


RESEARCH ARTICLE

Open Access



Molecular cloning of *SLC35D3* and analysis of its role during porcine intramuscular preadipocyte differentiation

Wentong Li^{1,2†}, Keliang Wu^{3†}, Ying Liu², Yalan Yang⁴, Wenwen Wang¹, Xiuxiu Li¹, Yanmin Zhang², Qin Zhang¹, Rong Zhou^{2*} and Hui Tang^{1*} 

Abstract

Background: Solute carrier family 35 (SLC35) is one of a large number of membrane transporter protein families. Member D3 of this family is thought to be involved in adipose deposition and metabolic control.

Results: We obtained 2238 bp cDNA of porcine *SLC35D3*, it contains a 1272 bp ORF, encoding a 423 amino acid polypeptide, and a 966 bp 3' UTR. BLAST results revealed that the amino acid sequence of porcine *SLC35D3* had the closest phylogenetic relationship with members of the genus *Ovis aries*. Further bioinformatics analysis showed that the *SLC35D3* protein contains 8 transmembrane domains, and that there is no signal peptide structure. The secondary structure of the protein mainly contains 37.12% α -helices, 7.8% in β -folds, and 33.57% random coils. mRNA expression analysis showed that *SLC35D3* is expressed in lung, liver, heart, spleen, kidney, longissimus dorsi muscle (LDM), leaf fat (LF), and subcutaneous adipose tissue (SAT). To examine the effects of *SLC35D3* expression on fat synthesis and catabolism, *SLC35D3*-siRNA was transfected into cultured intramuscular adipocytes. *SLC35D3* silenced cells showed increased expression of genes related to fat synthesis, and increased deposition of intramuscular fat (IMF), abundance of lipid droplets, and the level of free fatty acid (FFA) in the culture medium. In contrast, the siRNA decreased the expression genes involved in fat catabolism.

Conclusions: Our results demonstrate that silenced *SLC35D3* results in increased adipogenic processes in pig intramuscular adipocytes. These data represent the first exploration of *SLC35D3* expression in swine, and provide valuable insights into the functions of *SLC35D3* in adipocyte differentiation.

Keywords: *SLC35D3*, cDNA clone, Sequence characteristics, Tissue expression, Preadipocytes

Background

Pigs have long served as models in biomedical research because of their similarity to humans with regard to body size, physiological conditions, eating patterns, and fat deposition [1–4]. Pig breeds do vary in fat deposition and are characterized by differences in intramuscular fat content and backfat thickness. Some indigenous Chinese

breeds, such as the Yimeng Black pig, exhibit particularly high body fat mass [5, 6]. Excessive fat deposition, which can result in obesity and disorders of energy metabolism in humans, is an important predictor of metabolic abnormalities [7]. Thus, the pig is an ideal model for the study of obesity and metabolic syndrome (MetS) [8, 9].

Obesity is a complex disease, it is influenced by genetic, environmental, and phenotypic factors [10–12], but the underlying mechanisms are not well understood [13]. Obesity is mainly determined by genetic differences [14], thus identification of the genes involved in fat deposition is of great interest. The solute carrier (SLC) group is the second largest group of membrane transport proteins, with more than 400 members in more than 60 families [15]. These proteins participate in

* Correspondence: zhourong03@caas.cn; tanghui@sdau.edu.cn

[†]Wentong Li and Keliang Wu contributed equally to this work.

²The State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, People's Republic of China

¹Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, College of Animal Science and Technology, Shandong Agricultural University, 61 Daizong Street, Tai'an 271018, People's Republic of China

Full list of author information is available at the end of the article



numerous physiological processes including the transporting of inorganic ions, amino acids, sugars, lipids, neurotransmitters, and drugs [15]. Human *SLC35D3* is associated with fat deposition and is a candidate gene for MetS [16]. *SLC35D3* is also involved in the biogenesis of platelet dense granules, and its expression in the brain is limited to the expression of dopamine receptor D1, though not receptor D2 [16–18]. Other research indicates that *SLC35D3* is an important regulator of tissue-specific autophagy [19]. *SLC35D3* therefore offers an opportunity to understand the mechanisms of fat deposition, and may lead to therapeutic interventions for obesity.

In this study, we cloned the *SLC35D3* CDS from the Yimeng Black pig, analyzed its amino acid sequence, and studied its expression in selected tissues and organs. We then transfected cultured intramuscular adipocytes with an *SLC35D3*-siRNA and examined the cells for changes in fatty acid metabolism. The results establish a foundation for understanding the function of the *SLC35D3* gene in pigs.

