Porcine *perilipin* (*PLIN*) gene: Structure, polymorphism and association study in Large White pigs

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ABSTRACT: Perilipin, encoded by the *PLIN* gene, is a lipid droplet-associated phosphoprotein that functions as a key regulator of triacylglycerol storage and hydrolysis in adipocytes. In this study, structure and variability of the porcine *PLIN* gene were characterised. PCR fragments encompassing exons 1 to 9 and interspersed introns were sequenced and the obtained sequence was deposited in EMBL/GenBank/DDBJ databases (AM931171). Seven silent polymorphisms and one nonsynonymous polymorphism were detected in the coding sequence. The nonsynonymous polymorphism g.627G>A causing an amino acid substitution p.Val3Ile was found only in Meishan and Meishan × Piétrain cross. Associations were studied between loci g.4119A>G and g.7966T>C, and average daily gain (ADG), backfat thickness (BFT) or lean meat content (LMC) in 166 gilts from two herds. Due to supposed complete linkage disequilibrium between loci g.4119A>G and g.7966T>C only genotype combinations *AA-TT*, *AG-TC* and *GG-CC* were detected. A significant difference (P = 0.0290) between *GG-CC* and *AG-TC* genotype combinations for BFT in herd 2 and a suggestive difference (P = 0.0516) between *GG-CC* and *AA-TT* genotypes for ADG in herd 1 were detected.

Keywords: pig; perilipin; polymorphism; association analysis

Perilipin is a lipid droplet-associated phosphoprotein involved in the protein kinase A-mediated hormonal stimulation of lipolysis. Several studies in rats, mice and humans showed that perilipin is expressed mainly in adipocytes (Egan et al., 1990; Greenberg et al., 1991; Nishiu et al., 1998; Lu et al., 2001), where it plays important functions in regulating triacylglycerol storage and hydrolysis (Souza et al., 1998; Brasaemle et al., 2000) and in steroidogenic cells, where it regulates lipolysis of cholesteryl esters, precursors for steroid hormone synthesis (Servetnick et al., 1995; Lu et al., 2001). Perilipin-null mice have greater lean body mass and smaller adipocytes, show enhanced leptin expression and are resistant to diet-induced obesity (Martinez-Botas et al., 2000; Tansey et al., 2001).

In humans, the *PLIN* gene that encodes for perilipin was found to be overexpressed in the adipose tissue of obese individuals (Kern et al., 2004). Single nucleotide polymorphisms (SNPs) within the *PLIN* gene were found to be associated with percentage body fat (Qi et al., 2004b), lower body mass index, obesity risk (Qi et al., 2004a,b) and total cholesterol levels (Yan et al., 2004).

Tissue expression patterns of the porcine *PLIN* gene studied by Tao et al. (2008) revealed ubiquitous expression of *PLIN* mRNA, with the highest level found in adipose tissue and low level in other tis-

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sues (liver, kidney, heart, muscle, lung, spleen, pancreas, intestine, brain, stomach). Tao et al. (2008) mapped the *PLIN* gene by radiation hybrid analysis close to microsatellite *SWR1210*, which is located at position 82.3 cM on the USDA – USMARC linkage map of porcine chromosome 7 (http://www.marc.usda.gov/genome/swine/marker_list.html). In this region QTLs for backfat thickness (Malek et al., 2001; Kim et al., 2005; Ponsuksili et al., 2005), ham weight (Milan et al., 2002) and average daily gain (Nezer et al., 2002; Kim et al., 2006) were localised. Due to the aforementioned facts the *PLIN* gene may be considered as a candidate gene for fatness traits in pigs. This study was aimed at the analysis of structure and variability of the porcine *PLIN* gene and its association with growth and meat performance in Large White pigs.

MATERIAL AND METHODS

Sequencing of the porcine *PLIN* gene

Overlapping PCR fragments covering the porcine *PLIN* gene from exon 1 to exon 9 were amplified and sequenced. The forward primer for amplification of the PCR fragment containing 5´ end of the gene was designed from human *PLIN* mRNA (BC031084), the other primers (data not given) were designed from porcine *PLIN* mRNA (AY973170). To determine exon-intron boundaries, this sequence was aligned to the human and murine *PLIN* mRNA (NM_002666 and NM_175640) sequences, respectively.

