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Association analyses of porcine *SERPINE1* reveal sex-specific effects on muscling, growth, fat accretion and meat quality

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Summary

The *serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1* (*SERPINE1*) gene encodes plasminogen activator inhibitor type 1 (PAI), which is the major physiological inhibitor of tissue-type and urokinase-type plasminogen activators and plays a role in obesity and insulin resistance in women but not in men. We detected SNP FN396538:g.566G>A in intron 3 and a non-synonymous substitution NM_213910: c.612A>G in exon 3 (p.Ile159Val) and mapped the gene to position 8.4 cM on the linkage map of chromosome 3. Association analyses were conducted on the 12th–15th generation of the Meishan × Large White (MLW) cross (n = 565), with records for weight at the end of test, lifetime daily gain, test time daily gain, loin depth and backfat depth, as well as on a European wild boar × Meishan (W × M) F_2 population (n = 333) with 47 traits recorded for carcass composition and meat quality. Analyses performed across the entire MLW population or in the male animals did not show any trait significantly associated with the loci studied. In female animals, both SNPs were associated with loin depth at nominal $P < 0.05$ with adjusted P values equal to 0.051 (g.566) and 0.057 (c.612). Differences between homozygotes were up to 0.65 SD. In the entire W × M population and female animals, *SERPINE1* was significantly associated at adjusted $P < 0.05$ in descending order with muscling, growth and fat accretion and in male animals with meat quality (R-value). In the studied populations, allele effects were in opposite directions, which implies that the SNPs are markers that are in linkage disequilibrium with a causative mutation.

Keywords association study, fat deposition, meat quality, meatiness, pig, *SERPINE1*, sex-specific QTL.

The *serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1* (*SERPINE1*) gene encodes plasminogen activator inhibitor, type 1 (PAI), a member of the serine protease inhibitor family, which is the major physiological inhibitor of tissue-type and urokinase-type plasminogen activators. PAI plays a role in many patho-physiological processes including obesity and insulin resistance in women. PAI is expressed in murine as well as in human adipose tissue, and its expression in human adipose tissue is positively correlated with body mass index. Studies in mice and humans have demonstrated that PAI is linked to fat metabolism, insulin resistance and type 2 diabetes (reviewed by Lijnen 2005 and Alessi *et al.* 2007). Thus, the *SERPINE1* gene is an obvious candidate for fatness and carcass composition traits in pigs. The human *SERPINE1* gene, located on chromosome 7 at q21.3-q22 (Klinger *et al.* 1987), is approximately 12.2 kb long and consists of nine exons (Loskutoff *et al.* 1987). The porcine *SERPINE1* cDNA sequence consists of 131 bp of 5′ untranslated region, a 1206-bp open reading frame encoding a 402 amino acid protein, and a 1651-bp 3′ untranslated sequence. The porcine *SERPINE1* gene is closely related to the human gene, with nucleotide and amino acid identities 78% and 86% respectively (Bijnens *et al.* 1997).

The aim of this work was to search for polymorphisms within the porcine *SERPINE1* gene and to perform an association study in a segregating Meishan × Large White

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(MLW) cross population, allowing a comparison with a European wild boar × Meishan (W × M) F_2 population.

We detected SNP *FN396538:g.566G>A* (NCBI ss469105327) in intron 3, as well as detecting, by comparative sequencing of cDNA from m. longissimus lumborum et thoracis (m.l.l.t.) from two European wild boar and two Meishan animals, SNP *NM_213910:c.612A>G* (NCBI ss469105328) in exon 3 causing amino acid substitution p.Ile159Val (for PCR primers, PCR amplification conditions, cDNA cloning, sequencing and SNP genotyping see Appendix S1 and Table S1). Allele frequencies estimated in four breeds and European wild boar are given in Table S2. In all populations, allele frequencies were above 5%, except for allele *g.566G* in Duroc and European wild boar.