Results

Cloning and sequence analysis of the *SLC35D3* gene

Although the sequence of the porcine *SLC35D3* was predicted and annotated within the *Sus scrofa* genome (NC_010443.4), a cDNA version has not yet been cloned. To obtain a full-length cDNA, we designed PCR primers using the annotated sequence, and used total RNA extracted from the liver tissue of a Yimeng Black pig as a PCR template. The PCR product was cloned and sequenced, the fragment (2238 bp) (KY631756.1) contains a 1272 bp CDS encoding 423 amino acid residues, and a 966 bp 3' untranslated region (UTR). The molecular weight of the corresponding porcine *SLC35D3* protein is 44,653.9 Da and the isoelectric point is 6.94. Three potential O-glycosylation sites and 42 phosphorylation sites were found using functional site prediction algorithms. *SLC35D3* does not appear to possess a signal peptide structure and is therefore unlikely to be secreted from the cell. In the predicted secondary structure, 37.12% of the amino acids participate in α -helices, 7.8% in β -folds, and 33.57% in random coils (Fig. 1a). Eight transmembrane domains were predicted by TMHMM analysis (Fig. 1e). Analysis using ProtScale suggests that the protein has a distinct hydrophobic region (Fig. 1b). To determine the level of amino acid conservation in homologous proteins, a multiple sequence alignment analysis was performed (Fig. 1d), revealing highly conserved regions. SWISS-MODEL was used to predict the 3D structure (Fig. 1c). Homology modeling suggests that the structure of porcine *SLC35D3* is not highly homologous to human 5i20.1.A (PDB: D7A5Q8). Together, these results provide a

foundation for further study of the relationship between structure and function. Finally, we used neighbor-joining to construct a phylogenetic tree from the *SLC35D3* protein sequence of the Yimeng Black pig and other mammals (Fig. 1f). *SLC35D3* from the Yimeng Black pig has the highest homology to members of the genus *Ovis aries*.

Expression of *SLC35D3* in porcine tissues from different breeds and ages

SLC35D3 mRNA levels in tissues from 180-day old Yorkshire pigs were quantified by RT-qPCR and normalized using *18S* RNAs (Fig. 2a). mRNA was isolated from liver, kidney, lung, heart, spleen, LDM, LF, and SAT. *SLC35D3* expression was high in lung, LF, LDM, and SAT, suggesting that *SLC35D3* may play an important role in adipose deposition.

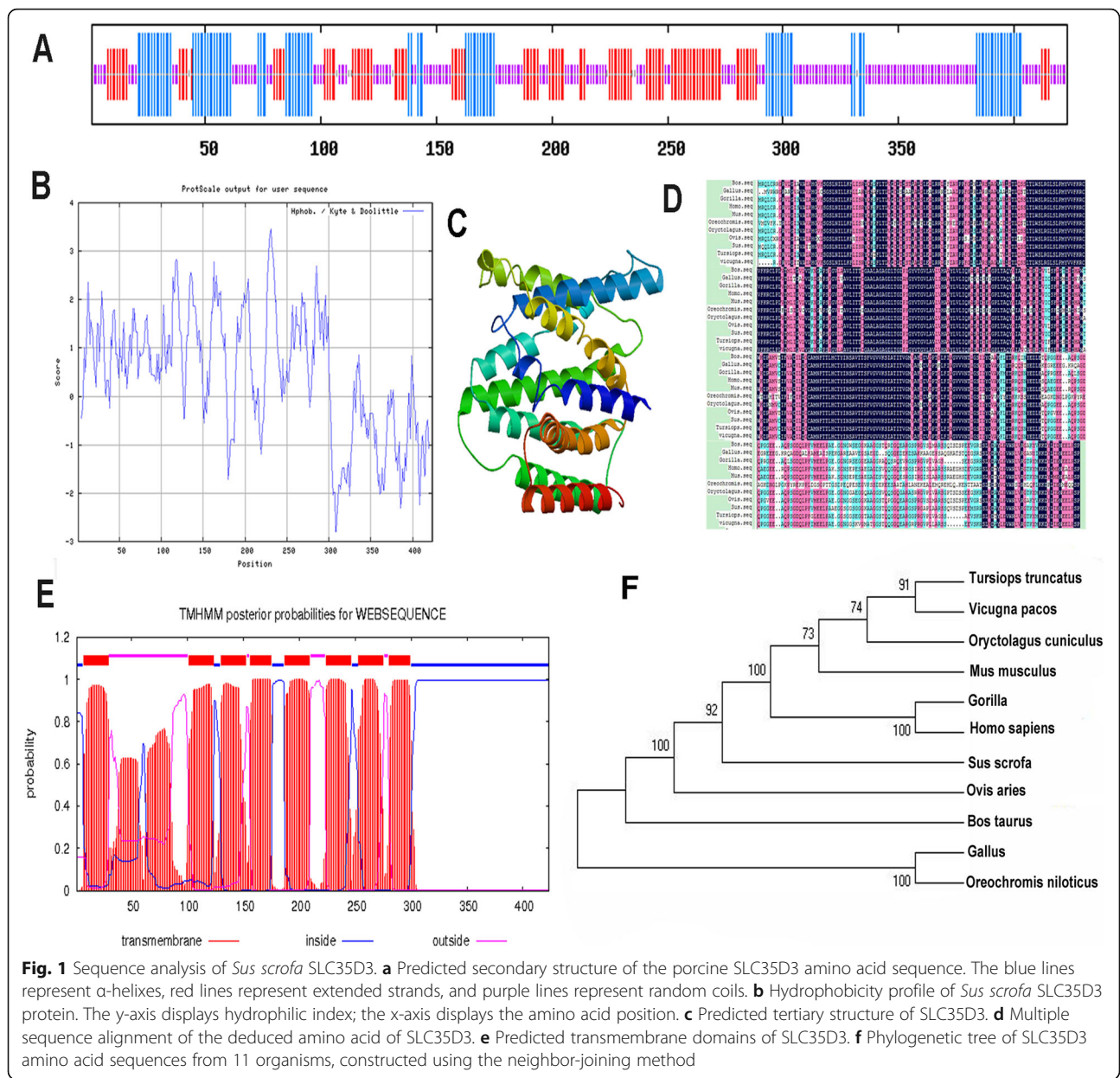
We also compared *SLC35D3* expression in the LDM in Yorkshire and Yimeng Black pigs. *SLC35D3* expression was higher in Yorkshire 7-day old pigs than in 180-day old pigs (Fig. 2b); between the 180-day old Yorkshire and Yimeng Black pigs, expression was higher in the Yorkshire breed (Fig. 2c).

