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Analysis of g.265T > C SNP of fatty acid synthase gene and expression study in skeletal muscle and backfat tissues of Italian Large White and Italian Duroc pigs

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ABSTRACT

Fat deposition is a crucial aspect of pig meat quality as fat content influences both organoleptic and nutritive characteristics of fresh meat, meat products and consumer acceptance. Among genes controlling fat metabolism, the gene encoding fatty acid synthase (*FASN*) was proposed as a candidate controlling body fat deposition as it is a central enzyme in lipogenesis. The main function of FASN enzyme is the catalysis of the biochemical process that induces the synthesis of palmitate from acetyl-CoA and malonyl-CoA, in the presence of NADPH. This work aims to study variability in the expression level of *FASN* gene mapped on SSc12 where different QTLs for fat composition and marbling were discovered. In particular, we analysed the SNP T265C for *FASN* gene identified by Muñoz et al. (2003). The association study conducted on 237 Italian Large White (ILW) sib tested pigs to determine whether this polymorphism affected meat productive traits did not allow finding any associations between the SNP and the reported traits. Regarding the Italian Duroc (ID) breed, no association study was performed because in this breed T allele was very rare. Differential expression between breeds of the target gene in *semimembranosus* muscle and in backfat tissue was evident comparing *FASN* transcription level between ID and ILW pigs. In particular, ID pigs have a higher expression level of the gene in skeletal muscle than ILW ($P=0.01$). In backfat tissue the Italian Large White samples showed higher gene expression level than Italian Duroc pigs with a tendency to significant difference ($P=0.08$). If further analyses will confirm this results on a large sample, in ID breed the transcriptional level of *FASN* gene could be considered as marker of fat deposition.

1. Introduction

Fat content influences both organoleptic and nutritive characteristics of fresh meat, meat products and consumer acceptance. Carcass and meat quality traits in pig are influenced by several factors as genetic, breed, nutrition, sex and age (Wood et al., 2004). Fat deposition is a crucial aspect of pig meat quality: a suitable marbling and a right balance between lean and fat meat in carcass are both essential features to produce high quality dry cured hams and other seasoned products (Čandek-Potokar and Škrlep, 2012; Pena et al., 2013). These traits are influenced by genetics, but little is known on genes responsible for phenotypic variation of fat deposition. Regulation of fatty acid (FA) metabolism is affected by changes in transcription, mRNA processing, mRNA stability, and activity of several transcription factors, some involved in FA oxidation and

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some involved in FA synthesis (Duran-Montgé et al., 2009). The identification of variants in porcine candidate genes with effects on adiposity can be helpful to explain the functional role of these genes in fatty acid metabolism and fat deposition. Differences among breeds in intramuscular fat content (IMF) and backfat thickness (BFT) have been reported but the molecular events underlying the different aptitudes are poorly known (Gao and Zhao, 2009). The analysis of the expression profile of lipogenic genes in muscle and fat tissues in different pig breeds can contribute to improve the knowledge on the control of the fat deposition in pig meat and can provide new insight into carcass adiposity (Cánovas et al., 2010; Ren et al., 2008). Among genes controlling fat metabolism, the gene encoding fatty acid synthase (*FASN*) was proposed as a candidate controlling body fat development as it is a central enzyme in lipogenesis. Protein encoded by *FASN* gene has a key role in the fatty acid biosynthesis and performs its activity mainly in liver and fat (Menendez et al., 2009). The main function of *FASN* enzyme is the catalysis of the biochemical process that is induced at the synthesis of palmitate (C16:0) from acetyl-CoA and malonyl-CoA, in the presence of NADPH (Wakil, 1989).

