1. Thus, the aim of this study was to investigate the potential association between SNPs in six candidate genes (*SCD, DGAT1, DGAT2, MTTP, FASN, H-FABP*) and the FA profiles of meat and fat in heavy pigs.

2. Materials & methods

2.1. Animals

White heavy pigs (n = 129) from four previously described commercial hybrids farmed for the production of San Daniele Ham (Renaville et al., 2012) were included in this study. Two reference hybrids from the Italian Swine National Association (ANAS) were obtained by mating Italian Duroc (ID) or Large White (LW) boars with Landrace x Large White (L x LW) crossbred sows, whereas two industrial lines were bred from Goland C21 (GOLAND) or Danline HD (DANBRED) boars with their own selected lines of sows. Pigs were randomly chosen during the slaughtering of 21 lots of heavy animals from different farms located in the North of Italy. Each farm comprised only one commercial hybrid. Commercial hybrid was produced by at least five different farms. A group of four to eight subjects was selected from each slaughtering lot, balanced by gender and carcass fatness, U and R classes following the European grading system, in order to represent the variability of production conditions in the PDO chain. All animals were fed according to the recommendations of the “San Daniele