

Molecular cloning, expression and polymorphism of the porcine apolipoprotein A5 gene in a Jinhua × Pietrain F₂ reference population

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As a newly described member of the apolipoprotein gene family, apolipoprotein A5 (APOA5) has been suggested to play a key role in the triglyceride metabolism in both human and mice. The aim of this study was to identify the porcine (Sus scrofa) APOA5 gene, determine its mRNA and its mutations that are associated with lipid accumulation. The porcine APOA5 cDNA was amplified by reverse transcriptase polymerase chain reaction using the information of the mouse or other mammals. It had been determined that the open reading frame of the porcine APOA5 gene consists of 1092 bp, which encodes a predicted protein composed of 363 amino acids with a similarity to bovine (80.43%) and to human (78.47%). The expression analysis indicated that the porcine APOA5 gene was expressed in hypophysis, fat and liver. Twelve single nucleotide polymorphisms (SNPs), including 4 SNPs in the 5' end, 1 SNP in second intron, 1 SNP in third exon and 6 SNPs in the 3' end, were identified in the porcine APOA5 gene and genotyped on the Jinhua × Pietrain F₂ reference population, it had revealed that the SNP of C1834T was significantly associated with average backfat thickness and leaf fat weight (P < 0.01 and P < 0.05, respectively). In conclusion, this study has got basic information of the porcine APOA5 gene and provides evidence that the APOA5 gene could be a potential candidate gene for fat deposition.

Keywords: porcine, APOA5 gene, cloning, expression, polymorphism

Implications

The pig meat is the major consuming meat in China. Native pig breed exhibited excessive body fat deposition, which was one of the main problems in the pig industry. High content of fat is not only to decrease the market value of pig meat, but also to increase the raise costs. We try to find genetic markers that cause to whole body fat deposition. Apolipoprotein A5 gene is a major candidate gene for our targets. This study is potential for improving the pig meat quality.

Introduction

In China, a long-term goal was to decrease body fat in pig production, because most of native pig breeds had excessive body fat deposition, which can decrease feed conversion rate and the whole quality of pig meat. Recently, studies had shown many genes are associated with the adipose traits in pig, such as *FTO*, *CMYA1* or *leptin* gene (Silveira *et al.*, 2008; Fontanesi *et al.*, 2009; Xu *et al.*, 2009).

The *apolipoprotein A5 (APOA5)* gene, a new member of the apolipoprotein gene family, is rapidly being recognized as a key regulator of serum triglyceride concentration (van Dijk *et al.*, 2004; Elosua *et al.*, 2006). *APOA5* can enhance lipoprotein lipase (LPL) activity to accelerate the rate of LPL-mediated triglyceride hydrolysis to regulate triglyceride metabolism (Schaap *et al.*, 2004; Rensen *et al.*, 2005). It had been found that several haplotypes of *APOA5* gene were related with the significant changes in triglyceride level (Grallert *et al.*, 2007; Nelbach *et al.*, 2008). In rat, the percentage of body fat was significantly correlated with serum triglyceride (Paik and Yearick, 1978), and in the young females and male pigs, the triglyceride level was positively correlated with backfat thickness (Mersmann and MacNeil, 1985). Martin *et al.* (1985) also noted that the high backfat Ossabaw sows had higher serum triglyceride levels. In healthy men, high fat content was associated with elevated total serum triglyceride (Walton *et al.*, 1995), and visceral abdominal fat was also positively associated with serum triglyceride level in Japanese (Taniguchi *et al.*, 2002). The above studies indicated that fat deposition can be

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influenced by the concentration of circulating triglyceride and *APOA5* may play an important role in fat deposition. To date, several mammalian *APOA5* cDNA sequences, such as human, cattle, rat and mouse have already been cloned, but porcine *APOA5* is still unknown.

At present, tagging single nucleotide polymorphisms (tagSNPs) are selected in genes to represent other co-related SNPs in linkage disequilibrium (LD) with the tagSNPs because of its high efficiency. This method has widely used to analyze the association in family study between genotype and traits in human disease or animal quantitative traits (Jiang *et al.*, 2006; Tang *et al.*, 2006; Hivert *et al.*, 2009). Based on the important role in the triglyceride homeostasis, *APOA5* is considered as a potential candidate gene for fat deposition in our study. In order to identify the porcine *APOA5* gene and the associations between *APOA5* and fat deposition, we had firstly cloned and sequenced the whole gene, then detected the SNPs of *APOA5*, and investigated the effects of tag SNPs of *APOA5* with fat deposition in the Jinhua × Pietrain F₂ reference population lastly.

