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Single-minded 1 Gene Mapping and Its Variants Association with Growth, Carcass Composition and Meat Quality Traits in the Pig

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ABSTRACT : Single-minded 1 gene (*SIM1*) is a homolog of Drosophila *SIM1* gene which plays a key role in the midline cell lineage of the central nervous system and is implicated in the regulation of feeding behavior and obesity in the human and mouse. In this study, porcine *SIM1* gene was firstly mapped to chromosome 1p13 using radiation hybrid (RH) mapping and two polymorphisms were detected at position 607 (A/G) in *SIM1* intron7 and position 780 (C/T) in *SIM1* exon8. The last substitution was genotyped in a 364 F2 animal-population and an association analysis of these genotypes was performed with growth, carcass and meat quality traits by the statistical animal model. The results showed the significant influence of the *SIM1* genotype on growth ($p<0.05$): live weight at birth, later period of growth and average daily gain; and effects on carcass composition ($p<0.05$): weight of head and buck kneed foreleg, backfat depth, loin eye area, carcass leaf fat and ham fat weights; and traits related to intramuscular fat content ($p<0.05$). (**Key Words :** *SIM1*, Radiation Hybrid Mapping, Carcass Composition, Meat Quality Traits, Pig)

INTRODUCTION

Single-minded 1 (*SIM1*) gene has been identified as a homolog of *Drosophila SIM* gene, which plays a key role in the midline cell lineage of the central nervous system (Crews et al., 1988). The *SIM* gene homologs have been isolated from a variety of species including human, mouse and zebrafish (Ema et al., 1996; Chrast et al., 1997; Wen et al., 2002). SIM proteins belong to a family of transcription factor called bHLH/PAS (basic helix-loop-helix/Period-Arnt-Sim). In mammals, *SIM1* is expressed in the developing kidney and central nervous system, and is also essential for the formation of the supraoptic and paraventricular (PVN) nuclei of the hypothalamus. PVN contains neuroendocrine cells that are critical for the regulation of several physiological processes, such as energy balance and blood pressure. Loss-of-function experiments in mice showed that the bHLH/PAS transcription factor *SIM1* is essential for the differentiation of PVN neurons (Michaud et al., 1998); in the absence of *SIM1* almost all PVN neurons failed to develop and homozygous *SIM1*-deficient mice died shortly after birth, but heterozygous *SIM1*-deficient mice survived and developed hyperphagia and obesity (Michaud et al., 2001). Therefore, it is believed that *SIM1* could control feeding behavior and be connected with obesity.

In the past few years, efforts were made mainly on the QTL (quantitative trait loci) scanning and identification of major genes (candidate genes) associated with traits of economic interest in the pig to chromosomal regions (Rohrer et al., 1998; Kim et al., 2005; Kim et al., 2007). Recently, establishing a relationship between candidate gene mutation and performance such as carcass weight, pH, meat color or intramuscular fat content of the carcass has been unfolded (Ruiz, 1996; Suzuki et al., 2003; Simek et al., 2004). Today, most pig producers have included DNA tests as selection tools in their production programs in order to improve meat quality to meet consumer needs (Tarrant et al., 1998; Visscher et al., 2000). For instance, the gene tests used to remove *HAL* mutations contributing to negative effects on meat quality (Fujii et al., 1991).

However, no comprehensive study has been performed on *SIM1* in the pig, which makes investigations of porcine *SIM1* effects on pig growth, carcass and meat characters increasingly important. The objective of the present study was to map *SIM1* in the pig and identify the polymorphisms in the *SIM1* gene, and then to analyze associations with porcine growth, carcass composition and meat quality.

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MATERIALS AND METHODS

SIM1 gene sequencing and mapping

A pair of primers (Forward: 5'-CGCTGCGCC CACCACCTGC-3'; and Reverse: 5'-ACCCAGACCCAGC CGCCGTG-3') was designed based on the published human *SIM1* gene sequence of exon 7 and exon 8 (GenBank NC_000006.10). PCR was conducted in a 25 μl reaction volume consisting of 50 ng of porcine genomic DNA, 1× PCR buffer, 0.3 μM of each primer, 0.1 mM of dNTPs, 1.5 mM $MgCl_2$ and 1 U *Taq* DNA polymerase (Sangon, Shanghai, China). Samples were denatured at 94°C for 4 min followed by 35 cycles under the following conditions: denaturation for 40 s at 94°C, annealing for 40 s at 60°C and extension for 40 s at 72°C, cooling at 4°C.