The sequence of the gene was determined by direct sequencing or sequencing of cloned PCR products amplified with DNA from Large White pig as template using BigDye Terminator v1.1 Cycle Sequencing Kit and ABI PRISM 3100 – Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), according to the procedure described by the manufacturer. Cloning of PCR products was performed using QIAGEN PCR Cloning^{Plus} Kit (Qiagen, Valencia, CA, USA).

Variability analysis

Search for polymorphisms was done by alignment of DNA sequences from one Landrace and one Meishan × Piétrain cross. Sequences of primers used for genotyping and conditions of the PCR are summarized in Table 1. PCR was carried out in 25 µl of reaction volume, containing 50 ng of porcine genomic DNA, 1 × PCR reaction buffer, 2.2mM $MgCl_2$, 200µM of each dNTP, 0.2µM of each forward and reverse primer and 1U LA DNA Polymerases Mix (Top-Bio, Prague, Czech Republic). A PCR thermal profile consisted of pre-denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 20 s, annealing at X°C for 20 s and elongation at 68°C for 20 s and final extension at 68°C for 7 min (X is the annealing temperature specific for the primer pair, see Table 1). Amplified fragments were

Table 1. Primer pairs, their annealing temperature and restriction enzymes (RE) for PCR-RFLP methods used for genotyping of polymorphisms detected in coding sequences of the porcine *PLIN* gene (AM931171)

Locus	Primers (5´ → 3´)	T_a	Length of amplicon	RE	Alleles and length of fragments
g.627G>A	F: TAG CTC ATC AGA CTC CAG GGA R: CTC TTC CTT CCC CCA TCA GT	56°C	238 bp	*Hinc*II	*A* – 238 bp *G* – 150, 88 bp
g.4119A>G	F: CCA GAA GAC CTA CAC CAG CAC R: TCT GGA TGC CCT TCT CGT AA	58°C	80 bp	*Hin*1I	*A* – 80 bp *G* – 44, 36 bp
g.7374G>A	F: GAG CTG AAG GAC ACC ATC TCC R: ACC TGA CTC TTC CTT GGC TG	60°C	258 bp	*Pst*I	*A* – 183, 75 bp *G* – 258 bp
g.7416C>T				*Mva*I	*C* – 141, 78, 39 bp *T* – 219, 39 bp
g.7966T>C	F: GAT CTG CTC TCC TTC CCT CC R: CTG TTT CAG AGC GCG AGA C	64°C	175 bp	*Nla*IV	*C* - 115, 60 bp *T* – 175 bp

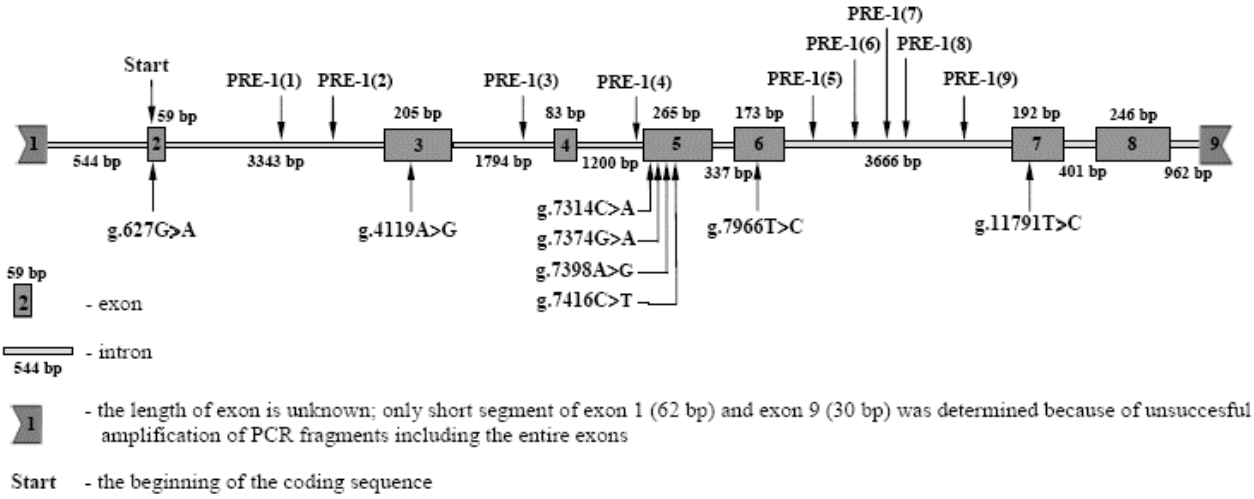

Figure 1. Structure and organisation of the porcine *PLIN* gene (AM931171)

digested with 1U of particular restrictase (Table 1) at 37°C overnight.

