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cDNA Cloning, Tissue Expression and Association of Porcine Pleiomorphic Adenoma Gene-like 1 (PLAGL1) Gene with Carcass Traits

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ABSTRACT : Pleiomorphic adenoma gene-like1 (*PLAGL1*) encodes a zinc-finger (ZF) protein with seven ZFs of the C2H2-type which is a regulator of apoptosis and cell cycle arrest, and also regulates the secretion of insulin. In both human and mouse, *PLAGL1* is a candidate gene for tumor suppressor and transient neonatal diabetes mellitus (TNDM). In this study, a 2,238 bp fragment covering the complete coding region was obtained and deposited to GenBank (accession number: DQ288899). The reverse transcriptase-polymerase chain reaction (RT-PCR) indicated that *PLAGL1* was expressed almost equally in heart, liver, spleen, lung, kidney, stomach, small intestine, skeletal muscle, fat, uterus and ovary. Comparing the sequences of Large White and Meishan pigs, a C-T transition in exon 6 was found. The polymorphism could be detected by *Taq*I and was genotyped in five purebreds (Large White, Landrace, Meishan, Tongcheng and Bamei). Association analysis was performed between the polymorphism and carcass traits in 276 pigs of a "Large White ×Meishan" F2 resource population. As a consequence, significant associations of the genotypes with shoulder backfat thickness (SFT) and internal fat rate (IFR) were observed. Pigs with TT genotype had low SFT and high IFR compared with TC or CC genotypes. (**Key Words :** *PLAGL1*, Porcine, Polymorphism, Carcass Traits)

INTRODUCTION

Pleiomorphic adenoma gene-like 1 (*PLAGL1*) gene encoding a C2H2 zinc finger protein and located on 6q24-6q25 in the human and chromosome 10 in the mouse (Abdollahi et al., 1997; Piras et al., 2000), is a regulator of apoptosis and cell cycle arrest (Spengler et al., 1997). Bilanges et al. (1999) reported that *PLAGL1* was a candidate gene for tumor suppressor. Moreover, *PLAGL1* is a transcriptional regulator of the type 1 receptor for pituitary adenylate cyclase-activating polypeptide, which is the most potent known insulin secretagogue and an important mediator of autocrine control of insulin secretion in the pancreatic islet (Kamiya et al., 2000). In humans, *PLAGL1* was paternal expressed (Abdollahi et al., 1997), and paternal duplication of it causes transient neonatal diabetes mellitus (TNDM) which presents with intrauterine growth retardation (Gardner et al., 1999; Ma et al., 2004). Arima et al. (2005) suggested that *PLAGL1*, *LIT1* (*KCNQ1OT1*) and *p57KIP2* (*CDKN1C*) were in an imprinted gene network that may play an important role in Beckwith-Wiedemann syndrome. *PLAGL1* regulated $p57^{KIP2}$ through *LIT1* and was part of a novel signaling pathway regulating cell growth. All these studies indicated that *PLAGL1* might play an important role in growth and carcass composition. However, there are no reported studies of the *PLAGL1* gene in pigs.

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MATERIALS AND METHODS

Experimental animals

In this study, 276 F2 hybrids of Large White×Meishan pigs, 47 Large White pigs, 44 Landrace pigs, and 75 Meishan pigs were obtained from the Experimental pig station of Huazhong Agricultural University. Thirty-nine Tongcheng pigs and 31 Bamei pigs were obtained from different research farms in China. Pigs for association analysis were fed as described by Zuo et al. (2004). The finishing animals were slaughtered in 2003 and 2004 and measurements made according to the method of Xiong and Deng (1999).