The PCR products were purified, cloned and sequenced. Comparisons of pig exon sequences from the PCR products with the corresponding human *SIM1* sequences in GenBank were done using the DNAMAN software to confirm that the expected pig sequences were isolated. The porcine PCR product was 787 bp including an intron of 621 bp, and it showed a high identity of 91% between human and porcine exon sequence (GenBank accession No. DQ646427).

Another pair of primers (Forward: 5'-CAGATTTTCA TGCAGCTCA-3'; and Reverse: 5'-ATGAACCTGCTTCT GAAC-3') for radiation hybrid mapping was designed based on the above sequence result of porcine *SIM1* gene. The IMpRH panel, consisting of 118 clones, was used for mapping (Yerle et al., 1998; Hawken et al., 1999). PCR was performed in a total reaction volume of 15 μl containing 50 ng of template DNA, 1×PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM of each primer, 200 μM dNTPs and 0.5 U *Taq* DNA polymerase, and the PCR procedure was the same as above. The controls consisted of porcine and hamster genomic DNAs and H_2O. Each clone in the RH panel was amplified twice in order to get reliable results. The presence and absence of the DNA fragment was scored in 2% agarose gel electrophoresis and the results were analyzed by the tools provided at http://imprh.toulouse.inra.fr/.

Table 1. Association between *SIM1* genotypes and recorded traits

Trait	Genotype						Mean±SE	
	CC		CT		TT			
	n	Mean±SE	n	Mean±SE	n	Mean±SE	Additive	Dominance
Birth weight (kg)	39	1.256±0.054^{A}	143	1.110±0.028^{B}	103	1.030±0.034^{B}	0.1±0.029**	-0.004±0.02
Live weight 30 d (kg)	14	6.947±0.589	60	6.676±0.286	51	6.429±0.313	-0.181±0.282	0.102±0.194
Weaning weight (kg)	34	8.350±0.408	100	8.853±0.239	82	8.011±0.265	0.148±0.233	-0.338±0.16*
Live weight 60 d (kg)	33	14.208±0.769	123	14.646±0.399	92	13.460±0.466	-0.275±0.424	-0.175±0.279
Live weight 90 d (kg)	36	26.339±1.116^{AB}	138	26.910±0.573^{A}	103	24.452±0.668^{B}	0.309±0.588	-0.912±0.395*
Live weight 120 d (kg)	37	39.914±1.479^{A}	142	39.475±0.758^{AB}	105	36.349±0.887	0.266±0.804	-0.974±0.57
Live weight 150 d (kg)	38	53.592±1.824^{AB}	134	55.025±0.977^{A}	92	50.542±1.186^{B}	0.104±1.1	-1.944±0.793*
Average daily gain (kg/d)	39	0.351±0.013^{AB}	142	0.356±0.007^{A}	105	0.322±0.008^{B}	0.028±0.007**	-0.013±0.005**
Head weight (kg)	40	6.021±0.082^{B}	154	5.927±0.042^{B}	106	6.263±0.051^{A}	-0.18±0.045**	0.122±0.042**
Ham weight (kg)	40	26.078±0.348	154	26.303±0.178	106	25.955±0.215	0.09±0.188	-0.199±0.25
Ham muscle weight (kg)	40	5.761±0.094	147	5.682±0.049	97	5.662±0.061	0.048±0.051	-0.029±0.05
Ham fat weight (kg)	40	1.901±0.054	147	1.979±0.028	97	2.022±0.035	-0.062±0.3*	0.008±0.029
Buck kneed foreleg weight (kg)	40	1.530±0.035^{B}	148	1.544±0.018^{B}	97	1.627±0.022^{A}	-0.038±0.019*	0.012±0.013
Carcass length (cm)	40	72.816±0.461	154	73.112±0.236	105	72.190±0.287	0.362±0.259	-0.378±0.218
Carcass Backfat S (mm)	40	44.358±1.11	154	45.433±0.568	105	46.024±0.691	-1.405±0.573*	0.269±0.454
Carcass Backfat P2 (mm)	40	21.355±0.954^{B}	154	23.570±0.488^{A}	105	24.067±0.594^{A}	-1.984±0.507**	0.077±0.385
Carcass Backfat GM (mm)	40	20.858±1.078	154	23.780±0.552^{A}	105	23.719±0.672^{AB}	-1.801±0.592**	-0.002±0.424
Carcass weight (kg)	40	50.653±0.747	154	52.087±0.382	106	51.361±0.463	-0.425±0.363	-0.556±0.498
Carcass leaf fat weight (kg)	13	0.650±0.057^{B}	68	0.760±0.028^{AB}	28	0.863±0.045^{A}	-0.086±0.034*	0.001±0.024
Longissimus drosi								
pH 45 m (°C)	40	6.223±0.056	154	6.200±0.029	105	6.128±0.035	0.051±0.028	-0.017±0.019
Temperature 45 m (°C)	40	38.983±0.220	154	38.844±0.113	106	38.810±0.137	-0.023±0.115	0.058±0.075
Protein content (%)	33	23.540±0.171	131	23.610±0.086	74	23.612±0.115	-0.035±0.093	0.007±0.06
Water content (%)	33	74.103±0.219^{A}	131	73.721±0.110^{A}	74	73.222±0.148	0.399±0.123**	-0.14±0.081
Intramuscular fat content (%)	33	2.376±0.195^{B}	131	2.501±0.098^{B}	74	3.037±0.131^{A}	-0.302±0.111**	0.178±0.071*
L^*	40	49.094±0.740	154	47.751±0.378	104	48.033±0.463	0.353±0.382	0.176±0.25
a^*	40	0.291±0.274	154	0.075±0.139	104	0.471±0.170	-0.189±0.139	0.184±0.091*
b^*	40	9.930±0.27	154	9.525±0.138	104	9.849±0.169	-0.01±0.142	0.143±0.093
c^*	40	9.829±0.254	153	9.655±0.130	104	10.063±0.159	-0.127±0.137	0.137±0.09
h^*	40	89.66±1.682	154	90.292±0.860	104	88.369±1.051	1.017±0.859	-0.927±0.561
Loin eye area (cm^2)	37	37.274±1.011^{A}	129	35.177±0.544^{A}	91	31.753±0.651	2.808±0.554**	-0.92±0.392*

