Association of the A-G Polymorphism in Porcine Adiponectin Gene with Fat Deposition and Carcass Traits

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ABSTRACT : The adiponectin gene is known to be involved in the regulation of energy homeostasis involving food intake, carbohydrate and lipid metabolism. Human adiponectin gene polymorphisms have been recently reported to be associated with obesity, insulin sensitivity and the risk of type 2 diabetes. The present study was carried out to investigate the porcine adiponectin gene as a candidate gene for fat deposition and carcass traits. A mutation of A178G of the porcine adiponectin gene that resulted in substitution of the amino acid Isoleucine to Valine was identified. *Acy*I PCR-RFLP was used to detect the polymorphism of the genotypes in five different pig populations (Large White, Landrace, Duroc, Chinese breeds Meishan and Qingping). The A allele frequency was significantly higher among subjects from Chinsese lard type breeds, while the G allele was the only one present in those from Western lean type breeds. To determine if there was an association of the polymorphism with phenotypic variation, the mutation was tested in 267 pigs of the "Large White×Meishan" F2 resource population. The results of association analyses showed significant associations of the genotypes with fat deposition and carcass traits. Allele G was significantly associated with increase in loin eye height, loin eye area and lean meat percentage and bone percentage, and decrease in fat mean percentage, ratio of lean to fat, shoulder fat thickness, 6-7 rib fat thickness, thorax-waist fat thickness and buttock fat thickness. The substitution of A178G (Ile60Val) happened to be located at amino acid 60 in the collagenous domain of porcine adiponectin which might affect the association into higher-order structures, and accordingly affect the posttranslational modifications and optimal biological activity of the multimeric forms. The identified functional polymorphism provides new evidence of adiponectin as an important candidate gene affecting fat deposition and carcass traits in pigs.
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Key Words : Adiponectin, Fat, Pig, Polymorphism, Gene

INTRODUCTION

Adiponectin is an abundantly expressed secretory protein exclusively synthesized in adipose tissue. The cDNA encoding adiponectin was first described as Acrp30 (adipocyte complement-related protein of 30 kDa) (Scherer et al., 1995), which subsequently reported by several laboratories independently: AdipoQ (Hu et al., 1996), adipose most abundant gene transcript (apM1) (Maeda et al., 1996), gelatin-binding protein 28 (GBP28) (Nakano et al., 1996) and adiponectin (Ouchi et al., 1999; Arita et al., 1999). In recent years, biochemical, genetic and animal studies have established a critical role for the adipokine adiponectin in the regulation of both lipid and carbohydrate metabolism, particularly by enhancing insulin sensitivity in muscle and liver, and by increasing fatty acid oxidation in muscle. Yamauchi et al. (2001) discovered that leptin and adiponectin were additive in their enhancement of the inhibition of glucose production by insulin in the liver of lipotrophic mice.

The adiponectin gene consists of three exons and two introns located on human chromosome 3q27, where a diabetes susceptibility locus has been mapped (Kissebah et al., 2000) and has been in hot research as a candidate gene for type 2 diabetes and weight loss curing (Perusse et al., 2004). Adiponectin gene polymorphisms have been associated with BMI, insulin sensitivity, and type 2 diabetes in some cross-sectional studies. A missense mutation (R112C) in exon3 (Takahashi et al., 2000) and a silent T94G exchange in exon 2 (Stumvoll et al., 2002) have been associated with increased circulating adiponectin levels, and the dose of allele G was associated with an increase of relative mRNA level and a reduction of approximately 1.12 kg/m^2 in BMI (Yang et al., 2003). Polymorphisms of G11391A and T45G were associated with BMI, waist-to-hip ratio and the risk of becoming hyperglycemic (Fumeron et al., 2004).

In vitro, porcine adiponectin also acts as an autocrine regulatory factor to regulate energy metabolism and antagonizes the incorporation of glucose carbon into lipid in the adipocyte (Jacobi et al., 2004). Porcine adiponectin sequence, which has an open reading frame of 732 bp in length and encodes 243 amino acids, shares approximately 88, 86, 85 and 83% homology with the dog, human, cow and mouse adiponectin respectively, and 79-83% similarity with dog, human, cow and mouse proteins at the amino acid level (Jacobi et al., 2004).