Associations between the SNPs and traits were analysed using the GLM procedure of SAS, release 8.2 (SAS Institute Inc.). These studies were conducted in the 12th–15th generation of the MLW cross (PIC) and in a W × M F_2 population. The segregating MLW cross was used for the association study because of its short extent of linkage disequilibrium (LD) (Goldstein & Weale 2001). In this population, as in other European Meishan crossbred lines, the level of LD is expected to be higher than that in purebred Chinese breeds, extending up to 0.05 cM. However, this is still lower than in most European breeds, extending up 2 cM, with a threshold of $r^2 = 0.3$ assuming that on average 1 cM is equivalent to 1 Mb (Du *et al.* 2007; Amaral *et al.* 2008). The W × M F_2 population was utilized for the comparison of results obtained on the MLW population and because of the great number of precisely measured traits despite the large extent of LD expected.

The MLW population consisted of 565 animals with 326 castrated male and 239 female animals genotyped for SNP g.566G>A. However, only 558 animals (323 castrated males and 235 females) were successfully genotyped for SNP c.612A>G. The following trait records were collected in the population: weight at the end of the test, lifetime daily gain, test time daily gain, loin depth and backfat depth (Appendix S1). Pigs were slaughtered at age 145 ± 4 days and live weight 81.3 ± 10.3 kg. Means and standard deviations of traits for the entire population and female animals are given in Table S3.

In the MLW population, the frequencies of *g.566G* and *c.612A* alleles were 0.40 and 0.54 in the whole population, 0.41 and 0.53 in male and 0.40 and 0.55 in female animals, respectively. The loci *g.566* and *c.612* were in LD with $r^2 = 0.52$ (for details see Du *et al.* 2007). The statistical model for association analyses performed in the population included the genotypes of one of the loci, sex and season of the test as discontinuous independent variables, and slaughter age as a continuous variable (for details see Appendix S1). Analyses performed across the entire MLW population or in the male animals did not show any trait significantly associated with the loci studied. In female animals, both SNPs were associated at nominal $P < 0.05$ with loin depth (Table 1), in which the *g.566A* and *c.612G* alleles were associated with higher muscling (the differences between homozygotes were 0.65 and 0.56 SD respectively). After adjustment for multiple testing, both associations became very close to significance at $P < 0.05$. To check the size of *SERPINE1* × sex interaction, both polymorphisms were analysed separately with the *SERPINE1* × sex interaction in the model (Appendix S1). The genotype × sex interaction effects were highly significant for loin depth with *P* values equal to 0.0035 and 0.0051 for the loci *g.566* and *c.612* respectively in the MLW population.

To compare results obtained in the MLW population and the W × M F_2 resource population (Müller *et al.* 2000; Geldermann *et al.* 2003) with a whole genome linkage map, the 47 traits recorded for carcass composition and meat

Table 1 Association between genotypes of the *SERPINE1* gene and performance in female animals of the Meishan × Large White synthetic (MLW) population. For each genotype, least squares mean ± standard error (LSmean ± SE) are given.

	F test						
Trait	*F* value	Nominal *P* value	Adjusted *P* value	PV (%)	*t* test (LSmean ± SE)		
					Genotype *g.566G>A*		
					AA (*n* = 71)	AG (*n* = 143)	GG (*n* = 25)
Loin depth (mm)	4.68	0. 0102*	0.0510	3.14	48.30 ± 0.73[a]	47.13 ± 0.57[1]	44.05 ± 1.22[1,a]
					Genotype c.612A>G (Ile159Val)		
					AA (*n* = 62)	AG (*n* = 133)	GG (*n* = 40)
Loin depth (mm)	4.57	0.0114*	0.0570	3.10	45.89 ± 0.78[a]	47.32 ± 0.60[1]	49.56 ± 0.97[1,a]

P = *P* value of the *F* test; *$P < 0.05$; Adjusted *P* value = *P* value of the *F* test adjusted for multiple testing (Benjamini & Hochberg 1995); PV (%) = the proportion of error variance reduction by inclusion of the independent variable genotype *SERPINE1* in the initial model. From the *t* test matching small letters indicate differences significant at level $P < 0.01$; matching numbers indicate that differences are significant at the level of $P < 0.05$.

quality listed in Table S4 were used. Pigs were slaughtered at the age 210 ± 6 days and live weight of 71.7 ± 13.8 kg.