Table 1. Primer sequences used in the amplification and genotyping the polymorphism in the porcine *PLAGL1* gene

Primers	Primer sequences	Annealing temperature (°C)	Product size (bp)	Template
CDS1F	AGCTTCCGAGAGGACTCC	60	698	cDNA
CDS1R	AGCCCAGCATGGTGTTG			
CDS2F	ACCTCCAAACCCACGACC	53	924	cDNA
CDS2R	GCCAGAAGCCCAACAGG			
CDS3F	CCTCATACTCCCCACTTG	55	934	cDNA
CDS3R	ATCCCTGAAAAGAAATACAC			
TaqIF	GCGACATACCAAGAAGACG	63	706	DNA
TaqIR	GCCCAGGCTCATAGTGC			
GAPDHF	ACCACAGTCCATGCCATCAC	60	480	cDNA
GAPDHR	TCCACCACCCTGTTGCTGT			

RNA isolation and cDNA synthesis

Total RNA from adult porcine skeletal muscle was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The first strand cDNAs were synthesized from 2 μg total RNA treated with DNAse I (TaKaRa, Tokyo, Japan) in a 20 μl reaction containing 5 μM oligo$(dT)_{16}$ primer, 1×M-MLV first-strand buffer, 40 U M-MLV reverse transcriptase, 1 mM dNTP and 8 U RNase inhibitor (Promega, Madison, WI, USA) at 42°C for 60 min.

Primer design and PCR conditions

The human *PLAGL1* cDNA sequence (GenBank: NM_006718) was used to search available ESTs in the 'EST-others' database with the standard BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The pig ESTs shared more than 80% homology with the human cDNA sequences were assembled to produce an EST-contig. Primers (Table 1) were designed based on the sequences of contig. PCR reactions were all amplified in 25-μl reaction volume consisting of 100 ng porcine genomic DNA or 50 ng cDNA, 1× PCR buffer, 0.5 μmole of each primer, 100 μmole of each dNTP, 2 mM $MgCl_2$ and 1 U *Taq* DNA polymerase (Promega, Madison, WI, USA). The PCR parameters were 4 min at 94°C, and then 45 s at 94°C, 50 s at optimal temperature and 1 min at 72°C for 35 cycles followed by a final extension of 10 min at 72°C.

Expression profile of porcine *PLAGL1* gene

Total RNA from heart, liver, spleen, lung, kidney, stomach, small intestine, skeletal muscle, fat, ovary and uterus of an adult Meishan pig were extracted and the synthesis of the first strand of cDNA were performed as described above. Primer pair CDS1F/CDS1R was used to determine the expressed status of *PLAGL1* gene in the eleven tissues. The house-keeping gene GAPDH was also amplified as internal control. PCR conditions for the expression profile were as follows: 94°C, 4 min; 28 cycles with 94°C, 45s; 60°C, 50 s, 72°C, 45 s; and a final extension at 72°C for 10 min.

Polymorphism screening and PCR-RFLP examination

RT- PCR products with primers CDS1F/CDS1R, CDS2F/CDS2R and CDS3F/CDS3R were purified with Wizard prep PCR purification system (Promega), cloned with pMD18-T easy vector (TaKaRa, Tokyo, Japan) and sequenced commercially. Compared sequences of Large White and Meishan breeds revealed one SNP(C/T) at position 1,428 (DQ288899) of the *PLAGL1* gene. A primer pair *Taq*IF/*Taq*IR (Table 1) was designed to distinguish the genotype well. PCR products (9 μl) with 3 U restriction enzyme and 1 μl standard buffer were incubated at 65°C for 4 h and then separated by 1.5% agarose gels.

Statistical analyses

The association between genotype and carcass trait was performed with the least square method (GLM procedure, SAS version 8.0). According to the method of Liu (1998), both additive and dominance effects were also estimated using the REG procedure of SAS version 8.0, where the additive effect was denoted as -1, 0 and 1 for TT, TC and CC, respectively, and the dominance effect represented as 1, -1 and 1 for TT, TC and CC, respectively. The statistical model was assumed to be: $T_{ijk} = \mu+S_i+Y_j+G_k+b_{ijk}X_{ijk}+e_{ijk}$, where T_{ijk} is the observed values of traits; μ is the least-square mean; S_i is effect of sex (i = 1 for male or 0 for female), Y_j is the effect of year (j = 1 for year 2003 or 0 for year 2004), G_k is the effect of genotype (K = CC, CT and TT), b_{ijk} is the regression coefficient of the slaughter weight, X_{ijk} is the slaughter weight, and e_{ijk} is the random residual.