Expression profile of *SLC35D3* during intramuscular preadipocyte differentiation

We performed RT-qPCR to monitor mRNA expression of *SLC35D3* and the marker genes *PPARY*, *FASN*, and *FABP4*, in cultured preadipocytes that were undergoing differentiation. Cells were cultured for 2 days after reaching a density of 90%, when they had reached confluency, then were induced to differentiate. Cells were assayed 0, 2, 4, 6, and 8 days after the induction of differentiation. *SLC35D3* mRNA expression gradually increased to its maximum level at day 4, and then decreased thereafter (Fig. 2e). Lipid droplet accumulation increased throughout the 8-day experiment (Fig. 2d).

Silencing of *SLC35D3* expression promotes adipogenesis during differentiation of porcine intramuscular preadipocytes

The inhibition efficiency of four candidate siRNAs (siRNA 1–4) was 28, 41, 50, and 24% respectively; the most effective, siRNA3, was used to inhibit the expression of *SLC35D3* gene during intramuscular adipocytes differentiation. As demonstrated by Oil Red O staining of *SLC35D3* silenced porcine intramuscular preadipocytes, adipogenesis was greatly increased over control cells at 8 days post induction (Fig. 3a); the level of free fatty acid (FFA) released into the culture medium also increased (Fig. 3b). As expected, relative expression of *SLC35D3* decreased significantly in cells transfected with siRNA3 (Fig. 4). RT-qPCR was also used to detect the

