Short communication

Identification of gene variation within porcine *PRDM16* gene and its association with fat and loin muscle area

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Abstract

The PR domain containing the 16 (*PRDM16*) gene, also known as the MDS1/EVI1-like gene (*MEL1*), may act as a bidirectional switch between brown fat and skeletal muscle in mice. The molecular characteristics and possible biological function of porcine *PRDM16* gene have been less reported. In this study, the mRNA expression profile, linkage mapping and association analyses of the *PRDM16* gene were carried out in the pig. The *PRDM16* mRNA was expressed widely in various tissues including fat and the *longissimus dorsi* muscle. One SNP c.-3284+171C>T in intron 14 was identified and that made the *PRDM16* gene being assigned between *SWR1130* and *SW122* on SSC6. The different genotypes of c.-3284+171C>T were significantly associated with backfat and loin muscle area in the ISU Berkshire × Yorkshire pig resource family, but such associations were not verified in another pig population. The discovery of additional mutations and association studies are warranted before the *PRDM16* gene can be recommended for marker assisted selection in the pig.

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The PR domain containing the 16 (*PRDM16*) gene, comprising of a PRD1-BF1-RIZ1 homologous (PR) domain and several zinc fingers, has been shown to turn on the expression of brown fat-selective genes and turn off the expression of white fat-enriched genes, and therefore acts as an critical regulator during adipose development (Seale *et al*., 2007; Kajimura *et al*., 2008). Furthermore, knockdown of the *PRDM16* gene from brown fat precursors led to muscle differentiation in mice (Seale *et al*., 2008). These findings imply that the *PRDM16* gene may play a role in the conversion and growth of fat and skeletal muscle tissues. The pig is one of the important livestock species for meat supplies, and it can also easily deposit fat during the growth period. This makes it interesting to explore if the *PRDM16* gene is involved in the development of fat and skeletal muscle tissues in pigs. In this study, mRNA expression patterns of the *PRDM16* gene were analyzed and further SNP discovery and association analyses were implemented to identify the potential genetic marker associated with fatness and muscle mass in the pig.

The tissues used for mRNA expression analysis were collected from three Yorkshire piglets, and included the heart, liver, spleen, lung, kidney, brain, *longissimus dorsi* muscle, interior fat, stomach, small intestine and testis. Total RNA was isolated following the instruction of TRIzol reagent kit (Tiangen Biotech, Beijing, China) and mRNA expression patterns were determined by the RT-PCR technique. The PCR mixture consisting of 2 μg total RNA and 5 μL oligo (dT) was incubated at 70 °C for 5 min to eliminate the RNA secondary structure. The reactions were chilled on ice for 2 min and the remaining reagents including 5 μL dNTPs (10 mM each dNTP), 10 μL 5 × buffer, 2.5 μL RNAase inhibitor with 300 U M-MLV reverse transcriptase (Promega, Madison, WI, USA) were added to a total volume of 50 μL. Reverse transcription reaction was proceeded for 1 h at 42 °C. Finally, the reverse transcriptase was inactivated by 5 min incubation at 90 °C. 2 μL single-stranded cDNA was amplified with a pair of primers (F1:5'-

CTGGCTGCACGTCTGTTACC-3' /R1: 5'-CCTGAAGGTCACGCCTAGAG-3'). The PCR programme was as follows, 4 min at 94 °C; 28 cycles of 30 s at 94 °C, 30 s at 60 °C, 20 s at 72 °C, and a final extension of 5 min at 72 °C. 5 μL PCR products were used to detect the expression profile in agarose gel, and the amplification of the *RPL32* gene (F2: 5'-CGGAAGTTTCTGGTACACAATGTAA-3'/R3:5'-TGGAAGAGACGTTGTGAGCAA-3') was performed as a positive control. The mRNA expression patterns of the porcine *PRDM16* gene in various tissues are shown in Figure 1. The *PRDM16* gene was highly expressed in the brain, lung, testis, interior fat and kidney, and it was moderately expressed in the heart, liver, spleen, *longissimus dorsi* muscle, stomach and small intestine. In humans it was found that the *PRDM16* gene is widely expressed in a variety of normal tissues, especially in the heart, lung, kidney and pancreas (Lahortigav *et al*., 2004). This is in agreement with the results presented in pigs. In addition, the expression patterns implied that the *PRDM16* gene has additional functions besides its roles on brown fat tissues. For instance, the up-regulation of *PRDM16* could initiate a leukemogenic cascade (Shing *et al*., 2007; Modlich *et al*., 2008), and the copy number of *PRDM16* molecules of patients with osteosarcoma was higher than that of the normal (Man *et al*., 2004).