RESULTS

Sequence analysis and tissue distribution of porcine *PLAGL1* gene

Primers CDS1F/CDS1R, CDS2F/CDS2R and CDS3F/CDS3R amplified a total of 2238bp (Genbank accession number DQ288899) of porcine *PLAGL1* gene from cDNA of skeletal muscle tissue. Comparing the porcine sequence to homologythe human *PLAGL1* cDNA sequence revealed 382 bp of 5' untranslated sequence, a complete open reading fragment (ORF) consisting of 463 amino acids and

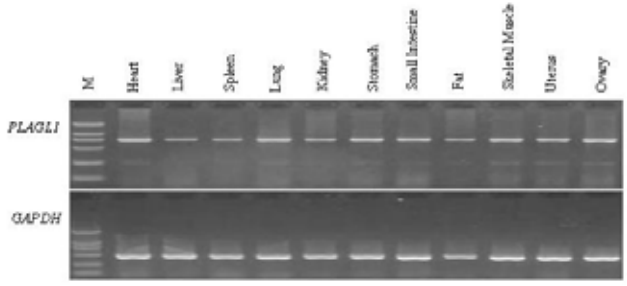

Figure 1. Tissues distribution of porcine *PLAGL1* gene in heart, liver, spleen, lung, kidney, stomach, small intestine, fat, skeletal muscle, uterus and ovary. M: DNA Ladder (2,000 bp, 1,000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

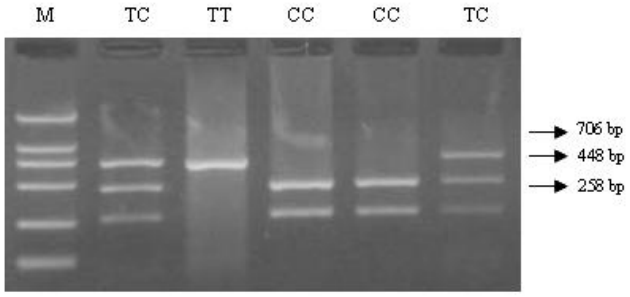

Figure 2. PCR-*Taq*I-RFLP of porcine *PLAGL1* gene. Lane M: DNA Ladder (2,000 bp, 1,000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

a 464 bp 3' untranslated sequence. Sequence analysis showed that compared to human and mouse it had, respectively, 90% and 83% homology in nucleotides or 91% and 68% homology in amino acids. RT-PCR of heart, liver, spleen, lung, kidney, stomach, small intestine, skeletal muscle, fat, uterus and ovary indicated that porcine *PLAGL1* gene was expressed almost equally in all the tissues examined (Figure 1).

SNP detection and allele frequencies

The SNP at position 1,428 (DQ288899) was in the coding region of exon 6 and was a samesense mutation. It could be detected by restriction enzyme *Taq*I (Takara). Primers *Taq*IF and *Taq*IR were used to distinguish the genotype TT (706 bp), TC (706 bp+448 bp+258 bp) and CC (448 bp+258 bp) (Figure 2). This SNP was typed in five breeds including two Western breeds (Landrace and Large White) and three Chinese ones (Meishan, Tongcheng and Bamei). Sample size of each breed and allele frequencies are listed in Table 2. From the table, we can conclude that allele frequencies of this polymorphism are significantly different between Chinese and Western pig breeds. The allele distribution revealed that the Chinese indigenous breeds Meishan, Tongcheng and Bamei had higher frequencies of the T allele, containing 73.3%, 55.1% and 61.7%, respectively. In comparison, allele C was the only allele present in Western breeds (Large White and Landrace).

Association analysis of genotypes and carcass traits

276 pigs of the "Large White×Meishan" F2 resource family were used to estimate the association between the polymorphism and carcass traits. At this locus, the number of genotypes TT, TC and CC were 24, 144 and 108, respectively. The detailed results of association analysis are listed in Table 3. Among the 12 carcass traits, statistically significant associations between PCR-*Taq*I-RFLP and shoulder backfat thickness (SFT) ($p<0.05$) and internal fat rate (IFR) ($p<0.05$) were found, but no significant conclusion can be made on other carcass traits. At this locus, pigs with the TT genotype had low SFT (-9.13% and -10.1%) and high IFR (8.77% and 10.56%) compared with pigs with CC and TC genotypes, respectively. The dominant effect was significant ($p = 0.0446$) on SFT but the additive effect was significant ($p = 0.0438$) on IFR.