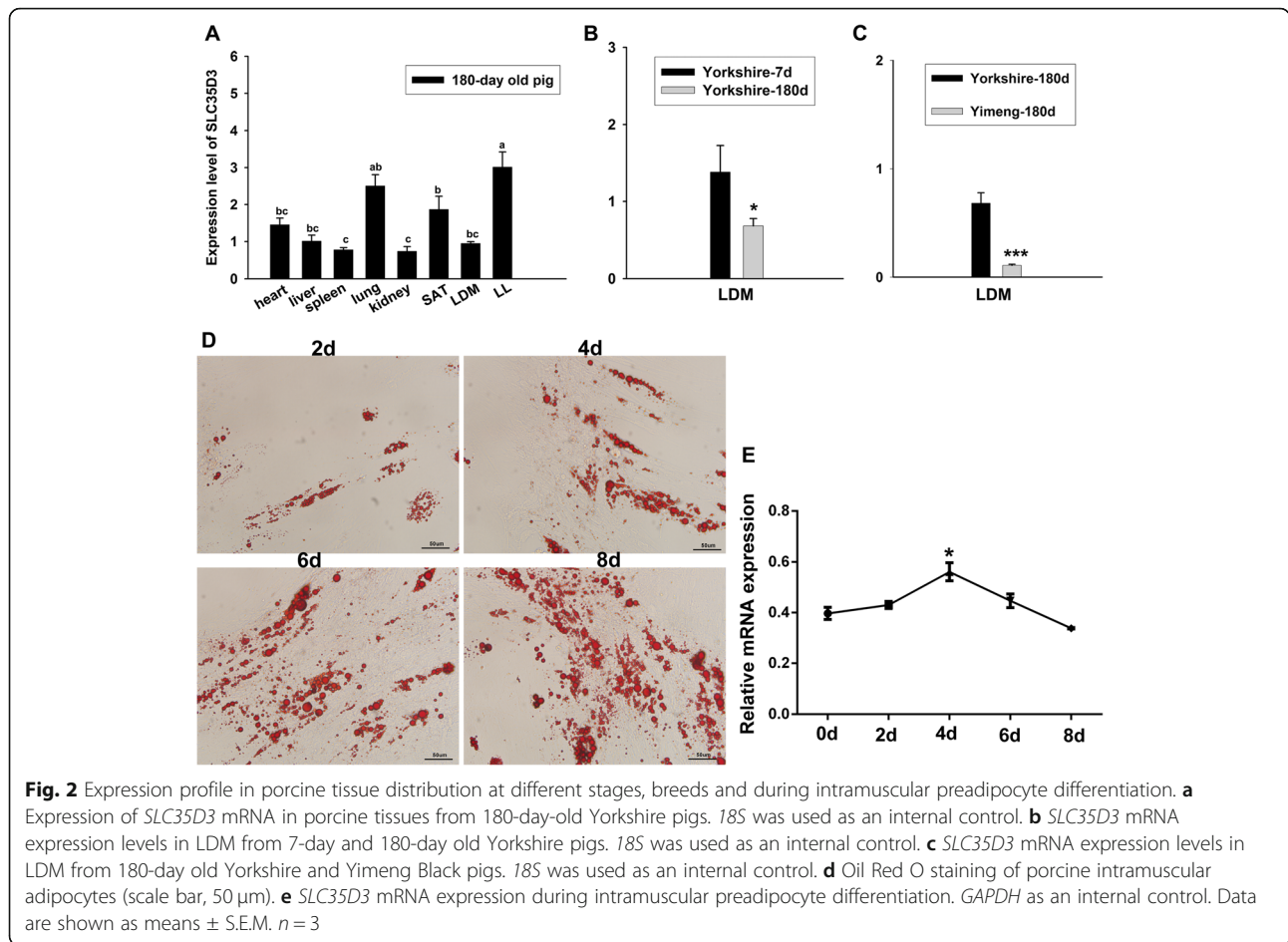


expression of five genes related to fat synthesis and catabolism (Fig. 4). Decreased expression was observed for *Sirt1* and *ATGL* at one or more time points. In mature adipocytes, *Sirt1* promotes fat mobilization through repression of *PPAR γ* [20]. *ATGL* is expressed in many tissues, particularly adipocytes, where it promotes both basal and stimulated lipolysis [21]. In contrast, increased expression was observed for *PPAR γ* , *C/EBP α* , and *aP2*, although the differences were not significant for *C/EBP α* . *PPAR γ* is expressed in white and brown adipose tissue, though it's expressed most highly in adipocytes and plays a key role in the regulation of adipogenesis, energy balance, and lipid biosynthesis [22]. *C/EBP α* is primarily expressed in fat, it is a key regulator at the adipogenic

initiation stage, and it synergistically triggers adipocyte-specific gene expression with *PPAR γ* after the growth arrest stage [23]. *aP2* is actively secreted from adipocytes, and is regulated by fasting- and lipolysis-related signals; circulating *aP2* levels are markedly elevated in obese mice and humans [24]. Overall, these results suggest that interference with *SLC35D3* gene expression promotes porcine intramuscular preadipocyte differentiation. We speculate that *SLC35D3* can inhibit the adipogenic process in porcine intramuscular adipocytes.