Though adiponectin gene polymorphisms have been reported to be associated with human obesity, it still has no

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Table 1. Primer sequence used for screening and genotyping polymorphisms in the porcine adiponectin gene

Name	Primer sequence (5' to 3')	Annealing temp. (°C)	cDNA (bp)	Genomic DNA (bp)
A1F	GGATGCTGTTGTTGGGAG	57	377	-1,861
A1R	AGGGACAGTGACCCGAGT			
A2F	AGGTCCCCGAGGTTTCCC	65	485	485
A2R	GAGTTGGCAGTGCTCATCATTC			
A3F	GGATGCTGTTGTTGGGAG	61	-	279
A3R	CCAAAATCTCTCCTCTACCCA			

report on porcine adiponectin gene polymorphism until now. In this study, polymorphisms in porcine adiponectin gene were identified, and the associations with phenotypic measurements were analyzed in pigs. The results will help understand the function of porcine adiponectin and suggest new candidate gene for marker assistant selection (MAS) of lean type pigs. As a rapidly emerging research model for obesity, it also implies the genetic control of pork industry and the potential application to human obesity.

MATERIALS AND METHODS

Animals

All pigs were chosen from purebred and crossbred populations established in Agricultural Ministry Key Laboratory of Pig Breeding and Genetics, Huazhong Agricultural University. Screening for polymorphisms in the porcine adiponectin gene was performed in a totall of 203 pigs of Large White (n = 37), Landrace (n = 31), Duroc (n = 35), Qingping (n = 59), and Meishan (n = 41) breeds. For association study, fat deposition and carcass traits were recorded in 267 pigs of the "Large White×Meishan" F2 resource population. All the F2 pigs were given twice daily diets formulated according to age under a standardized feeding regimen and free access to water. The finishing animals were slaughted in the year of 2003 and 2004, and measured according to the method of Xiong and Deng (1999). The estimated values of important economic traits involving fat deposition and carcass traits were obtained as follows: bone percentage (BP), fat meat percentage (FMP, %), lean meat percentage (LMP, %), ratio of lean to fat (RLF), shoulder fat thickness (SFT, cm), 6-7th rib fat thickness (RFT, cm), thorax-waist fat thickness (TFT, cm), buttock fat thickness (BFT, cm), average backfat thickness (ABT, cm), leaf fat weight (LFW, kg), caul fat weight (CFW, kg), loin eye height (LEH, cm), loin eye area (LEA, cm^2).

Genomic DNA was isolated from blood of all the 470 pigs using phenol/chloroform purification-based protocols (Smbrook et al., 2001).

Sequence analyses and polymorphism screening

To screen polymorphisms in the porcine adiponectin gene, oligonucleotide primers (A1F, A1R; and A2F, A2R; Table 1) were designed from pig adiponectin mRNA sequence (AY135647 and NM_214370) revealing 2 overlapping PCR fragments which covered the whole protein-coding regions in exon2 and exon3 of the porcine adiponectin gene using the genomic DNA from five breeds of pigs (two animals per breed). PCR products were gel purified, cloned and sequenced. The nucleotide sequences of each animal were compared to find out polymorphisms in porcine adiponectin gene.

PCR/*Acy*I-PCR-RFLP

To genotype the polymorphic site in the porcine adeponectin gene, PCR-RFLP was performed. Oligonucleotide primers (A3F, A3R; Table 1) were used to amplify the fragment from genomic DNA. It gives a 278 bp PCR product including polymorphic locus for A178G which makes an amino acid substitution of I60V.

PCR reaction was carried out in a 25 μl volume containing 2.5 μl 10×PCR buffer (with NH_4^+), 1.5 μl 25 mM $MgCl_2$, 1.5 μl 2 mM each dNTP, 0.5 μl each primer (10 μM), 0.5 U *Taq* DNA polymerase (Biostar Internationa, Toronto, ON, Canada) and 50 ng DNA. PCR cycling program was 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 40 s and final extension at 72°C for 8 min. PCR products (278 bp) were digested (10 μl reaction solution containing 3 U restriction enzyme) with *Acy*I followed by electrophoresis on 2% agarose gels stained with ethidium bromide. Because the A→G change creates a *Acy*I site, the uncut 278-bp band was designated as A allele, whereas the presence of 100 bp and 178 bp bands was indicative of the G allele.