In the W × M population, both SNPs were in complete LD with haplotypes fixed in the founder populations. The *g.566A–c.612G* haplotype was fixed in European wild boar, while the *g.566G-c.612A* haplotype was fixed in Meishan F_0 animals. Frequencies of the *g.566A–c.612G* haplotype in the entire F_2 (n = 333), F_2 female (n = 165) and F_2 male (n = 168) populations were 0.51, 0.53 and 0.49 respectively.

Linkage mapping performed in the W × M population with the SNP *g.566G>A* placed the *SERPINE1* gene 11.9 Kosambi cM in front of the most proximal marker *SW72* on the porcine chromosome 3 (SSC3) linkage map of the W × M family (Beeckmann *et al.* 2003). After correction for map length, this corresponds to position 8.4 cM on the USDA–USMARC linkage map of SSC3 (Rohrer *et al.* 1996). The human gene is at position 100.7 Mb on chromosome 7 (Ensembl Human – assembly GRCh37.p2, August 2010). This position is located between *SW2021* (12.4 cM) and *SW2429* (17.3 cM) on the USDA–USMARC linkage map of SSC3 (Meyers *et al.* 2005).

In the W × M family, the statistical model for the association analyses included fixed effects of *SERPINE1* haplotype, season (2-month interval), sex, litter number (one or two) and slaughter age as a continuous linear effect in the model. For separate analyses of male and female animals, variable sex was omitted. For the *SERPINE1* × sex interaction, the haplotypes were analysed with the *SERPINE1* × sex interaction in the model (for the models see Appendix S1).

In the W × M population, the *SERPINE1* gene was associated at nominal $P < 0.05$ with four growth and fattening traits, seven fat deposition traits, five muscling traits and two meat quality traits in the entire W × M population (Table S5), with four growth and fattening traits, three fat deposition traits, four muscling traits and three another traits in females (Table S6) and with three meat quality traits in males (Table S7). Traits significantly associated in the entire population, females and males, after correction for multiple testing (Benjamini & Hochberg 1995) at adjusted $P < 0.05$, are listed in Table 2. Both in the entire population and in female animals, the *g.566G–c.612A* haplotype that originated with the Meishan breed was associated with higher growth and fattening, fat deposition and muscling compared to animals with the *g.566A–c.612G* haplotype that originated with European wild boar. In female animals, *SERPINE1* explained approximately twice the proportion of phenotypic variance reduction (PV) compared to the entire population, with up to 9.95% PV for ham meat weight (Table 2). Moreover, in female animals, the *g.566G–c.612A* haplotype was associated with heavier heads and hearts and longer carcasses (Table S6). The SNPs were associated at nominal $P < 0.05$ with meat quality traits in the entire W × M population (cooling loss and pH24, m.l.l.t) (Table S5) and in male animals (*R*-value, pH24, m.l.l.t and glycolytic potential) (Table S7). After correction for multiple tests, only the *R*-value in males was significant with adjusted $P < 0.05$. For all associated traits in female and male animals, the genotype × sex interaction effects were highly significant with P values equal to 0.0002–0.001 for muscling traits, 0.0001–0.0016 for growth and fattening traits, 0.0001–0.0048 for fat deposition traits, 0.0019–0.0216 for other traits and 0.0013 for *R*-value.

Analyses performed in the W × M population corroborated our findings obtained on the MLW population that the *SERPINE1* gene is associated with muscling in female but not in male animals. In the entire and female populations, the *SERPINE1* gene was associated in descending order of magnitude with muscling, growth and fattening, and fat deposition. However, allele effects for both loci were reversed in the MLW and W × M populations, which implies that the studied SNPs are markers in LD with a causative mutation either in the promoter of the gene or in a *cis*-regulatory region near the *SERPINE1* gene and that the linkage phase between the markers and causative mutation is opposite in MLW and W × M populations.