Material and methods

Animal and tissue collection, RNA extraction and cDNA synthesis

The tissue samples of kidney, hypophysis, heart, hypothalamus, spleen, small intestine, muscle, adipose and liver were derived from crossbred of Landrace sire × Yorkshire of 210-day old. Total RNA from the tissues was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration of total RNA was measured at 260 nm using the NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Aliquots of total RNA were reverse transcribed using random primers and the Improm-II reverse transcriptase (Promega, Madison, WI, USA), and finally the product was stored at -20°C.

RT-PCR

The porcine expressed sequence tag (EST) databases were searched by Basic Local Alignment Search Tool (BLAST; <http://www.ncbi.nlm.nih.gov/blast/>) based on the sequences of cDNAs of human and rat *APOA5* gene (accession numbers: NM_052968 and NM_080576), then 9 porcine ESTs (accession numbers: BP443998, BP446485, DY418089, CJ000488, BP446061, BP446847, DB808886, CJ000039 and BP442824) were selected, which assembled into a contig, with which a set of primers was designed for PCR amplification and sequencing. The cycling conditions comprised denaturation at 94°C for 3 min, 40 cycles at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 1 min, and last extension at 72°C for 10 min.

Rapid amplification of cDNA 3' end

The 3' end of cDNA was amplified with the 3' Takara full RACE Kit (Otsu, Shiga, Japan) according to the manufacture's protocol. First-stand cDNA was synthesized using the adaptor

primer, then two rounds of 3' RACE-PCRs were performed using the amplification primer, 3' RACE outer primer and gene specific outer primer for first round; 3' RACE inner primer and gene specific inner primer for the nested PCR (Table 1). The PCR was performed using the following conditions: 32 cycles of (94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min) for first PCR; and 32 cycles of (94°C for 30 s, 60°C for 30 s and extension at 72°C for 1 min) for nested PCR.

Amplification and sequencing of genomic region

Genomic BLAST programs at National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/blast/>) and Sanger institute (<http://www.sanger.ac.uk/DataSearch/blast.shtml/>) were used for homology searches. Genomic DNA was extracted from blood using standard phenol protocol. All PCRs were performed by 36 cycles of 94°C for 30 s, annealing at 55°C to 68°C for 30 s and extension at 72°C for 1 min, and last extension at 72°C for 10 min. All products were ligated into the pGEM-T easy vector system (Takara, q58 T6(v)4ii 0 TD1(m) -1.