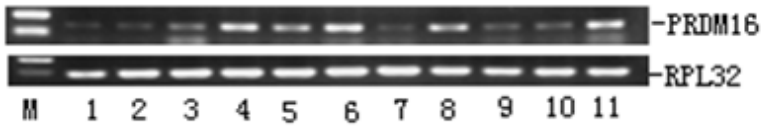

Figure 1 mRNA expression analyses of the porcine *PRDM16* gene using the RT-PCR method. Lanes 1~ 11 indicate heart, liver, spleen, lung, kidney, brain, *longissimus dorsi* muscle, interior fat, stomach, small intestine and testis tissues, respectively. The amplification product of the *RPL32* gene was used as a control.

The human *PRDM16* transcript (ENST00000378389) retrieved from the Ensemble database (http://www.ensembl.org/index.html) was used as an entry to search for a homologous pig sequence (http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig). The pig genome sequence fragments showing above 85% identity with the entry sequence, were obtained. The pairs of primers were designed for PCR amplification from the pig genomic DNA. PCR products were commercially sequenced. SNP discovery was implemented by sequencing the pooled PCR products amplified from six DNA samples and each two were from Yorkshire, Landrace and Tongcheng pigs. Three SNPs, c.-3284+171C>T, c.-3284+207C>T, c.-3284+208C>T, in intron 14 were identified and then deposited into a dbSNP database (dbSNP acc. no. ss136268357, ss136268362 and ss136268366). The SNP c.-3284+171C>T could be distinguished by the restriction enzyme, *Aci*I. A PCR-*Aci*I RFLP technique was developed using the redesigned primers F3/R3 (F3: 5'-AGAGCTAGGGCCACAGAG-3'/R1: 5'-CCAACAGCGAGATGAACC-3'), with allele T revealing a 280bp and allele C revealing a 215bp and a 70bp fragment. The PCR-RFLP procedure for SNP genotying was as follows: PCR mixture (10 μL) included 1×PCR buffer, 0.2 μM each primer, 150 μM each dNTP, 1.5 mM $MgCl_2$, 2U Taq DNA polymerase (Takara Company, Dalian, China) and 12.5 ng genomic DNA; PCR reaction comprised of the initial denature at 95 °C for 5 min, 30 cycles with 94 °C for 30 s, 60 °C for 30 s, 72 °C for 15 s, followed by a final extension at 72 °C for 5 min; RFLP reaction mixture (10 μL) consisted of 1 μL 10×buffer, 1U restricted enzyme *Aci*I (NEB, Ipswich, MA, USA) and 3 μL PCR products. Samples were incubated overnight at 37 °C. SNP genotyping was performed in two different pig populations. Population A (n = 205) included the Yorkshire (Y, n = 26), Landrace (L, n = 26) and Tongcheng (T, n = 49) breeds, and crossbreds L×(Y×T, n = 54) and Y × (L × T, n = 50). The association analysis was implemented using the mixed procedure (SAS, 2006) and this model treated population/population combination, sex, slaughter date and marker genotyping as fixed effects, dam as random effect and body weight as covariate (Tang *et al*., 2008). The genotyping was also performed in the ISU Berkshire × Yorkshire (B×Y) pig resource family comprising of 515 F2 animals (Malek *et al*., 2001). The association analyses were implemented using the mixed model procedure, including sex, slaughter date and marker genotypes as fixed effects, dam (litter) as random effect and body weight as covariate. In population A, fat traits including average backfat, backfat at shoulder, 6 - 7^{th} rib, loin, last rib and rump were used for association analysis, as well as muscle mass traits including lean meat ratio and loin eye area. The details about the measurement of

these traits were described by Tang *et al.* (2008). In population B, the analyzed traits included average backfat, backfat at 10th rib, lumber and last rib and loin eye area (Malek *et al*., 2001).