Prior QTL analyses conducted in the W × M population using the linkage map with *SW72* located at position 17.8 cM on the USDA MARC linkage map as the most proximal marker (http://www.marc.usda.gov/genome/htmls/MarkerSearch.jsp?MarkerName=SW72&Species=sus) revealed QTL for abdominal fat weight and average daily gain at positions 17.8 cM and 23.1 cM on the USDA USARS linkage map of SSC3 (Beeckmann *et al.* 2003; Hu & Reecy 2007). Among other QTL detected in the proximal region of SSC3 are QTL for birth weight at positions 17.8 and 23.0 cM (Quintanilla *et al.* 2002; Liu *et al.* 2007), paternally expressed QTL for average daily gain at position 17.8 cM (De Koning *et al.* 2001), and QTL for average backfat thickness at position 0 cM detected in an experiment performed with gilts only (Kuehn *et al.* 2007). To the best of our knowledge, sex-specific QTL for muscling and meat quality traits have not been detected previously in the proximal region of SSC3.

Studies using a nutritionally induced obesity model in transgenic mice support a role for the *SERPINE1* gene in adipogenesis and obesity. However, the role of PAI in the development of adipose tissue remains enigmatic (reviewed by Lijnen 2005, 2009, 2011). The effects of the *SERPINE1* gene may be mediated through the ability of PAI to interfere with insulin signalling and/or through inhibition of protein convertase furin, which is involved in transforming growth factor, beta 1 activation and insulin receptor processing (reviewed by Alessi *et al.* 2007). In humans, association studies carried out with the *SERPINE1* −675 4G/5G promoter polymorphism showed that the presence of the *4G* allele was positively correlated with PAI protein levels and that protein levels were associated with increased insulin resistance and overall and central

Table 2 Association between haplotypes of the *SERPINE1* gene and performance traits in males and females in the W × M F_2 population. For each haplotype, least squares mean ± standard error (LSmean ± SE) are given.