DISCUSSION

Imprinted genes are preferentially expressed from either the maternally inherited allele or the paternally inherited allele (Khatib, 2004). In mammals in particular, imprinted genes have an important function in the regulation of fetal growth, development, function of the placenta and postnatal behavior (Reik et al., 2003). In addition, imprinted QTLs affecting carcass composition and growth on chromosome 2 have so far been reported in pigs (Andersson et al., 1994; Alexander et al., 1996; Nezer et al., 1999). Koning et al. (2000) found five QTLs affecting body composition, of which four were imprinted with an experimental cross between Chinese Meishan pigs and commercial Dutch pigs. Lee et al. (2003) genotyped F2 pigs from crosses between Korean native and Landrance pigs at 24 microsatellite

Table 2. The genotype frequencies and allele frequencies of porcine *PLAGL1* gene in five breeds

Breed	No. of animals	Genotype			Allele frequency	
		TT	TC	CC	T	C
Landrace	44	0	0	44	0	1
Large White	47	0	0	47	0	1
Meishan	75	43	24	8	0.7333	0.2667
Tongcheng	39	11	21	7	0.5513	0.4487
Bamei	31	15	12	4	0.6774	0.3226

Table 3. Association of *PLAGL1* genotypes and carcass traits

Trait[1]	Genetype (Lsmean±SE)[2]			Effect (mean±SE)	
	TT (n = 24)	TC (n = 144)	CC (n = 108)	Additive	Dominance
DP (%)	0.718±0.009	0.732±0.004	0.731±0.005	0.0058±0.0054	-0.0037±0.0033
FMP (%)	0.226±0.009	0.242±0.004	0.235±0.005	0.0032±0.0055	-0.0057±0.0035
LMP (%)	0.553±0.007	0.541±0.003	0.546±0.003	-0.0018±0.0047	0.0041±0.0029
IFR (%)	0.0335±0.001[a,3]	0.0308±0.001[b]	0.0303±0.001[b]	-0.0017±0.0009*	0.0006±0.0005
RLF	2.532±0.148	2.386±0.061	2.462±0.070	-0.0121±0.0863	0.0566±0.0538
SFT (cm)	3.276±0.148[a]	3.644±0.061[b]	3.605±0.070[b]	0.1404±0.0878	-0.1042±0.0547*
RFT (cm)	2.653±0.120	2.867±0.0497	2.786±0.057	0.0510±0.0695	-0.0753±0.0433
TFT (cm)	1.994±0.114	2.094±0.047	2.033±0.054	0.0044±0.0664	-0.0420±0.0414
BFT (cm)	1.956±0.142	2.032±0.058	1.952±0.067	-0.0106±0.0794	-0.0397±0.0495
ABT (cm)	2.470±0.1106	2.656±0.0456	2.593±0.0523	0.0457±0.0645	-0.0636±0.0402
CL (cm)	91.931±0.854	91.552±0.349	91.631±0.403	-0.0723±0.4815	0.1221±0.3002
LEA (cm^2)	30.1467±0.947	29.975±0.387	29.760±0.447	-0.0733±0.5454	0.0011±0.3401

[1] DP, Dressing percentage; FMP, Fat meat percentage; LMP, Lean meat percentage; IFR, Internal fat rate; RLF, Ratio of lean to fat; SFT, Shoulder fat thickness; RFT, 6-7 rib fat thickness; TFT, Thorax-waist fat thickness; BFT, Buttock fat thickness; ABT, Average backfat thickness; CL, Carcass length; LEA, Loin eye area.

[2] Least square mean values (±SE).

[3] Different letters denoting significant difference between groups: [a, b,] * $p<0.05$.

markers covering chromosomes 2, 6 and 7, and found two imprinted QTLs affecting growth traits. Therefore, imprinted genes may have an important effect on performance in pigs.