The SNP c.-3284+171C>T showed high polymorphism in both populations A and B. In population B c.-3284+171C>T was highly significantly (P <0.01) associated with loin eye muscle area and was significantly (P <0.05) associated with lumbar backfat, carcass weight and body length. It was also suggestively significantly (P <0.1) associated with average backfat and last rib backfat (Table 1). The zinc-finger PR domain-containing protein encoded by the *PRDM16* gene can induce expression of a number of genes associated with white and brown fat (such as adipokine resistin, PGC-1α and UCP-1), mitochondrial biogenesis and oxygen consumption. Most recently the *PRDM16* gene has been shown to interact with either *PGC-1α* or *CtBPs* to activate brown genes or to suppress white gene expression, respectively (Seale *et al*., 2007; Kajimura *et al*., 2008). *PRDM16* binds and co-activates the transcriptional function of *PPAR-γ*, which is known to be involved in adipose tissue development. In addition, loss of *PRDM16* from brown fat cells could cause an increase in myogenic gene expression and bona fide skeletal muscle differentiation (Seale *et al*., 2008), but the exact mechanisms need to be further addressed. It is possible that in pigs the *PRDM16* gene functions in fat and lean meat growth with the same molecular mechanisms. However, there was no significant association between SNP c.-3284+171C>T genotypes and the analyzed traits in population A (data not shown), which suggested that additional SNP mining and association analyses in pig populations with different genetic backgrounds are necessary.

Table1 Association of c.-3284+171C>T within the *PRDM16* gene with the analyzed traits in pigs

Population	Trait*	LSM (s.e.)**			P <
		TT	TC	CC	
B	LEA	35.1[a](0.71)	35.8[a](0.55)	37.7[b](0.75)	0.01
	Average BF	3.35(0.07)	3.22(0.05)	3.35(0.08)	0.1
	Last rib BF	3.25(0.07)	3.10(0.04)	3.19(0.07)	0.1
	Lumbar BF	3.64[a](0.09)	3.48[b](0.06)	3.67[a](0.09)	0.05
	Carcass BW	86.68[a](0.27)	87.18[ab](0.17)	87.66[b](0.30)	0.05

* LEA - loin eye muscle area (cm^2); AVBF - average back fat (cm); Last rib BF - last rib back fat (cm), Lumbar BF - lumbar back fat (cm); Carcass BW - carcass body weight (kg).

** LSM (s.e.) represents least squares means and their standard errors. Means with superscripts a and b differ significantly (P <0.05).

The human *PRDM16* gene locates at HSA 1p36.23-p33, where it corresponds to the SSC6 of pigs (https://www-lgc.toulouse.inra.fr/pig/compare/compare.htm). The SNP c.-3284+171C>T was used for linkage mapping in the B×Y population. The mapping procedures were performed with two-point linkage analyses using an improved CRIMAP (Ver 2.5) developed by Evans I and Maddox J (16 Park Square Port Melbourne, 3207 Australia, E-mail: jillm@rubens.its.unimelb.edu.au). The *PRDM16* gene was mapped on SSC6 and the order of makers was *SWR1130* (10.6cM)-*PRDM16*-(3.1cM) *SW122*. Numbers of QTL related to fat and loin muscle area have been mapped around this region (http://www.animalgenome.org/cgi-bin/QTLdb; Paszek *et al*., 2001; Yue *et al*., 2003; Edwards *et al*., 2008). Combing the association analyses and linkage mapping promote the porcine *PRDM16* gene as a candidate gene for fat deposit and muscle production. Further work on causative mutation discovery and function

analysis of *PRDM16* gene in pigs is required.