	F-test				*t*-test (LSmean ± SE)		
					Haplotype *FN396538:g.566-NM_213910:c.612*		
Population/Trait	*F*-value	Nominal *P*-value	Adjusted *P*-value	PV (%)	A-G /A-G	A-G/G-A	G-A/G-A
W × M (♂ + ♀)					(*n* = 90)	(*n* = 160)	(*n* = 83)
Growth and fattening							
Carcass weight cold (kg)	7.65	0.0006***	0.0056**	3.97	51.71 ± 1.27^{1,A}	54.65 ± 1.03^{1,2}	58.27 ± 1.32^{2,A}
Live weight at slaughter (kg)	7.24	0.0008***	0.0063**	3.73	67.62 ± 1.51^{A}	70.94 ± 1.22^{1}	75.15 ± 1.56^{1,A}
Food consumption (kg)	6.46	0.0018**	0.0106*	3.28	187.87 ± 3.93^{A}	193.26 ± 3.20^{a}	205.86 ± 4.07^{a,A}
Food conversion ratio (kg/kg)	4.41	0.0130*	0.0460*	2.07	4.10 ± 0.08^{1,a}	4.31 ± 0.06^{1}	4.39 ± 0.08^{a}
Fat deposition							
Abdominal fat weight (kg)	4.97	0.0075**	0.0353*	2.41	0.85 ± 0.04^{a}	0.90 ± 0.03^{1}	1.03 ± 0.04^{1,a}
Back fat weight (kg)	4.79	0.0089**	0.0380*	2.30	2.13 ± 0.09^{a}	2.29 ± 0.07	2.48 ± 0.09^{a}
Fat area on m.l.l.t. at 13th/14th rib (cm^2)	4.52	0.0115*	0.0450*	2.14	23.61 ± 0.70^{a}	24.29 ± 0.57^{1}	26.26 ± 0.73^{1,a}
Ham external fat weight (kg)	4.35	0.0137*	0.0460*	2.04	2.15 ± 0.07^{a}	2.27 ± 0.06^{1}	2.43 ± 0.08^{1,a}
Muscling							
Shoulder meat weight (kg)	10.26	<0.0001***	0.0024**	5.44	2.24 ± 0.05^{a,A}	2.39 ± 0.04^{1,a}	2.54 ± 0.05^{1,A}
Ham meat weight (kg)	9.81	<0.0001***	0.0024**	5.19	4.18 ± 0.08^{1,A}	4.40 ± 0.07^{1,a}	4.66 ± 0.08^{a,A}
Loin and neck meat weight (kg)	8.96	0.0002***	0.0031**	4.70	3.59 ± 0.08^{1,A}	3.80 ± 0.06^{1,2}	4.01 ± 0.08^{2,A}
Ham weight (kg)	7.83	0.0005***	0.0056**	4.07	6.88 ± 0.16^{1,A}	7.23 ± 0.13^{1,2}	7.70 ± 0.16^{2,A}
W × M (♀)					(*n* = 48)	(*n* = 80)	(*n* = 37)
Growth and fattening							
Carcass weight cold (kg)	7.04	0.0012**	0.0094**	7.23	50.31 ± 1.75^{A}	53.26 ± 1.48	59.49 ± 2.00^{A}
Live weight at slaughter (kg)	6.84	0.0014**	0.0094**	7.00	65.86 ± 2.05^{A}	69.38 ± 1.74^{a}	76.51 ± 2.35^{a,A}
Food consumption (kg)	6.02	0.0030**	0.0162*	6.09	180.82 ± 4.99^{a}	185.57 ± 4.23^{b}	204.09 ± 5.70^{a,b}
Fat deposition							
Back fat weight (kg)	4.26	0.0158*	0.0619	4.19	2.04 ± 0.11^{a}	2.17 ± 0.10^{1}	2.5 ± 0.13^{1,a}
Shoulder external fat weight (kg)	4.91	0.0085**	0.0400*	4.00	0.92 ± 0.04^{1}	0.96 ± 0.04^{2}	1.10 ± 0.05^{1,2}
Muscling							
Ham meat weight (kg)	9.43	0.0001***	0.0047**	9.95	4.06 ± 0.12^{A}	4.35 ± 0.10^{a}	4.77 ± 0.13^{a,A}
Shoulder meat weight (kg)	8.70	0.0003***	0.0071**	9.29	2.18 ± 0.07^{A}	2.37 ± 0.06^{1}	2.60 ± 0.08^{1,A}
Loin and neck meat weight (kg)	7.85	0.0006***	0.0085**	8.02	3.55 ± 0.10^{A}	3.76 ± 0.09^{a}	4.13 ± 0.12^{a,A}
Ham weight (kg)	7.41	0.0009***	0.0085**	7.64	6.70 ± 0.22^{A}	7.11 ± 0.18^{a}	7.88 ± 0.25^{a,A}
Other traits							
Weight of head (kg)	7.43	0.0008***	0.0085**	7.66	3.50 ± 0.11^{A}	3.84 ± 0.09	4.11 ± 0.13^{A}
Weight of heart (g)	5.98	0.0031**	0.0161*	6.04	195.62 ± 5.06^{a}	202.36 ± 4.29^{b}	219.80 ± 5.78^{a,b}
Carcass length (cm)	4.65	0.0110*	0.0470*	4.49	75.95 ± 0.82^{A}	77.71 ± 0.70	79.52 ± 0.94^{A}
W × M (♂)					(*n* = 42)	(*n* = 80)	(*n* = 46)
Meat quality							
R-value	7.36	0.0009***	0.0423*	8.27	0.98 ± 0.02^{a}	0.97 ± 0.02^{A}	1.06 ± 0.02^{a,A}

P = *P*-value of the *F* test; *$P < 0.05$; **$P < 0.01$;***$P < 0.001$; Adjusted *P*-value = *P*-value of the *F* test adjusted for multiple testing (Benjamini & Hochberg 1995); PV (%) = the proportion of error variance reduction by inclusion of the independent variable genotype *SERPINE1* in the initial model. From the *t* test matching capital letters indicate differences significant at the level of $P < 0.001$; matching small letters indicate differences significant at the level of $P < 0.01$; matching numbers indicate that the differences are significant at the level of $P < 0.05$.