Statistical analysis

The association between genotypes and traits recorded was performed with the general linear model (GLM) procedure (SAS Institute Inc. Cary, NC, USA). Both additive and dominance effects were estimated using the REG procedure. The additive effect was defined as -1, 0 and 1 for AA, AG and GG, respectively, and the dominance effect represented as 1, -1 and 1 for AA, AG and GG, respectively. The statistical model was assumed to be: $T_{ijk} = \mu+S_i+Y_j+G_k+b_{ijk}X_{ijk}+e_{ijk}$ (Liu, 1998), 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 2 for female), Y_j is the effect of year (j = 1 for year 2003 or 2 for year 2004), G_k is the effect of genotype (K = AA, AG and GG), b_{ijk} is the

Table 2. Genotypic frequencies for the A→G substitution in the adiponectin gene in five populations

Breed	Number of pigs	Genotype and frequency			Allele frequency	
		AA	AB	BB	A	B
Large White	37	0	0	37 (100)	0	100
Landrace	31	0	0	31 (100)	0	100
Duroc	35	0	0	35 (100)	0	100
Qingping	59	28 (47.5)	27 (45.8)	4 (6.7)	70.4	29.6
Maishan	41	37 (90.3)	3 (7.3)	1 (2.4)	93.95	6.05

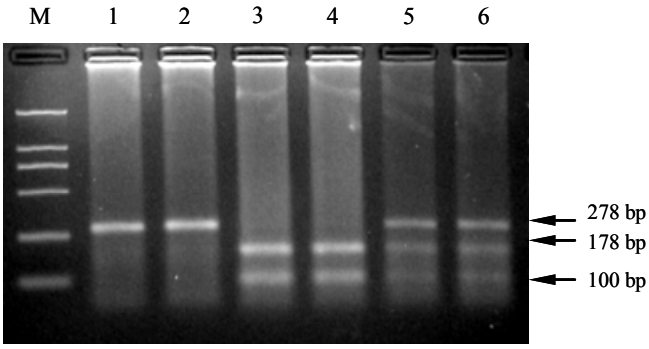

Figure 1. *Acy* I PCR-RFLP of porcine adiponectin gene. Lane 1-2: genotype AA; Lane 3-4: genotype GG; Lane 5-6: genotype AG; Lane M: DNA Ladder (2,000 bp, 1,000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

regression coefficient of the slaughter weight, X_{ijk} is the slaughter weight, and e_{ijk} is the random residual.

RESULTS

Identification of mutation in porcine adiponectin gene

The sequences of protein-coding regions in exon2 and 3 of the porcine adioponectin gene from three pig breeds have been deposited in the GenBank database under the accession numbers: DQ164207 (Large White), DQ164208 (Landrace) and DQ164209 (Meishan). By comparing the sequence of these different pig breeds, five putative polymorphisms were found. An A/G exchange in nucleotide 178 (exon2) results in a missense mutation (Ile60Val). Polymorphism was designated according to its location relative to the A of the ATG of the initiator methionine (Met) of adiponectin. The A/G transversion can be detected by *Acy*I PCR-RFLP.

A/G Polymorphism and allelic frequency of the porcine adiponecin gene

Genotypes marked with AA (278bp), AB (278 bp+100 bp+178 bp) and BB (100 bp+178 bp) (Figure 1). The allele frequency analysis included 203 unrelated animals of five different pig breeds (Table 2). Allele frequencies of this polymorphism are significantly different among Chinese pig breeds and Western commercial pig breeds. The allele distribution revealed that the Chinese indigenous Meishan and Qingping breeds had higher frequencies of the A allele, sharing 93.95% and 70.4% respectively. In comparison, allele G was the only allele present in Western commercial pig breeds (Large White, Duroc and Landrace). Thus, the genotype frequencies of all the five pig breeds did not conform to Hardy-Weinberg expectation.