Table 1 Primer sets designed for porcine *APOA5* gene

| Set | Position | Sense/antisense | Function | PCR (T_m) | Size (bp) |
|-----|---------------------|---|------------------------------------|---------------|--|
| 1 | CDs | F: 5'-ATGGCAAGCGTGGCTGTAGTCTG-3' R: 5'-TTAGGGCTCCCCAGATGGTTCGAG-3' | cDNA cloning and Tissue expression | 60 | 1092 |
| 2 | GSP1 | R: 5'-AGAGAAGGGAAGAAGGAAGAAACT-3' | 3'RACE | 55 | 309 |
| | GSP2 | R: 5'-ATATCCCTGTTGGCCGATGCTGGT-3' | | 60 | 248 |
| 3 | 5'region | F: 5'-ATCCAGGATACATTTGAAGCAA-3' R: 5'-GCTGGAAGTTGATAGGATGAAGAT-3' | Sequencing | 62 | 240 |
| 4 | 5'region | F: 5'-TGCCTCTCATTATGTCATCTTCAT-3' F: 5'-CTGGGAAAAGTCTGAGGTATCCTG-3' | Sequencing and SNP | 63 | 709 |
| 5 | 5'region | F: 5'-GTCTGGGCAGTTGAGTAAGAGC-3' R: 5'-ATGGAATGTGGACCTCAAATATT-3' | Sequencing and SNP | 65 | 637 |
| 6 | Exon1,2 and intron1 | F: 5'-TGGGAATATGAACTAAGGACCTC-3' R: 5'-GAGTTGAGGAGTTGGGTAGTG-3' | Sequencing and SNP | 65 | 796 |
| 7 | Intron2 | F: 5'-GCAAGTCTCCTGAGAGATGTC-3' R: 5'-CAGGAAGTGTCCATATTGTTGAG-3' | Sequencing | 66 | 892 |
| 8 | Exon3 | F: 5'-AAGGCCCTCACTCTTGGTTC-3' R: 5'-GCTGGTCCAAGTTTTCTGAAT-3' | Sequencing and SNP | 65 | 691 |
| 9 | Exon3 and 3'region | F: 5'-ATTGGGCACCACGTGCAGGAG-3' R: 5'-TGGCAACAGGCTTGACCAGGT-3' | Sequencing and SNP | 63 | 825 |
| 10 | 3'region | F: 5'-ATTTGTGGGAAGACATCAACTACA-3' R: 5'-TATATAAGGAAATCCAGGCTCCAG-3' | Sequencing | 64 | 670 |
| 11 | 3'region | F: 5'-AAACAAATGGTATGGGTTGTGA-3' R: 5'-CCCCTGTGGCATGTAGAAGT-3' | Sequencing | 60 | 196 |
| 12 | Intron2 | Forward inner primer (T allele): 5'-TAGTTCTGCCCGCTATCCTGGCCCGCT-3' Reverse inner primer (C allele): 5'-GCTGCCTGCAGAGAGTCTAAACAGCCAAG-3' Forward outer primer: 5'-TTCAACCACCCAGGCAGGAAAGGCTT-3' Reverse outer primer: 5'-TCTGTGCAAGAAAAGCACGGAGGCACCT-3' | ARMS-PCR for SNP | 69 | 203 (T allele) 281 (C allele) 427 (from two outer primers) |
| 13 | Exon3 | Forward inner primer (C allele): 5'-TGCAGATGCTCTCGACAAGCTCACGGTC-3' Reverse inner primer (G allele): 5'-AATGTGCGCGTGCAGGGCCTTGCCATC-3' Forward outer primer: 5'-CTGCGCGTGGTCGGAGAGGACACCAAGG-3' Reverse outer primer: 5'-TGTCGTGGCGGAAAGCCTGGAGTCGCTG-3' | ARMS-PCR for SNP | 60 | 199 (C allele) 288 (G allele) 430 (from two outer primers) |
| 14 | 3'region | F: 5'-ATTTGTGGGAAGACATCAACTACA-3' R: 5'-TATATAAGGAAATCCAGGCTCCAG-3' | RFLP for SNP | 65 | 670 |
| 15 | GAPDH (AF017079) | F: 5'-ATGGTGAAGGTCGGAGTGAAC-3' R: 5'-TACTCCTTGAGGCCATGTG-3' | Control for tissue expression | 60 | 1002 |

CD = coding sequence; GSP1 = gene specific primer1; F = forward; R = reverse; SNP = single nucleotide polymorphism; ARMS-PCR = amplification refractory mutation system; RFLP = restriction fragment length polymorphism; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

primers 4 to 6 and 8 to 9 were also used to detect the polymorphisms on all animals. Additionally, genotyping was performed with the products of primers 12 to 13 by ARMS-PCR (amplification refractory mutation system) (Ye *et al.*, 2001), while a *BsrBI* site for restriction fragment length polymorphism in the products of primer 14.

Statistical analysis

The Hardy–Weinberg equilibrium of each mutation and LD among mutations in porcine *APOA5* gene was estimated using the HAPLOVIEW program (Barrett *et al.*, 2005).

Association analysis of the SNPs with the traits in reference population was performed using the MIXED model procedure of SAS v9.0 (SAS institute Inc., Cary, NC, USA) with the following model: $Y_{ijklmno} = \mu + bW_i + B_j + S_k + D_l + G_m + Li_n + e_{ijklmno}$, where $Y_{ijklmno}$ is the dependent variable (traits); μ is the general mean; W_i is live weight (kg) as a covariate; B_j the birth year; S_k the sex; D_l the age (days); G_m the genotype of SNPs; Li_n the random effect of litter and $e_{ijklmno}$ the random error. P -value < 0.05 or < 0.01 was considered statistically significant or highly statistically significant after Bonferroni correction.