Least square means (LSM) estimated for each polymorphism is indicated with its standard error (SE).

Significant differences (within a trait) between the genotype classes are indicated with different capital superscripts at $p<0.05$.

* $p<0.05$ and ** $p<0.01$. n = number of individuals.

Animals and phenotypic records

A resource family was developed from a cross between two purebred Pietrain sires and two Jinhua dams (Jinhua pig, a Chinese domesticated pig, mainly inhabit eastern China, Zhejiang province), four F1 boars and ten F1 sows were used to produce 527 F2 animals, 364 of which were used in this study.

On the day before slaughter, the animals were weighed at the farm of origin with a minimum of 50 kg and a maximum of 104 kg (average = 81.99 kg). Average daily gain was calculated as the weight gain divided by the number of days elapsed between the two measurements. After slaughter, at 45 min postmortem, individual carcass weight was recorded and backfat thickness was measured by ruler on the left side of each carcass at three locations (P2, *gluteus medius* and scapula).

Muscle pH and muscle temperature at 45 min were taken on the *longissimus dorsi* (LD) muscle using a HANNA (HI 8424) pH meter fitter with a glass electrode and an Ama-digit meter (German). A sample of *longissimus dorsi* muscle at the fourth rib was taken from each animal and used for the determination of intramuscular fat, protein and water content, loin eye area (LEA) and for the instrumental analysis of color. LEA of LD muscle was traced on an acetate film between the 3rd and 4th last ribs and was subsequently determined by planimetry. Intramuscular fat, protein and water content of LD muscle were determined by a Meat Analyzer, Antaris (Thermo Electron, USA), working in the wavelength range 780-2,500 nm by the Near Infrared Transmission (NIT) principle. The color parameters (CIE system: lightness, L*; redness, a*, yellowness, b*, chroma, c*, and hue angle, h*) were determined using an X-rite (sp60, USA). Carcass length was measured from the anterior edge of the symphysis pubis to the vascular impression on the anterior edge of the first rib. Information on the recorded traits is included in Table 1.