Association of the polymorphism with fat deposition and carcass traits

For association analyses, 267 pigs of the "Large White×Meishan" F2 resource population were used. Among them, the number of genotypes AA, AG and GG were 86, 115 and 66 respectively, and the genotype distribution can conform to the expected 1:2:1 ratio (χ^2 = 3.81, d.f. = 2, p>0.05).

The detailed statistic analysis is listed in Table 3. Highly significant associations between *Acy*I PCR-RFLP genotype and 6-7 rib fat thickness (p = 0.0041), buttock fat thickness (p = 0.0034) and loin eye area (p = 0.0076) were observed. Significant associations were observed with bone percentage (p = 0.0232), fat meat percentage (p = 0.0141), lean meat percentage (p = 0.0407), ratio of lean to fat (p = 0.0367), shoulder fat thickness (p = 0.0307), thorax-waist fat thickness (p = 0.0248) and loin eye height (p = 0.0185). Although it did not reach statistical significance (p = 0.0598), there was a dose-dependent decrease in average backfat thickness for the G allele in 276 pigs. No significances were observed as for caul fat or leaf fat weight and abdominal fat weight, however, there was a tendency that allele G had less thickness. This locus seemed to be significantly additive in action and allele A was associated with increase of fat deposition. Pigs with AA genotype had more fat mean percentage (+1.552%), less lean meat percentage (-1.134%) and ratio of lean to fat (-0.232) than pigs with GG genotype.

DISCUSSION

To our knowledge, it is the first time to report the polymorphism of porcine adiponectin gene and reveal an important functional SNP (A178G) causing amino acid change of I60V.

In this study, analysis of the A/G polymorphism revealed great differences between lean type breeds and lard type breeds, for a much higher frequency of allele A in two Chinese indigenous breeds whereas allele G only present in

Table 3. Association between porcine adiponectin *Acy*I PCR-RFLP and fat deposition and carcass traits in 267 pigs of the "Large White ×Meishan" F2 resource population

Traits[1]	Genetype (Lsmean±SE)[2]			Effect (mean±SE)	
	AA (n = 86)	AG (n = 115)	GG (n = 66)	Additive	Dominance
BP (%)	12.151±0.244^{a,3}	12.589±0.211^{ab}	13.001±0.279^{b}	0.187±0.189	-0.028±0.285
FMP (%)	24.251±0.509^{a}	23.749±0.439^{a}	22.335±0.582^{b}	-0.776±0.392*	0.472±0.591
LMP (%)	54.045±0.366^{a}	54.562±0.315^{ab}	55.191±0.418^{b}	0.567±0.279*	-0.089±0.421
RLF	2.345±0.075^{a}	2.472±0.064^{ab}	2.585±0.085^{b}	0.115±0.057*	0.001±0.086
SFT (cm)	3.694±0.077^{a}	3.612±0.066^{ab}	3.439±0.088^{b}	0.072±0.059	0.036±0.089
RFT (cm)	2.895±0.065^{A}	2.813±0.056^{a}	2.608±0.074^{Bb}	-0.054±0.050	0.084±0.076
TFT (cm)	2.135±0.069^{a}	2.017±0.052^{ab}	1.928±0.068^{b}	0.010±0.046	-0.010±0.070
BFT (cm)	2.109±0.076^{A}	1.963±0.065^{AB}	1.767±0.087^{B}	-0.051±0.059	0.021±0.089
ABT (cm)	2.691±0.204	2.983±0.175	2.434±0.231	0.003±0.155	0.424±0.234
LFW (kg)	0.841±0.031	0.802±0.027	0.765±0.036	-0.021±0.024	-0.007±0.036
CFW (kg)	1.162±0.031	1.170±0.027	1.134±0.036	-0.017±0.024	0.029±0.036
LEH (cm)	7.347±0.072^{a}	7.491±0.062^{ab}	7.610±0.083^{b}	-0.001±0.056	0.026±0.085
LEA (cm^2)	28.725±0.505^{A}	29.966±0.436^{AB}	30.797±0.577^{B}	0.130±0.394	0.229±0.594

[1] BP: Bone percentage; FMP: Fat meat percentage; LMP: Lean meat percentage; 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; LFW: Leaf fat weight; CFW: Caul fat weight; LEH: Loin eye height; LEA: Loin eye area.