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References

Edwards, D.B., Ernst, C.W., Raney, N.E., Doumit, M.E., Hoge, M.D. & Bates, R.O., 2008. Quantitative trait loci mapping in a F2 Duroc × Piétrain resource population. I. Growth traits. J. Anim. Sci. 86, 241-253.

Kajimura, S., Seale, P., Tomaru, T., Erdjument-Bromage, H., Cooper, M.P., Ruas, J.L., Chin, S., Tempst, P., Lazar, M.A. & Spiegelman, B.M., 2008. Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. Genes Dev. 22, 1397-1409.

Lahortiga, I., Agirre, X., Belloni, E., Vázquez, I., Larrayoz, M.J., Gasparini, P., Coco, F.L., Pelicci, P.G., Calasanz, M.J. & Odero, M.D., 2004. Molecular characterization of a t(1;3)(p36;q21) in a patient with MDS. MEL1 is widely expressed in normal tissues, including bone marrow, and it is not overexpressed in the t(1;3) cells. Oncogene 23, 311-316.

Malek, M., Dekkers, J.C., Lee, H.K., Baas, T.J. & Rothschild, M.F., 2001. A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. I. Growth and body composition. Mamm. Genome 12, 630-636.

Man, T.K., Lu, X.Y., Jaeweon, K., Perlaky, L., Harris, C.P., Shah, S., Marc Ladanyi, M., Gorlick, R., Lau, C.C. & Rao, P.H., 2004. Genome-wide array comparative genomic hybridization analysis reveals distinct amplifications in osteosarcoma. BMC Cancer 4, 45.

Modlich, U., Schambach, A., Brugman, M.H., Wicke, D.C., Knoess, S., Maetzig, T., Rudolph, C., Schlegelberger, B. & Baum, C., 2008. Leukemia induction after a single retroviral vector insertion in Evi1 or Prdm16. Leukemia 22, 1519-1528.

Paszek, A.A., Wilkie, P.J., Flickinger, G.H., Miller, L.M., Louis, C.F., Rohrer, G.A., Alexander, L.J., Beattie, C.W. & Schook, L.B., 2001. Interval mapping of carcass and meat quality traits in a divergent swine cross. Anim. Biotechnol. 12, 155-165.

SAS, 2006. Statistical Analysis Systems user's guide, Release 9.1. SAS Institute, Inc., Cary, N.C., USA.

Seale, P., Kajimura, S., Yang, W., Chin, S., Rohas, L.M., Uldry, M., Tavernier, G., Langin, D. & Spiegelman, B.M., 2007. Transcriptional control of brown fat determination by PRDM16. Cell Metab. 6, 38-54.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scimè, A., Devarakonda, S., Conroe, H.M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M.A., Beier, D.R. & Spiegelman, B.M., 2008. PRDM16 controls a brown fat/skeletal muscle switch. Nature 454, 961-968.

Shing, D.C., Trubia, M., Marchesi, F., Radaelli, E., Belloni, E., Tapinassi, C., Scanziani. E., Mecucci, C., Crescenzi, B., Lahortiga, I., Odero, M.D., Zardo, G., Gruszka, A., Minucci, S., Di Fiore, P.P. & Pelicci, P.G., 2007. Overexpression of sPRDM16 coupled with loss of p53 induces myeloid leukemias in mice. J. Clin. Invest. 117, 3696-3707.

Tang, Z.L., Peng, Z.Z., Liu, B., Fan, B., Zhao, S.H., Li, X.P., Xu, S.P. & Li, K., 2008. Effect of breed, sex and birth parity on growth, carcass and meat quality in pigs. Front Agric. China 2, 331-337.

Yue, G., Stratil, A., Kopecny, M., Schröffelova, D., Schröffel, J., Hojny, J., Cepica, S., Davoli, R., Zambonelli, P., Brunsch, C., Sternstein, I., Moser, G., Bartenschlager, H., Reiner, G. & Geldermann, H., 2003. Linkage and QTL mapping for Sus scrofa chromosome 6. J. Anim. Breed. Genet. 120, 45-55.