PCR amplification of a pig *SIM1* gene fragment and SSCP analysis

The porcine *SIM1* exon8 single strand conformational polymorphism was detected in the fragment which was used to design the PCR primers (Forward: 5'-ATT GAT TTG GGC CGG TTC AG-3'; and Reverse: 5'-TGA GGA CGT AGT TGA CGC TGA C-3'). Amplification was performed in 15 μl reactions containing 50 ng of template DNA, 1×PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM of each primer, 200 μM dNTPs and 0.5 U *Taq* DNA polymerase. Amplification procedure consisted of denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, with a final extension step at 72°C for 5 min. PCR products were visualized by electrophoresis in 1% agarose gels using 1×TBE buffer.

To screen for polymorphism in the targeted region of *SIM1*, all amplicons were subjected to SSCP analysis. A 3 μl aliquot of each amplicon was mixed with 6 μl of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol), and after denaturation at 95°C for 5 min, samples were rapidly cooled on wet ice and then loaded on 16×18 cm, 12% acrylamide:bisacrylamide (29:1) (Bio-Rad) gels. Electrophoresis was performed using Power Pac 3000 (Bio-Rad) at 400 V for 18 h at 4°C in 0.5×TBE buffer and gels were silver-stained according to the method of Bassam (Bassam et al., 1991).

Statistical analysis

The association between genotypes and recorded performance were evaluated by the least squares method using the GLM procedure (SAS, 1999); both additive and dominant effects were also estimated using the REG procedure of SAS, where the additive effect was denoted as 1, 0 and -1 for genotypes CC, CT and TT, respectively, and the dominance effects represented as 1, -1 and 1 for CC, CT and TT, respectively. The model used to analyze the data was assumed to be

$$y = X\beta + Zu + e$$

Where y is a vector of trait records, β, u and e are vectors of fixed, additive genetic and residual effects respectively, and X and Z are known incidence matrices. Fixed effect in X included birth-season (1, 2, 3 and 4 for different season), effects of sex (1 for male or 0 for female), *SIM1* genotypes with values 1, 0 and -1 for the genotypes CC, CT and TT, respectively; the slope of this covariate estimates the additive effects., Additional analyses were performed fitting a model with dominance effects, with values 1, -1, 1 for the genotypes CC, CT and TT, respectively. Other covariates depended on the analysed traits: carcass weight for body composition traits; the age at slaughter for growth traits.

RESULTS

Porcine *SIM1* gene mapping

Two-point analysis showed that *SW301* and *SW781* were significantly linked markers with *SIM1* on chromosome 1 (LOD = 4.98 and 3.86). The most significantly linked marker *SW301* was at a distance of 74 cR (retention frequency was 32%). This location corresponded to position SSC1p13 (Figure 1), and agrees with the comparative mapping data of pig-human. (http://www.toulouse.inra.fr/lgc/pig/compare/SSCHTML/SSC1S.HTM).

Identification of polymorphism in the porcine *SIM1*

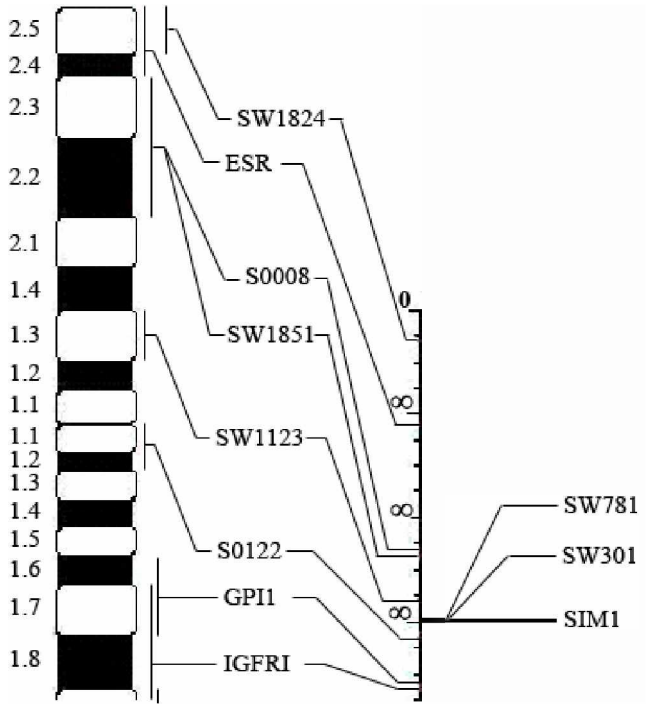

Figure 1. The *SIM1* gene mapped on chromosome 1 of porcine by *Mp*RH panel.