[2] Least square mean values (±SE).

[3] Different letters denoting significant difference between groups: $^{a, b,}$ * $p<0.05$; $^{A, B}$ $p<0.01$.

Duroc, Large White and Landrace pigs. It is therefore inferred that there were real differences among specific breeds by long-term and different background of breeding and selection, or another possible reason is that the number of animals in this study was limited. Anyway, it is the fact that there are significantly different allele frequencies among Chinese lard breeds and Western lean pig breeds.

Association analysis revealed that adiponectin gene *Acy*I PCR-RFLP polymorphism had significant effects on traits related to fat deposition in Large White×Meishan F2 resource family. The GG genotype was associated with less fat thickness and more loin meat area when comparing with the AA genotype. Thus increasing the frequency of the favorable allele G might be beneficial in breeding to accelerate the genetic improvement of these traits. Analysing more animals is necessary to confirm the association between the *Acy*I PCR–RFLP genotype and fat deposition and carcass traits in crossbreds and purebreds.

Adiponectin is a collagen-like plasma protein specifically secreted only by differentiated adipocytes and circulates in blood. Plasma adiponectin concentrations are decreased in obesity whereas it is adipose-specific. According to the report of Scherer et al. (1995) and the comparison among other species, porcine adiponectin is composed of four distinct domains: a signal peptide at the N terminus (1-18aa), a short variable region (19-40aa), a collagenous domain (41-106aa), and a C-terminal globular domain homologous to C1q (107-243aa). They usually have a variable number of "Gly-X-Y" (where X and Y represent any amino acid) collagenous repeats. The basic building block of adiponectin is a tightly associated trimer, which is formed by association between three monomers at the globular domains. Monomeric (30-kDa) adiponectin has not been observed in the circulation. Four to six trimers associate through their collagenous domains to form higher order structures, or oligomers (Scherer et al., 1995; Arita et al., 1999; Berg et al., 2002). It may have putative sites of post-translational modification for oligomer formation (Chandran et al., 2003).Without the collagenous domain, the globular domain of adiponectin still trimerizes but does not associate into higher-order structures (Berg et al., 2002). Both the globular and the collagenous domains are important for ensuring the stability and activity of the multimeric forms (Chandran et al., 2003). It is probable that the variant amino acid residue of the adiponectin mutation causes a significant change of the adiponectin function. The substitution of A178G results in the change of amino acid Isoleucine to Valine, which happened to be located at 60 aa in the collagenous domain. The change of collagenous repeat "Gly-Ile-Pro" to "Gly-Val-Pro" might affect the association of trimers into higher-order structures, thus affect the posttranslational modifications and optimal biological activity of the multimeric forms.

IMPLICATIONS

According to the results obtained, further genetic and functional studies are required to evaluate the significance of the porcine adiponectin Ile60Val substitution. Because the gene has been implicated in obesity and diabetes in humans, and this study suggests associations with fat related traits, further research on the gene in pigs may

provide useful information on genetic factor underlying lean pork production and suggestions on obesity research as well.

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REFRENCES

Arita, Y., S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi and Y. Matsuzawa. 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem. Biophys. Res. Commun. 257:79-83.

Berg, A. H., T. P. Combs and P. E. Scherer. 2002. Acrp30/ adiponectin: an adipocytokine regulating glucose and lipid metabolism. Trends Endocrinol. Metabolism. 13:84-89.

Chandran, M., S. A. Phillips, T. Ciaraldi and R. R. Henry. 2003. Adiponectin: More Than Just Another Fat Cell Hormone? Diabetes Care. 26:2442-50.

Fumeron, F., R. Aubert, A. Siddiq, D. Betoulle, F. Pean, S. Hadjadj, J. Tichet, E. Wilpart, M. C. Chesnier, B. Balkau, P. Froguel and M. Marre for the Epidemiologic Data on the Insulin Resistance Syndrome (DESIR) Study Group. 2004. Adiponectin gene polymorphisms and adiponectin levels are independently associated with the development of hyperglycemia during a 3-year period: the epidemiologic data on the insulin resistance syndrome prospective study. Diabetes 53:1150-1157.