Results

Cloning of porcine APOA5 gene

On the basis of sequences of *APOA5* cDNA from other species, conserved regions of porcine *APOA5* gene were identified. Further analysis revealed that the cDNA of the porcine *APOA5* gene was composed of 1883 bp and were then submitted to the Genbank database (Accession numbers: FJ810861). The ORF was 1092 bp, with a predicted protein composed of 363 amino acid residues, which is 80.43% and 78.47% homologous to bovine and human *APOA5*, respectively. The information of genomic sequence released by the Porcine Genome Project, and the gene located in chromosome 9, CH242-243D19, CU582845. Genomic sequence of porcine *APOA5* was obtained by PCR. The exon-intron boundaries were further identified by the cDNA and genomic DNA alignment by DNAMAN software. The gene is distributed among three exons spanning of 2.482 kb of genome. All the exon-intron boundaries are consistent with the GT-AG splicing rule (Figure 1). Using the house-keeping gene GAPDH as internal control, RT-PCR analysis showed that the porcine *APOA5* gene was expressed in hypophysis, adipose and liver (Figure 2).

Single and multiple nucleotide polymorphisms

In the porcine *APOA5* gene, twelve SNPs were detected (Figure 3). The promoter region (spaning -1200 to +8 bp) harbors four SNPs, while the exons and introns contain two SNPs (G1295C and C400T). The remaining six SNPs are in the 3' end region. No missense mutation occurs in the

coding region. All mutations belonged to porcine genomic DNA contig (CU582845).

Haplotype analysis

The analysis of genotype data of all F₂ progeny indicated that two SNPs in the promoter region: G-769T and G-323A form two haplotypes GG and TA. No historical recombination status between these two SNPs was confirmed by HAPLOVIEW. In the 3' end region, HAPLOVIEW also indicated that C1696T, T1697A, A1810G and T1940G have no historical recombination by forming two haplotypes of CTAT and TAGG. Therefore eight tagging SNPs, G-1013A, G-769T, G-458T, C400T, G1295C, A1810G, C1834T and G2107T, were used in the association analysis.

Association analysis of APOA5 gene with ABF and LF

The results of MIXED procedure revealed that the SNP of C1834T was significantly associated with ABF and LF in the population ($P = 0.0018$ and $P = 0.0213$, respectively) (Table 2). The CC animals had 0.306 cm of ABF less than the TT animals and 0.211 cm less than the CT heterozygote. Animal with GG genotypes had 0.09 kg of LF less than animals with TT. No any significance level was found to associate the other tag SNPs with ABF or LF.

Discussion

In this study, we described the identification and characterization of the full length of porcine *APOA5* cDNA and *APOA5* gene. The ortholog of porcine *APOA5* is related to its human and bovine counterparts based on cDNA and amino acid sequence comparisons. In human and rat, the expression of *APOA5* is mainly restricted to liver (van der Vliet *et al.*, 2001). The porcine *APOA5* is weakly expressed in adipose and over expressed in liver.

A total of 12 mutations were detected in porcine *APOA5* gene region including a multiple nucleotide polymorphism in the promoter region. No any polymorphism association with ABF and LF were detected in the region of promoter, intron or coding regions. In the 3' end region, the SNP of C1834T yielded strong association with ABF and LF ($P < 0.01$ and $P < 0.05$, respectively) (Table 2). In human, several SNPs within the *APOA5* locus (-1131T > C, -3A > G, S19W, IVS3 + 476G > A, 1259T > C and 1764C > T) have been identified, and their alleles are associated with triglyceride homeostasis in different populations (Nabika *et al.*, 2002; Lai *et al.*, 2004; Klos *et al.*, 2005; Moreno-Luna *et al.*, 2007). At the same time, two major haplotypes (-1131T > C and c.56C >