gene and association analysis between polymorphism with growth, carcass composition and meat quality traits

Under the established conditions, three unique SSCP banding patterns could be detected (Figure 2a). The alignment of the sequences from individual animals that were 11 and 22 homozygote identified a single nucleotide substitution (T→C; Figure 2b), and the SNP presented in exon8 at position 780 (GenBank accession No. DQ646427). Genotyping of this mutation on 364 animals of the study with phenotypes found CC, CT and TT genotype frequencies were 0.13, 0.51 and 0.36 respectively.

The least square analyses of genotype effect (Table 1) and the analyses using the statistical model revealed significant effects of *SIM1* on growth, carcass composition and meat quality. The results showed that the effect of *SIM1* genotype on live weight at birth, 90, 120 and 150 days and average daily gain had significance ($p<0.05$), but *SIM1* had no significant effect in any other growth period. This site seemed to be significantly additive ($p<0.01$) in action for birth weight and allele C was associated with increase of the trait. For average daily gain, the *SIM1* genotypes showed a significant effect ($p<0.05$), of which the additive and dominance effect were both highly significant ($p<0.01$). For carcass composition, the genotype of *SIM1* had significant effect on head and buck kneed foreleg weight and on loin eye area ($p<0.05$); allele T significantly increased head weight, buck kneed foreleg weight but decreased loin eye area. This site had a significant additive effect on weight of head and buck kneed foreleg and LEA ($p<0.01$) and, furthermore, it was significantly dominant for head weight and LEA at $p<0.01$ and $p<0.05$, respectively.

With fat measures analysis the effects of *SIM1* genotype were statistically significant on carcass leaf fat weight and carcass backfat at P2 and GM ($p<0.05$). Genotype CC decreased the carcass leaf fat weight and backfat thickness at P2 and GM with significantly additive effect ($p<0.05$).

Significant effects of *SIM1* polymorphism were also estimated on LD muscle quality (Table 1). The loins of CC animals exhibited higher percentages of water but lower intramuscular fat content (IMF) than those of TT animals with significant additive effects ($p<0.01$), but with significant dominance for IMF only ($p<0.05$). However, other meat quality traits, such as protein content, pH 45 m, temperature 45 m and muscle color, were scarcely affected by *SIM1*.

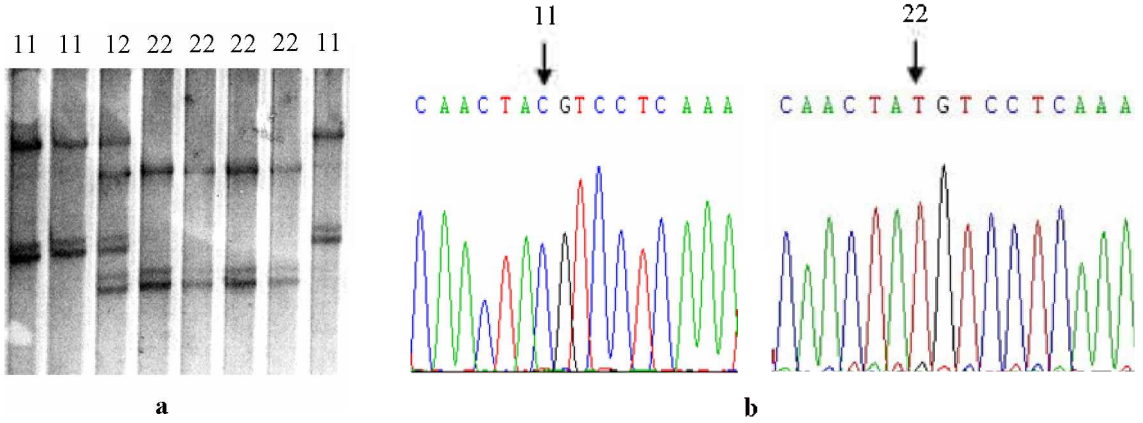

Figure 2. (a) PCR-single-strand conformational polymorphism of the *SIM1* gene. Representative swine for the three unique SSCP patterns corresponding to three allelic sequences 11, 12, and 22 are shown. (b) sequencing of 11 and 22 allelic sequence where there was a T→C substitution.