obesity (Bensen *et al.* 2004). Hoffstedt *et al.* (2002) concluded that the −675 4G/5G polymorphism was strongly linked to obesity and that increased risk of obesity is associated with the *4G* allele in homozygotes. Similar results were obtained in men and women. Contrary to this, Bouchard *et al.* (2005) found that the −675 4G/5G polymorphism was strongly associated with body mass index ($P < 0.01$) and fat mass ($P < 0.05$) in women. The −675 4G/5G polymorphism and *c.43G>A* (p.Ala15Thr) variant within exon 1 were associated with abdominal visceral fat only in post-menopausal women, and no association was observed in men. The −675 4G/5G polymorphism was also found to be significantly associated with myocardial infarction only in women (Ahmed *et al.*

2010). Bouchard *et al.* (2005) explained the sex-specific effects of *SERPINE1* polymorphism by hypothesizing that oestrogen could modulate the effects of *SERPINE1* genotypes on fat mass and visceral fat accretion. This is in agreement with the finding of oestrogen response elements in the promoter of the *SERPINE1* gene (Smith *et al.* 2004).

In conclusion, analyses of SNPs within the porcine *SERPINE1* gene have revealed sex-specific effects with influence on muscling, growth and fattening, which affect fat deposition in female animals and meat quality traits in male animals. However, these effects should be verified in other populations ahead of any application in the pig industry.

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References

Ahmed W., Malik M., Saeed I., Khan A.A., Sadeque A., Kaleem U., Ahmed N., Ajmal M., Azam M. & Qamar R. (2010) Role of tissue plasminogen activator and plasminogen activator inhibitor polymorphism in myocardial infarction. *Molecular Biology Reports* **38,** 2541–8.

Alessi M.C., Poggi M. & Juhan-Vague I. (2007) Plasminogen activator inhibitor-1, adipose tissue and insulin resistance. *Current Opinion in Lipidology* **18,** 240–5.

Amaral A.J., Megens H.J., Crooijmans R.P.M.A., Heuven H.C.M. & Groenen M.A.M. (2008) Linkage disequilibrium decay and haplotype block structure in the pig. *Genetics* **179,** 569–79.

Beeckmann P., Schröffel J. Jr, Moser G., Bartenschlager H., Reiner G. & Geldermann H. (2003) Linkage and QTL mapping for *Sus scrofa* chromosome 3. *Journal of Animal Breeding and Genetics* **120** (Suppl 1), 20–7.

Benjamini Y. & Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B* **57,** 289–300.

Bensen J.T., Hsu F.-C., Brown W.M. *et al.* (2004) Association analysis of the plasminogen activator inhibitor-1 4G/5G polymorphism in Hispanic and African Americans: the IRAS family study. *Human Heredity* **57,** 128–37.

Bijnens A.P., Knockaert I., Cousin E., Kruithof E.K. & Declerck P.J. (1997) Expression and characterization of recombinant porcine plasminogen activator inhibitor-1. *Journal of Thrombosis and Haemostasis* **77,** 350–6.

Bouchard L., Mauriege P., Vohl M.C., Bouchard C. & Pérusse L. (2005) Plasminogen-activator inhibitor-1 polymorphisms are associated with obesity and fat distribution in the Quebec Family Study: evidence of interactions with menopause. *Menopause* **12,** 136–43.

De Koning D.J., Ratting A.P., Harlizius B., Groenen M.A.M., Brascamp E.W. & van Arendonk J.A.M. (2001) Detection and characterization of quantitative trait loci for growth and reproduction traits in pigs. *Livestock Production Science* **72,** 185–98.

Du F.X., Clutter A.C. & Lohuis M.M. (2007) Characterization of linkage disequilibrium in pig populations. *International Journal of Biological Sciences* **3,** 166–78.

Geldermann H., Müller E., Moser G. *et al.* (2003) Genome wide linkage and QTL mapping in porcine F_2 families generated from Pietrain, Meishan and Wild Boar crosses. *Journal of Animal Breeding and Genetics* **120,** 363–93.

Goldstein D.B. & Weale M.E. (2001) Population genomics: linkage disequilibrium holds the key. *Current Biology* **11,** R576–9.

Hoffstedt J., Andersson I.L., Persson L., Isaksson B. & Arner P. (2002) The common −675 4G/5G polymorphism in the plasminogen activator inhibitor-1 gene is strongly associated with obesity. *Diabetologia* **45,** 584–7.