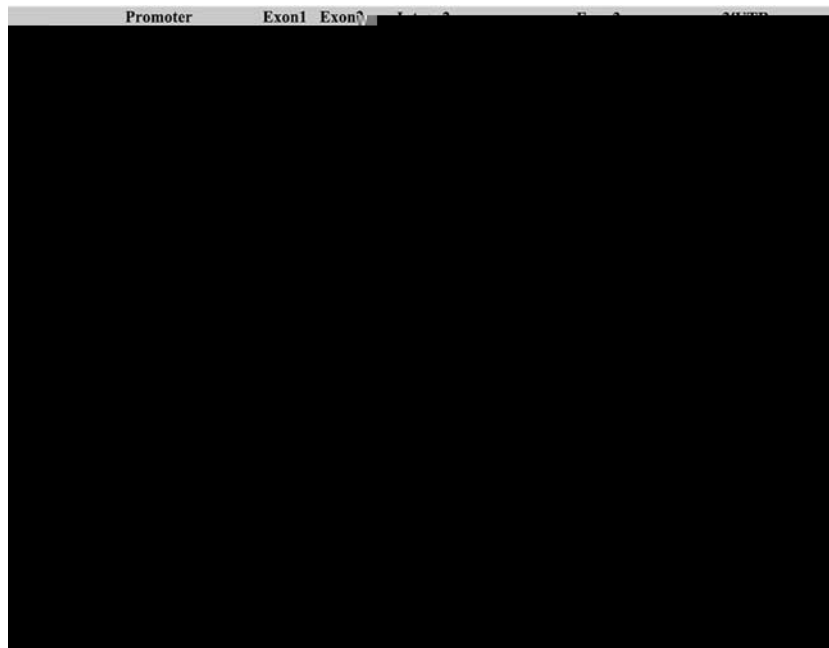


Figure 3 Genomic and haplotype analysis in the porcine *APOA5* gene. Pairwise linkage disequilibrium relationship for 12 mutations is noted based on r^2 measurements.

Table 2 Associations of *APOA5* gene polymorphism with ABF and LF

| Marker | Genotype | n | Carcass ABF thickness | | Carcass LF weight | |
|---------|----------|-----|----------------------------------|--------|---------------------------------|--------|
| | | | LSM \pm s.e. | P | LSM \pm s.e. | P |
| G-1013A | GG | 175 | 3.224 \pm 0.073 ^a | 0.6152 | 0.839 \pm 0.025 ^a | 0.1257 |
| | GA | 58 | 3.190 \pm 0.099 ^a | | 0.850 \pm 0.035 ^a | |
| | AA | 2 | 3.505 \pm 0.346 ^a | | 1.103 \pm 0.130 ^a | |
| G-769T | GG | 166 | 3.190 \pm 0.079 ^a | 0.3679 | 0.839 \pm 0.027 ^a | 0.7725 |
| | GT | 69 | 3.266 \pm 0.088 ^a | | 0.848 \pm 0.031 ^a | |
| G-458T | GG | 211 | 3.247 \pm 0.076 ^a | 0.1410 | 0.847 \pm 0.024 ^a | 0.3900 |
| | GT | 20 | 3.176 \pm 0.148 ^a | | 0.817 \pm 0.053 ^a | |
| | TT | 4 | 2.706 \pm 0.271 ^a | | 0.710 \pm 0.103 ^a | |
| C400T | CC | 161 | 3.225 \pm 0.074 ^a | 0.7768 | 0.838 \pm 0.025 ^a | 0.5226 |
| | CT | 74 | 3.202 \pm 0.096 ^a | | 0.857 \pm 0.034 ^a | |
| G1295C | GG | 160 | 3.227 \pm 0.075 ^a | 0.6900 | 0.840 \pm 0.025 ^a | 0.7118 |
| | GC | 75 | 3.197 \pm 0.094 ^a | | 0.850 \pm 0.033 ^a | |
| A1810G | AA | 63 | 3.172 \pm 0.089 ^a | 0.6271 | 0.811 \pm 0.031 ^a | 0.1769 |
| | AG | 126 | 3.236 \pm 0.080 ^a | | 0.843 \pm 0.027 ^a | |
| | GG | 46 | 3.249 \pm 0.094 ^a | | 0.877 \pm 0.033 ^a | |
| C1834T | CC | 85 | 3.057 \pm 0.085 ^{Aa} | 0.0018 | 0.793 \pm 0.030 ^a | 0.0213 |
| | CT | 113 | 3.268 \pm 0.079 ^{ABb} | | 0.857 \pm 0.027 ^{ab} | |
| | TT | 37 | 3.363 \pm 0.097 ^{Bb} | | 0.883 \pm 0.035 ^b | |
| G2107T | GG | 44 | 3.063 \pm 0.096 ^a | 0.0509 | 0.786 \pm 0.034 ^a | 0.0615 |
| | TG | 128 | 3.267 \pm 0.078 ^a | | 0.858 \pm 0.027 ^a | |
| | TT | 63 | 3.265 \pm 0.088 ^a | | 0.864 \pm 0.031 ^a | |