PLAGL1 regulates cell proliferation similarly to *IGF2* which is the first imprinted gene found in pigs and affects fat deposition (Jeon et al., 1999; Thomsen et al., 2004; Estelle et al., 2005). Yang et al. (2005) indicated that a SNP on the *PLAGL1* gene had a significant effect on the content of heart high density lipoprotein 3-cholesterol (HDL3-C). Another report by Kamiya et al. (2000) showed that the human *PLAGL1* gene was an important mediator of insulin secretion. These observations suggest that *PLAGL1* gene may be involved in fat metabolism similarly to *IGF2*. Therefore, porcine *PLAGL1* was selected as a candidate gene affecting fat deposition and carcass traits in our study.

In this research, we isolated the complete coding region of the porcine *PLAGL1* gene. It is well known that gene sequence especially in the coding region is very important for the investigation of gene expression and function. The high sequence similarity of porcine *PLAGL1* gene in amino acid sequences and its counterparts in other mammals indicated the significance and conservation of their biological function during evolution. Tissue distribution revealed that it was expressed almost equally in heart, liver, spleen, lung, kidney, stomach, small intestine, skeletal muscle, fat, uterus and ovary. Although RT-PCR may not accurately quantify expression in different tissues, at least it showed that the gene is expressed in these tissues.

A C/T transition was found in the coding region. Association analysis showed that the polymorphic site had a significant effect on shoulder back fat thickness. Pigs with the TT genotype had low SFT (-9.13%) compared with pigs with CC and TC genotypes (-10.1%). Thus, increasing the favourable allele T may be useful in pigs breeding. The C/T transition was a samesense mutation and does not directly change the amino acid or the structure of protein, but it may alter gene function by affecting translation with codon bias, changing the stability of the mRNA or controlling of transcription of the gene (Zhang et al., 2005). The polymorphism site could be a useful molecular marker for backfat thickness and carcass traits. However, the number of pigs analyzed was limited and other closely linked genes might have affected the observed results. Further investigation is required among other populations of pigs to confirm the association between the PCR-*Taq*I-RFLP and carcass traits.

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REFRENCES

Abdollahi, A., D. Roberts, A. K. Godwin, D. C. Schultz, G. Sonoda, J. R. Testa and T. C. Hamilton. 1997. Identification of a zinc-finger gene at 6q25: a chromosomal region implicated in development of many solid tumors. Oncogene. 14:1973-1979.

Alexander, L. J., D. L. Troyer, G. A. Rohrer, T. P. Smith, L. B. Schook and C. W. Beattie. 1996. Physical assignments of 68 porcine cosmid and lambda clones containing polymorphic microsatellites. Mamm. Genome. 7:368-372.

Andersson, L., C. S. Haley, H. Ellegren, S. A. Knott, M. Johansson,

K. Andersson, L. Andersson-Eklund, I. Edfors-Lilja, M. Fredholm and I. Hansson. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Sci. 263:1771-1774.

Arima, T., T. Kamikihara, T. Hayashida, K. Kato, T. Inoue, Y. Shirayoshi, M. Oshimura, H. Soejima, T. Mukai and N. 2005. ZAC, LIT1 (KCNQ1OT1) and p57KIP2 (CDKN1C) are in an imprinted gene network that may play a role in Beckwith-Wiedemann syndrome. Nucleic. Acids. Res. 33:2650-2660.

Bilanges, B., A. Varrault, E. Basyuk, C. Rodriguez, A. Mazumdar, C. Pantaloni, J. Bockaert, C. Theillet, D. Spengler and L. Journot. 1999. Loss of expression of the candidate tumor suppressor gene ZAC in breast cancer cell lines and primary tumors. Oncogene. 18:3979-3988.

Estelle, J., A. Mercade, J. L. Noguera, M. Perez-Enciso, C. Ovilo, A. Sanchez and J. M. Folch. 2005. Effect of the porcine IGF2-intron3-G3072A substitution in an outbred Large White population and in an Iberian×Landrace cross. J. Anim. Sci. 83:2723-2728.

Gardner, R. J., A. J. Mungall, I. Dunham, J. C. Barber, J. P. Shield, I. K. Temple and D. O. Robinso. 1999. Localisation of a gene for transient neonatal diabetes mellitus to an 18.72 cR3000 (approximately 5.4 Mb) interval on chromosome 6q. J. Med. Genet. 36:192-196.