Hu Z.L. & Reecy J.M. (2007) AnimalQTLdb: beyond a repository – a public platform for QTL comparisons and integration with diverse types of structural genomic information. *Mammalian Genome* **18,** 1–4.

Klinger K.W., Winqvist R., Riccio A. *et al.* (1987) Plasminogen activator inhibitor type 1 gene is located at region q21.3-q22 of chromosome 7 and genetically linked with cystic fibrosis. *Proceedings of the National Academy of Sciences of the United States of America* **84,** 8548–52.

Kuehn L.A., Rohrer G.A., Nonneman D.J., Thalmann R.M. & Leymaster K.A. (2007) Detection of single nucleotide polymorphism associated with ultrasonic backfat depth in a segregating Meishan x White Composite population. *Journal of Animal Science* **85,** 1111–9.

Lijnen H.R. (2005) Pleiotropic functions of plasminogen activator inhibitor-1. *Journal of Thrombosis and Haemostasis* **3,** 35–45.

Lijnen H.R. (2009) Role of fibrinolysis in obesity and thrombosis. *Thrombosis Research* **123** (Suppl 4), S46–9.

Lijnen H.R. (2011) Murine models of obesity and hormonal therapy. *Thrombosis Research* **127** (Suppl 3), S17–20.

Liu G., Jennen D.G.J., Tholen E. *et al.* (2007) A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc-Pietrain resource population. *Animal Genetics* **38,** 241–52.

Loskutoff D.J., Linders M., Keijer J., Veerman H., van Heerikhuizen H. & Pannekoek H. (1987) Structure of the human plasminogen activator inhibitor 1 gene: nonrandom distribution of introns. *Biochemistry* **26,** 3763–8.

Meyers S.N., Rogatcheva M.B., Larkina D.M., Yerle M., Milan D., Hawken R.J., Schook L.B. & Beever J.E. (2005) Piggy-BACing the human genome II. A high-resolution, physically anchored comparative map of the porcine autosomes. *Genomics* **86,** 739–52.

Müller E., Moser G., Bartenschlager H. & Geldermann H. (2000) Trait values of growth, carcass and meat quality in Wild Boar, Meishan and Pietrain pigs as well as their crossbred generations. *Journal of Animal Breeding and Genetics* **117,** 189–202.

Quintanilla Q., Milan D. & Bidanel J.-P. (2002) A further look at quantitative trait loci affecting growth and fatness in a cross between Meishan and Large White pig populations. *Genetics Selection Evolution* **34,** 193–210.

Rohrer G.A., Alexander L.J., Hu Z., Smith T.P., Keele J.W. & Beattie C.W. (1996) A comprehensive map of the porcine genome. *Genome Research* **6,** 371–91.

Smith L.H., Coats S.R., Qin H., Petrie M.S., Covington J.W., Su M., Eren M. & Vaughan D.E. (2004) Differential and opposing regulation of PAI-1 promoter activity by estrogen receptor alpha and estrogen receptor beta in endothelial cells. *Circulation Research* **95**, 269–75.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Materials and methods.

Table S1 Description of porcine *SERPINE1* primers.

Table S2 Allele frequencies at loci *FN396538g.566G>A* and *NM_213910:c.612A>G* of *SERPINE1*gene in various populations of unrelated animals.

Table S3 Overall means and phenotypic standard deviation (SD) of age at slaughter (independent trait) and five dependent traits studied in entire population and females of Meishan × Large White (MLW) cross.

Table S4 Traits used for association analysis of *SERPINE1*genotypes in European wild boar × Meishan (W × M) F_2 family.

Table S5 Association between haplotypes of SERPINE1 gene and performance traits for both female and male animals of European wild boar × Meishan F_2 (W × M) population.

Table S6 Association between haplotypes of *SERPINE1* gene and performance traits for female animals of European wild boar × Meishan F_2 (W × M) population.

Table S7 Association between haplotypes of *SERPINE1* gene and performance traits in male animals of European wild boar × Meishan F_2 (W × M) population.

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