ABF = average backfat; LF = leaf fat; LSM = least significant mean; s.e. = standard error; n = number of animals genotyped. Different superscript letters are significant differences (capital letters: $P < 0.01$; lowercase letters: $P < 0.05$).

APOA5 is located in the *ApoA1/C3/A4* gene cluster (van der Vliet *et al.*, 2001). Polymorphisms in this cluster have been linked to the human diseases in lipid metabolism (Mar *et al.*, 2004; Olivier *et al.*, 2004; Shanker *et al.*, 2008). Obviously the *APOA5* gene should be a strong candidate

gene for the triglyceride metabolism. In *APOA5*-knockout mice, the triglyceride concentration increased fourfold (van der Vliet *et al.*, 2001), while the serum triglyceride concentrations decreased by 50% to 70% in transgenic mice with the human *APOA5* (Baroukh *et al.*, 2004). *APOA5* may

enhance LPL-mediated triglyceride hydrolysis *in vitro* and modulate hepatic very low density lipoprotein-triglyceride synthesis to affect triglyceride levels (Fruchart-Najib *et al.*, 2004; Schaap *et al.*, 2004; Merkel *et al.*, 2005). Additionally, *APOA5* has a unique association with cellular lipid droplets, which it may be involved in the storage or mobilization of intracellular lipids (Shu *et al.*, 2007 and 2008). In chicken, the significant association between SNPs in T635C and abdominal fat weight in F₂ cross of White Plymouth Rock × Silkies were found (Yao *et al.*, 2008). Our results confirmed the significant relationship of SNPs in *APOA5* gene and body fat deposition in porcine. Although the exact reasons for these finding are not clear, it speculated that *APOA5* gene may be a functional factor in fat deposition. Our research also provided a foundation for further investigation on function of *APOA5* gene.

In conclusion, we cloned the porcine *APOA5* gene using a comparative bioinformatics approach and developed a total of 12 genetic markers in this gene. Genotyping these markers on ~250 Jinhua × Pietrain F₂ reference population indicated that porcine *APOA5* gene is significantly associated with ABF and LF weight. We propose *APOA5* as a candidate gene for fat deposition, and further studies are needed to evaluate the effects of this gene in other pig breeds and investigate the regulating mechanism of *APOA5* in triglyceride metabolism.

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References

Baroukh N, Bauge E, Akiyama J, Chang J, Afzal V, Fruchart JC, Rubin EM, Fruchart-Najib J and Pennacchio LA 2004. Analysis of apolipoprotein A5, c3, and plasma triglyceride concentrations in genetically engineered mice. *Arteriosclerosis, thrombosis, and Vascular Biology* 24, 1297–1302.

Barrett JC, Fry B, Maller J and Daly MJ 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21, 263–265.

Elosua R, Ordovas JM, Cupples LA, Lai CQ, Demissie S, Fox CS, Polak JF, Wolf PA, D’Agostino Sr RB and O’Donnell CJ 2006. Variants at the *APOA5* locus, association with carotid atherosclerosis, and modification by obesity: the Framingham Study. *Journal of Lipid Research* 47, 990–996.

Fontanesi L, Scotti E, Buttazzoni L, Davoli R and Russo V 2009. The porcine fat mass and obesity associated (FTO) gene is associated with fat deposition in Italian Duroc pigs. *Animal Genetics* 40, 90–93.