The complete coding sequence of *FASN* in pig has a length of 8044 nt (NM_001099930) and the corresponding polypeptide includes 2512 aminoacids (NP_001093400). *FASN* gene sequence is highly conserved between mammals (goat, horse, cow and human 93% of homology) and mRNA sequences are available for different species in GeneBank. The highest similarity at the protein level ($>80\%$) reported in the UniGene card (http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Ssc&CID=18175&ALLPROT=1) of porcine *FASN* gene is seen in horse, cow, mouse, human and rat. Muñoz et al. (2003) found a SNP g.265T > C (sequence ID AY183428) in the fourth exon of the gene and assigned *FASN* locus by physical and linkage mapping on Sscr12p1.5 in the region between 0 and 4.7 cM, near the chromosome marker S0143. Analysing the most recent porcine maps (*Sus Scrofa* assembly version 10.2), *FASN* gene maps near the marker *AIP4* (placed at 2 cM on the genetic map of SSC12) (http://www.ncbi.nlm.nih.gov/projects/mapview), in the same chromosome where other genes involved in fatty acid biosynthesis, as ATP citrate lyase, *ACLY* (Zambonelli et al., 2009), acetyl Co-A carboxylase alpha, *ACACA* (Calvo et al., 2000) and enolase 3 (beta, muscle) *ENO3* (Larkin et al., 2001) were previously mapped. On this chromosome several QTLs for fat deposition, fatty acid composition, and marbling are reported in the region flanking *FASN* gene (Clop et al., 2003; Edwards et al., 2008; Muñoz et al., 2007; Rohrer et al., 2012; Yue et al., 2003) but the QTG contributing to phenotype is not determined yet. Moreover, Muñoz et al. (2007) starting from human and mouse genomic information sequenced the porcine mRNA of *FASN* gene and found some polymorphisms of the gene. Analysing some of these SNPs either Muñoz et al. (2007) or Rohrer et al. (2012) found no significant association with fat related traits.

There are only few studies on porcine *FASN* gene, but the interest on this candidate locus is strong. In 1991, Mildner and Clarke found that *FASN* mRNA is expressed mainly in fat tissue and in liver (Mildner and Clarke, 1991). Ponsuksili et al. (2007) found an overregulation of *FASN* and other genes (acyl-CoA synthetase short-chain family member 2, *ACSS2*; *ACACA*) involved in the lipid metabolism in abdominal fat in German Landrace piglets compared to Pietrain piglets. Other researchers reported that *FASN* transcriptional level is influenced by hormonal signals, environmental and dietary factors and its regulation depends on several conditions, as age, sex and genetic variants at the locus (Gondret and Lebret, 2007; Guillerm-Regost et al., 2006; McNeil et al., 2005).

In the present study, *FASN* porcine gene was selected as a functional and positional candidate gene for fatness related traits because of its functional role as key lipogenic enzyme. This work aims to (i) study the SNP g.265T > C in the fourth exon of *FASN* gene in a population of sib tested Italian Large White (ILW) and Italian Duroc (ID) pigs to perform an association study with some productive traits; (ii) analyse the expression level of the gene in different tissues and in particular in skeletal muscle and backfat tissue; and (iii) search for an association between gene transcriptional level and the genotypes at *FASN* locus.

2. Material and methods

2.1. Animals and sampling

In this study different groups of pigs were considered: (1) First population: composed by 50 ILW, 66 ID, 55 Italian Landrace (IL), 27 Belgian Landrace (BL), 11 Meishan (M), 28 Hampshire (H), and 42 Pietrain (P). These animals were used to determine the allelic frequencies of the selected *FASN* SNP. (2) Second population: 100 freeze-dried blood samples of ILW pigs (69 females and 31 castrated males from 71 different sires) with extreme and divergent estimated breeding values (EBV) for BFT (50 with the highest and 50 with the lowest EBV) were chosen among 3591 ILW pigs evaluated in the period 1996–1999. Another group of 100 ID freeze-dried blood samples of pigs (58 females and 42 castrated males from 62 different sires) were selected among 1225 groups of siblings from this breed evaluated in the period 1996–1999, according to a selective genotyping approach based on extreme EBV for visible intermuscular fat (VIF; 50 with the most negative and 50 with the most positive values). (3) Third population: 114 ID (81 females and 33 castrated males) and 237 ILW sib tested pigs (150 females and 87 castrated males) were slaughtered in seven different days in the year 2003 in collaboration with the National Association of Pig Breeders (ANAS, www.anas.it). For each pig samples skeletal muscle tissue and backfat were collected and frozen in liquid nitrogen within 1 h after slaughtering. From the third population we selected a subset of samples of ID and ILW pigs divergent for BFT or VIF values to investigate on *FASN* gene expression level in backfat and skeletal muscle tissues. On the whole, RNA was extracted from 57 skeletal muscle (25 ID and 32 ILW) and 38 backfat samples (12 from ID and 26 from ILW breed) for a comparison between breeds.

For all pigs, EBV for ADG (calculated from 30 to 155 kg of live weight with a quasi ad libitum feeding level), feed