DISCUSSION

To our knowledge, this is the first time the location of *SIM1* on the chromosome has been confirmed by radiation hybrid (RH) mapping and for the association with the polymorphism to be analyzed based on a standard animal model with growth, carcass composition and meat traits in the pig.

To date, many studies have reported QTL affecting pig economic traits using a genome-wide scan on porcine chromosomes including SSC1, where *SIM1* was located. In this region, Koning et al. (2001) demonstrated a QTL for testing growth rate and, subsequently Carine Nezer et al. (2002) found a QTL for backfat and suggestive evidence for average daily gain by whole genome scan in a Pietrain× Large White intercross. Furthermore, Beekmann et al. (2003) reported a QTL in this area affecting head weight and feed intake. Results from all these studies have included the *SIM1* location that we estimated, therefore, it was inevitable that some correspondence was present reflecting the *SIM1* gene effect.

The T/C substitution locus is also different in human and mouse *SIM1* gene exon8 sequences (GenBank accession No. NC_000006 and NC_000076). In relation to growth traits, the birth weight and average daily gain were significantly affected by porcine *SIM1* SNP, with allele C being favorable for these traits. Regarding carcass composition, the loin eye area and weight of head, buck kneed foreleg, carcass leaf fat and ham fat were all affected by the polymorphism. These results correspond with the effect of *SIM1* in human medical genetic research. Patients with chromosome abnormalities involving the region where the *SIM1* gene is located present with some Prader-Willi syndrome-like clinical and behavioral features such as hyperphagia, severe obesity, abnormal head circumference and small hands and feet (Varela et al., 2006), which suggested early-onset obesity and hyperphagia were determined by the *SIM1* gene. This is also expected for the mouse, as Michaud et al. (2001) indicated that *SIM1* influences the development of hyperphagia and early-onset obesity in mice, with normal metabolism and energy expenditure, and Holder et al. (2004) detected *SIM1* expression in the paraventricular and supra-optic nuclei in wild-type adult mice. On the other hand, another study revealed that the supra-optic nuclei, where melanocortin-4 receptor (*MC4R*) is expressed and which appear to be a target of the alpha melanocyte-stimulating hormone, are involved in the regulation of body weight through the inhibition of food intake (Kalra et al., 1999). *MC4R* was regarded as a candidate gene that plays an important role in the control of energy homeostasis and fat mobilization associated with growth and fat deposition in pigs and its effect has been clearly associated with porcine backfat depth, feed intake and growth rate (Óvilo et al., 2006). The sense of the estimated effect is similar to that observed in the present work. Our findings of the polymorphism of *SIM1* and its effect on growth, backfat thickness, IMF in LD, leaf and ham fat traits demonstrate that *SIM1* is also an important factor for energy balance and fat deposition in pig. Thus, we suggest *SIM1* is a candidate gene for important economic traits in the porcine

Pork quality comprises a set of key fresh meat quality, processing and sensory characteristics that are important for the future profitability and competitiveness of the swine industry, hence many researchers have been dedicated to improving meat quality. However, breeding and selection by traditional methods is time consuming and genetically difficult, but easier if the genes responsible for meat quality are identified and mapped. Compared to other genomic approaches such as QTL scan, the association analysis of allele variants on candidate genes with porcine economic traits is easier and of practical use in marker assisted selection for breeding. The results presented here indicate that selection of the C allele will lead to heavier pigs with higher birth weight and average daily gain, but exhibiting a lower fat deposition and negative backfat thickness. Nevertheless, a higher frequency of allele T in the population would increase these traits, especially intramuscular fat content which is important for flavor and to aid slow dehydratation during the curing process in manufacture of dry-cured products. Consequently, the investigation of *SIM1* polymorphism related to fat and meat quality is perhaps the most meaningful finding, although the results obtained are preliminary. One immediate goal is to obtain the complete CDs of *SIM1* in an attempt to identify the meaning of T/C substitution and to find other polymorphisims.

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