Fruchart-Najib J, Bauge E, Niculescu LS, Pham T, Thomas B, Rommens C, Majd Z, Brewer B, Pennacchio LA and Fruchart JC 2004. Mechanism of triglyceride lowering in mice expressing human apolipoprotein A5. *Biochemical and Biophysical Research Communications* 319, 397–404.

Grallert H, Sedlmeier EM, Huth C, Kolz M, Heid IM, Meisinger C, Herder C, Strassburger K, Gehringer A, Haak M, Gianni G, Kronenberg F, Wichmann HE, Adamski J, Paulweber B, Illig T and Rathmann W 2007. *APOA5* variants and metabolic syndrome in Caucasians. *Journal of Lipid Research* 48, 2614–2621.

Hivert MF, Manning AK, McAteer JB, Dupuis J, Fox CS, Cupples LA, Meigs JB and Florez JC 2009. Association of variants in *RETN* with plasma resisting

levels and diabetes-related traits in the Framingham Offspring Study. *Diabetes* 58, 750–756.

Jiang Z, Michal JJ, Williams GA, Daniels TF and Kunej T 2006. Cross species association examination of UCN3 and CRHR2 as potential pharmacological targets for antiobesity drugs. *PLoS One* 1, e80.

Klos KL, Hamon S, Clark AG, Boerwinkle E, Liu K and Sing CF 2005. *APOA5* polymorphisms influence plasma triglycerides in young, healthy African Americans and whites of the CARDIA Study. *Journal of Lipid Research* 46, 564–571.

Lai CQ, Arnett DK, Corella D, Straka RJ, Tsai MY, Peacock JM, Adiconis X, Parnell LD, Hixson JE, Province MA and Ordovas JM 2007. Fenofibrate effect on triglyceride and postprandial response of apolipoprotein A5 variants: the GOLON Study. *Arteriosclerosis, thrombosis, and Vascular Biology* 27, 1417–1425.

Lai CQ, Demissie S, Cupples LA, Zhu Y, Adiconis X, Parnell LD, Corella D and Ordovas JM 2004. Influence of the *APOA5* locus on plasma triglyceride, lipoprotein subclasses, and CVD risk in the Framingham Heart Study. *Journal of Lipid Research* 45, 2096–2105.

Mar R, Pajukanta P, Allayee H, Groenendijk M, Dallinga-Thie G, Krauss RM, Sinsheimer JS, Cantor RM, de Bruin TW and Lusis AJ 2004. Association of the apolipoprotein A1/C3/A4/A5 gene cluster with triglyceride levels and LDL particle size in familial combined hyperlipidemia. *Circulation Research* 94, 993–999.

Martin RJ, Ramsay TG, Campion DR and Hausman GJ 1985. Fetal hormone and metabolite levels in lean and obese pigs. *Growth* 49, 400–407.

Merkel M, Loeffler B, Kluger M, Fabig N, Geppert G, Pennacchio LA, Laatsch A and Heeren J 2005. Apolipoprotein AV accelerates plasma hydrolysis of triglyceride-rich lipoproteins by interaction with proteoglycan-bound lipoprotein lipase. *Journal of Biological Chemistry* 280, 21553–21560.

Mersmann HJ and MacNeil MD 1985. Relationship of lipid concentrations to fat deposition in pigs. *Journal of Animal Science* 61, 122–128.

Moreno-Luna R, Perez-Jimenez F, Marin C, Perez-Martinez P, Gomez P, Jimenez-Gomez Y, Delgado-Lista J, Moreno JA, Tanaka T, Ordovas JM and Lopez-Miranda J 2007. Two independent apolipoprotein A5 haplotypes modulate postprandial lipoprotein metabolism in a healthy Caucasian population. *Journal of Clinical Endocrinology and Metabolism* 92, 2280–2285.

Nabika T, Nasreen S, Kobayashi S and Masuda J 2002. The genetic effect of the apoprotein AV gene on the serum triglyceride level in Japanese. *Atherosclerosis* 165, 201–204.

Nelbach L, Shu X, Konrad RJ, Ryan RO and Forte TM 2008. Effect of apolipoprotein A-V on plasma triglyceride, lipoprotein size and composition in genetically engineered mice. *Journal of Lipid Research* 49, 572–580.