Jeon, J. T., O. Carlborg, A. Tornsten, E. Giuffra, V. Amarger, P. Chardon, L. Andersson-Eklund, K. Andersson, I. Hansson, K. Lundstrom and L. Andersson. 1999. A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. Nat. Genet. 21:157-158.

Kamiya, M., H. Judson, Y. Okazaki, M. Kusakabe, M. Muramatsu, S. Takada, N. Takagi, T. Arima, N. Wake, K. Kamimura, K. Satomura, R. Hermann, D. T. Bonthron and Y. Hayashizaki. 2000. The cell cycle control gene ZAC/PLAGL1 is imprinted-a strong candidate gene for transient neonatal diabetes. Hum. Mol. Genet. 9:453-460.

Khatib, H. 2004. Imprinting of *Nesp55* gene in cattle. Mamm. Genome. 15:663-667.

Lee, H. K., S. S. Lee and T. H. Kim. 2003. Detection of imprinted quantitative trait loci (QTL) for growth traits in pigs. Asian-Aust. J. Sci. 16:1087-1092.

Koning, D. J., A. P. Rattink, B. Harlizius, J. A. Arendonk, E. W. Brascamp and M. A. Groenen. 2000. Genome-wide scan for body composition in pigs reveals important role of imprinting. Proc. Natl. Acad. Sci. USA. 97:7947-7950.

Liu, B. H. 1998. Statistical genomics: linkage, mapping, and QTL analysis. CRC Press, LLC.

Ma, D., J. P. Shield, W. Dean, I. Leclerc, C. Knauf, R. R. Burcelin, G. A. Rutter and G. Kelsey. 2004. Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM. J. Clin. Invest. 114:339-348.

Nezer, C., L. Moreau, B. Brouwers, W. Coppieters, J. Detilleux, R. Hanset, L. Karim, A. Kvasz, P. Leroy and M. Georges. 1999. An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. Nat. Genet. 21:155-156.

Pira, G., A. El Kharroubi, S. Kozlov, D. Escalante-Alcalde, L. Hernandez, N. G. Copeland, D. J. Gilbert, N. A. Jenkins and C. L. Stewart. 2000. Zac1 (Lot1), a potential tumor suppressor gene, and the gene for epsilon-sarcoglycan are maternally imprinted genes: identification by a subtractive screen of novel uniparental fibroblast lines. Mol. Cell. Biol. 20:3308-3315.

Reik, W., F. Santos and W. Dean. 2003. Mammalian epigenomics: reprogramming the genome for development and therapy. Theriogenol. 59:21-32.

Spengler, D., M. Villalba, A. Hoffmann, C. Pantaloni, S. Houssami, J. Bockaert and L. Journot. 1997. Regulation of apoptosis and cell cycle arrest by Zac1, a novel zinc finger protein expressed in the pituitary gland and the brain. EMBO. J. 16:2814-2825.

Thomsen, H., H. K. Lee, M. F. Rothschild, M. Malek and J. C. Dekkers. 2004. Characterization of quantitative trait loci for growth and meat quality in a cross between commercial breeds of swine. J .Anim. Sci. 82:2213-2228.

Xiong, Y. Z. and C. Y. Deng. 1999. Technical testing rule of lean pig. Chinese Agricultural Press, Beijing.

Yang, Q., C. Q. Lai, L. Parnell, L. A. Cupples, X. Adiconis, Y. Zhu, P. W. Wilson, D. E. Housman, A. M. Shearman, R. B. D'Agostino and J. M. Ordovas. 2005. Genome-wide linkage analyses and candidate gene fine mapping for HDL3 cholesterol: the Framingham Study. J. Lipid. Res. 46:1416-1425.

Zhang, Z. B., M. G. Lei, C. Y. Deng, Y. ZH. Xiong, B. Zuo and F. E. Li. 2005. Lipoprotein lipase gene and productive traits in pig resource family. Asian-Aust. J. Anim. Sci. 18:458-462.

Zuo, B., Y. Z. Xiong, C. Y. Deng, Y. H. Su, J. Wang, M. G. Lei, F. E. Li, S. W. Jiang and R. Zheng. 2004. cDNA cloning, genomic structure and polymorphism of the porcine FHL3 gene. Anim. Genet. 35: 230-233.