ASSOCIATION ANALYSIS

The association between *PLIN* genotypes and meat performance was studied in two different herds of Large White gilts: herd 1 (95 animals) and herd 2 (71 animals) with records for average daily gain (ADG), backfat thickness (BFT) and lean meat content (LMC). Average daily gain was measured from birth to slaughter and adjusted for 100 kg of live weight; backfat thickness and lean meat content were measured with ultrasound (Piglog 105, SFK, Soborg, Denmark).

The computation was performed using a mixed linear model in SAS for Windows 9.1.3 (procedure REML):

$$y_{ijklm} = \mu + PLIN_i + F_j + M_k + MB_l + YB_m + e_{ijklm}$$ for herd 1

$$y_{ijk} = \mu + PLIN_i + F_j + M_k + e_{ijk}$$ for herd 2

where:

$y_{ijk(lm)}$ = the phenotypic value of the analysed trait
μ = the population mean
$PLIN_i$ = the fixed effect of the i^{th} genotype
MB_l = the fixed effect of the month of gilt's birth
YB_m = the fixed effect of the year of gilt's birth
F_j and M_k = random effects of the gilt's father and the gilt's mother, respectively
$e_{ijk(lm)}$ = the random error effect of each observation

The analysed traits were average daily gain (ADG), backfat thickness (BFT) and lean meat content (LMC).

RESULTS AND DISCUSSION

Structure of the porcine *PLIN* gene

Eight overlapping PCR fragments of the porcine *PLIN* gene were amplified using DNA from Large White as template and sequenced. The obtained ~13.5 kb sequence was deposited in the EMBL/GenBank/DDBJ databases under accession number AM931171. The obtained porcine sequence resembled a human and murine exon-intron organi-

Table 2. SNPs detected in the coding sequence of the porcine *PLIN* gene (AM931171)

Exon	Polymorphism	Feature
2	g.627G>A	p.Val3Ile
3	g.4119A>G	silent
5	g.7314C>A	silent
5	g.7374G>A	silent
5	g.7398A>G	silent
5	g.7416C>T	silent
6	g.7966T>C	silent
7	g.11791T>C	silent

Table 3. Allele frequencies of five loci within the porcine *PLIN* gene in five breeds

Locus	Allele	Breed				
		L n = 13	LW n = 14	D n = 12	H n = 3	M n = 4
g.627G>A	*A*	0.00	0.00	0.00	0.00	1.00
	G	1.00	1.00	1.00	1.00	0.00
g.4119A>G	*A*	0.50	0.82	0.88	0.67	0.00
	G	0.50	0.18	0.12	0.33	1.00
g.7374G>A	*A*	0.00	0.00	0.00	0.00	1.00
	G	1.00	1.00	1.00	1.00	0.00
g.7416C>T	*C*	1.00	1.00	1.00	1.00	0.00
	T	0.00	0.00	0.00	0.00	1.00
g.7966T>C	*C*	0.50	0.18	0.12	0.33	1.00
	T	0.50	0.82	0.88	0.67	0.00

breed: L = Landrace; LW = Large White; D = Duroc; H = Hampshire; M = Meishan; n = number of animals

sation and encompassed complete coding exons 2 (with the 5′ end of the CDS) to 8, introns 1 to 8 and part of exons 1 and 9 (Figure 1). The 3′ end of the coding sequence (in exon 9) was not included, because of unsuccessful amplification of the PCR fragment including this part.

Nine PRE-1 sequences (Singer et al., 1987), designated PRE-1 (1–9), were revealed in introns 2: PRE-1(1), PRE-1(2); intron 3: PRE-1 (3); intron 4: PRE-1(4) and intron 6: PRE-1 (5–9) (Figure 1). Except the PRE-1(1), PRE-1(5) and PRE-1(9), which are truncated, these sequences contain all consensus parts: an RNA polymerase III split promoter, two short direct repeats and a poly A tail of variable length (Singer et al., 1987). PRE-1(1), PRE-1(6) and PRE-1(7) are of inverted (complementary) orientation. The similarity between all nine sequences ranged from 40 to 91%. The comparison with the consensus sequence published by Singer et al. (1987) showed a similarity 77–87%. The great number of PRE-1 in our sequence is not surprising, because it is the major SINE of the porcine genome (Singer et al., 1987; Yasue et al., 1991).