Olivier M, Wang X, Cole R, Gau B, Kim J, Rubin EM and Pennacchio LA 2004. Haplotype analysis of the apolipoprotein gene cluster on human chromosome 11. *Genomics* 83, 912–923.

Paik HS and Yearick ES 1978. The influence of dietary fat and meal frequency on lipoprotein lipase and hormone-sensitive lipase in rat adipose tissue. *The Journal of Nutrition* 108, 1798–1805.

Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC, Krauss RM and Rubin EM 2001. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science* 294, 169–173.

Rensen PC, van Dijk KW and Havekes LM 2005. Apolipoprotein AV: low concentration, high impact. *Arteriosclerosis, thrombosis, and Vascular Biology* 25, 2445–2447.

Schaap FG, Rensen PC, Voshol PJ, Vriens C, van der Vliet HN, Chamuleau RA, Havekes LM, Groen AK and van Dijk KW 2004. ApoAV reduces plasma triglycerides by inhibiting very low density lipoprotein-triglyceride (VLDL-TG) production and stimulating lipoprotein lipase-mediated VLDL-TG hydrolysis. *Journal of Biological Chemistry* 279, 27941–27947.

Shanker J, Perumal G, Rao VS, Khadrinarasimhiah NB, John S, Hebbagodi S, Mukherjee M and Kakkar VV 2008. Genetic studies on the *APOA1-C3-A5* gene cluster in Asian Indians with premature coronary artery disease. *Lipids in Health and Disease* 7, 33.

Shu X, Chan J, Ryan RO and Forte TM 2007. Apolipoprotein A-V association with intracellular lipid droplets. *Journal of Lipid Research* 48, 1445–1450.

Shu X, Ryan RO and Forte TM 2008. Intracellular lipid droplet targeting by apolipoprotein A-V requires the carboxyl-terminal segment. *Journal of Lipid Research* 49, 1670–1676.

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- Silveira AC, Antunes RC, Almeida JF, Braga TF, Freitas PF, César AS and Guimarães EC 2008. Obese gene polymorphism in Pietrain and Large White pigs after a divergent selection. *Genetics and Molecular Research* 7, 1217–1222.
- Tang NL, Pharoah PD, Ma SL and Easton DF 2006. Evaluation of an algorithm of tagging SNPs selection by linkage disequilibrium. *Clinical Biochemistry* 39, 240–243.
- Taniguchi A, Nakai Y, Sakai M, Yoshii S, Hamanaka D, Hatae Y, Kawata M, Yamanouchi K, Okumura T, Doi K, Tokuyama K, Nagasaka S and Fukushima M 2002. Relationship of regional adiposity to insulin resistance and serum triglyceride levels in nonobese Japanese type 2 diabetic patients. *Metabolism* 51, 544–548.
- van der Vliet HN, Sammels MG, Leegwater AC, Levels JH, Reitsma PH, Boers W and Chamuleau RA 2001. Apolipoprotein A-V: a novel apolipoprotein associated with an early phase of liver regeneration. *Journal of Biological Chemistry* 276, 44512–44520.
- van Dijk KW, Rensen PC, Voshol PJ and Havekes LM 2004. The role and mode of action of apolipoproteins CIII and AV: synergistic actors in triglyceride metabolism? *Current Opinion in Lipidology* 15, 239–246.
- Walton C, Lees B, Crook D, Worthington M, Godsland IF and Stevenson JC 1995. Body fat distribution, rather than overall adiposity, influences serum lipids and lipoproteins in healthy men independently of age. *American Journal of Medicine* 99, 459–464.
- Xu XL, Xu XW, Pan PW, Li K, Jiang ZH, Yu M, Rothschild MF and Liu B 2009. Porcine skeletal muscle differentially expressed gene CMYA1: isolation, characterization, mapping, expression and association analysis with carcass traits. *Animal Genetics* 40, 255–261.
- Yao JF, Zhang Y, Wu GQ, Zheng JX, Deng XM and Yang N 2008. Polymorphisms of chicken *APOA5* gene and association with carcass traits of chickens. *Yi Chuan* 30, 607–612 (In Chinese).
- Ye S, Dhillon S, Ke X, Collins AR and Day IN 2001. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Research* 29, E88–8.