Hu, E., P. Ling and B. M. Spigelman. 1996. AdipoQ is a novel adipose specific gene dysregulated in obesity. J. Biol. Chem. 271:10697-10703.

Jacobi, S. K., K. M. Ajuwon, T. E. Weber, J. L. Kuske, C. J. Dyer and M. E. Spurlock. 2004. Cloning and expression of porcine adiponectin, and its relationship to adiposity, lipogenesis and the acute phase response. J. Endocrinol. 182:133-144.

Kissebah, A. H., G. E. Sonnenberg, J. Myklebust, M. Goldstein, K. Broman, R.G. James, J. A. Marks, G. R. Krakower, H. J. Jacob, J. Weber, L. Martin, J. Blangero and A. G. Comuzzie. 2000. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc. Natl. Acad. Sci. USA. 97:14478-14483.

Liu, B. H. 1998. Statistical Genomics: Linkage, Mapping and QTL Analysis. CRC Press LLC. Boca Raton. pp. 404-409.

Maeda, K., K. Okubo, I. Shimomura, T. Funahashi, Y. Matsuzawa and K. Matsubara. 1996. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (Adipose Most abundant Gene transcript 1). Biochem Biophys. Res. Commun. 221:286-289.

Nakano, Y., T. Tobe, N. H. Choi-Miura, T. Mazda and M. Tomita. 1996. Isolation and characterization of GBP28, a novel gelatinbinding protein purified from human plasma. J. Biochem. 120:803-812.

Ouchi, N., S. Kihara, Y. Arita, K. Maeda, H. Kuriyama, Y. Okamoto, K. Hotta, M. Nishida, M. Takahashi, T. Nakamura, S. Yamashita, T. Funahashi and Y. Matsuzawa. 1999. Novel modulator for endothelial adhesion molecules: adipocyte derived plasma protein adiponectin. Circulation. 100:2473-2476.

Perusse, L., T. Rankien, A. Zuberi, Y. Chagnon, S. J. Weisnagel, G. Argyropoulos, B. Walts and E. E. Bouchard. 2005. The human obesity gene map: the 2004 update. Obesity Res. 13:381-490.

Smbrook, J. and D. Russell. 2001. Molecular Cloning: A Laboratry Manual. 3rd Ed. Cold Spring Harbor Laboratory Press, New York.

Saito, K., T. Tobe, S. Minoshima, S. Asakawa, J. Sumiya, M. Yoda, Y. Nakano, N. Shimizu and M. Tomita. 1999. Organization of the gene for gelatin-binding protein (GBP28). Gene. 229:67-73.

Scherer, P. E., S. Williams, M. Fogliano, G. Baldini and H. F. Lodish. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. J. Biol. Chem. 270:26746-26749.

Stumvoll, M., O. Tschritter, A. Fritsche, H. Staiger, W. Renn, M. Weisser, F. Machicao and H. Haring. 2002. Association of the T-G Polymorphism in Adiponectin (Exon 2) With Obesity and Insulin Sensitivity. Diabetes. 51:37-41.

Takahashi, M., Y. Arita, K. Yamagata, Y. Matsukawa, K. Okutomi, M. Horie, I. Shimomura, K. Hotta, H. Kuriyama, S. Kihara, T. Nakamura, S. Yamashita, T. Funahashi and Y. Matsuzawa. 2000. Genomic structure and mutations in adipose-specific gene, adiponectin. Int. J. Obes. Relat. Metab. Disord. 24:861-868.

Xiong, Y. Z. and C. Y. Deng. 1999. Principle and method of swine testing. Chinese Agriclulture Press, Beijing.

Yamauchi, T., J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C. Vinson, M. L. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura, M. Tomita, P. Froguel and T. Kadowaki. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat. Med. 7:941-946.

Yang, W. S., P. L. Tsou, W. J. Lee, D. L. Tseng, C. L. Chen, C. C. Peng, K. C. Lee, M. J. Chen, C. J. Huang, T. Y. Tai and L. M. Chuang. 2003. Allele-specific differential expression of a common adiponectin gene polymorphism related to obesity. J. Mol. Med. 81:428-434.