Table 4. Association between the g.4119A>G and g.7966T>C genotypes and the growth and meat performance in 2 herds of Large White gilts

Herd	Trait	Genotype combinations at loci g.4119A>G and g.7966T>C					
		GG-CC		*AG-TC*		*AA-TT*	
1	ADG (g)	n = 44	494.39 ± 15.45*	n = 40	524.22 ± 10.62	n = 11	528.59 ± 9.85*
	BFT (mm)		10.34 ± 0.32		10.02 ± 0.38		9.85 ± 0.60
	LMC (%)		59.44 ± 0.60		59.45 ± 0.38		59.08 ± 0.32
2	ADG (g)	n = 32	591.59 ± 14.20	n = 33	589.02 ± 14.04	n = 6	611.41 ± 17.72
	BFT (mm)		10.37 ± 0.35**		9.62 ± 0.33**		10.65 ± 0.59
	LMC (%)		58.76 ± 0.43		59.50 ± 0.41		58.63 ± 0.69

n = number of animals of the given genotype; ADG = average daily gain; BFT = backfat thickness; LMC = lean meat content; data are given in LSM ± SE (least-square mean ± standard error); *P = 0.0516; **P = 0.0290

Polymorphisms in the coding sequence of the porcine *perilipin* gene

In the coding sequence, eight single nucleotide substitutions were identified (Figure 1, Table 2). Seven of them are silent mutations and one (in exon 2) is the nonsynonymous mutation causing the substitution of Val with Ile at position 3 (p.Val3Ile). For five (g.627G>A, g.4119A>G, g.7374G>A, g.7416C>T and g.7966T>C) of the eight SNPs, PCR-RFLP assays were developed (Table 1). Allele frequencies of these SNPs in five breeds are given in Table 3.

The g.627G>A, g.7374A>G and g.7416C>T polymorphisms were monomorphic in all tested breeds and only *g.627G*, *g.7374G* and *g.7416C* alleles were present in Landrace, Large White, Duroc and Hampshire. Alleles *g.627A*, *g.7374A* and *g.7416T* were detected only in Meishan and Meishan × Piétrain cross. In all breeds the complete linkage disequilibrium between AM931171:g.4119A>G and AM931171:g.7966T>C loci was observed with *AA-TT*, *AG-TC* and *GG-CC* genotype combinations detected.

Association analysis

Large White gilts (n = 166) from 2 different herds were genotyped for AM931171:g.4119A>G and AM931171:g.7966T>C polymorphisms. The complete linkage disequilibrium between these polymorphic loci was observed in both herds and therefore only three genotype combinations *AA-TT*, *AG-TC* and *GG-CC* were found. The results of association analysis are summarised in Table 4. In herd 1, suggestive differences between genotypes *GG-CC* and *AA-TT* (P = 0.0516) similarly like between *AA-TT* and *AG-TC* (P = 0.0877) were observed for ADG, but non-significant differences between genotypes were found for BFT and LMC. In herd 2, significant difference (P = 0.0290) between *GG-CC* and *AG-TC* genotypes and a suggestive difference (P = 0.0880) between genotypes *AA-TT* and *AG-TC* was observed for BFT. A suggestive difference (P = 0.0658) was also found between genotypes *GG-CC* and *AG-TC* for LMC. Non-significant differences between genotypes were found for ADG. Investigated polymorphisms are synonymous and could be considered as markers within *PLIN* gene. The nonsynonymous polymorphism g.627G>A causing an amino acid substitution p.Val3Ile did not segregate in Large White under the association study. This polymorphism is located outside the central 25% of the amino acid sequence, which is necessary to target and anchor the protein to lipid droplets (Garcia et al., 2003; Subramanian et al., 2004) as well as outside the amino terminus (between amino acids 112 and 122) and the carboxyl terminus of 112 amino acids that are unique to perilipin and facilitate the storage of triacylglycerols (Garcia et al., 2004). Further association study is needed in a population in which AM931171: g.627G>A SNP segregates to investigate whether this mutation affects fat metabolism.

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