Discussion

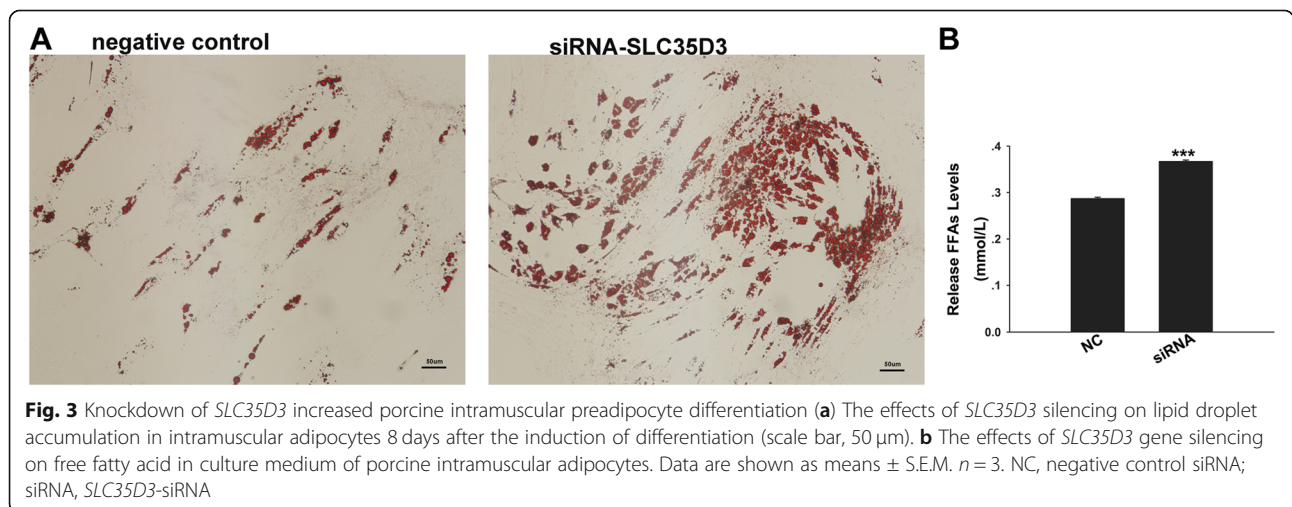
SLC35D3, a recently discovered gene, is involved in metabolic control, and is a candidate gene for MetS [16].

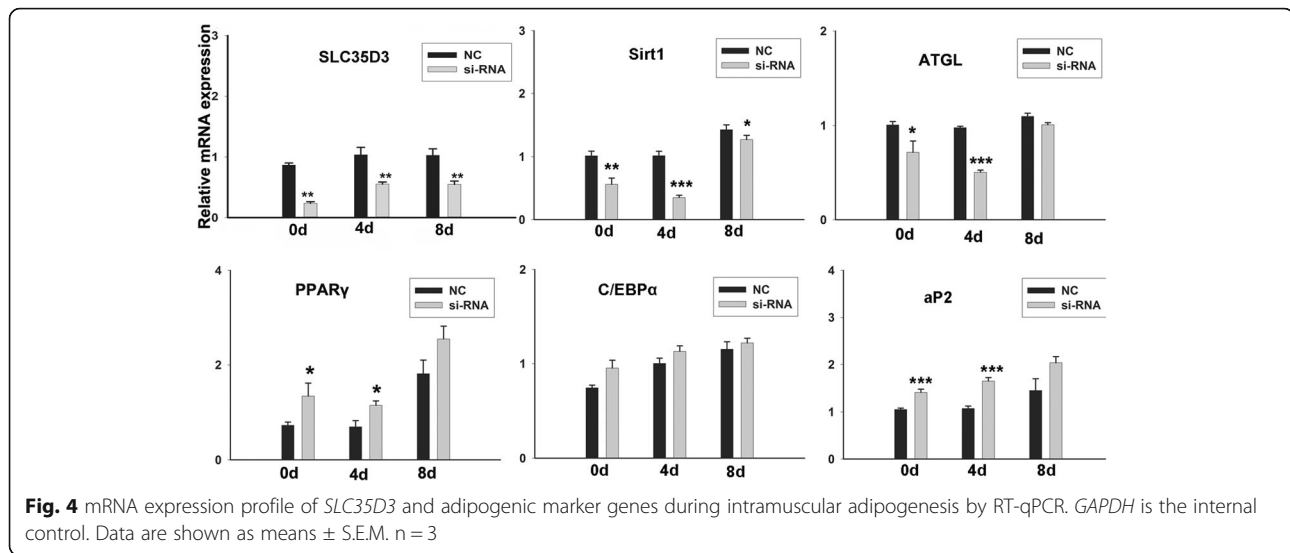


In this study we cloned, sequenced, and characterized the porcine *SLC35D3* gene. The porcine *SLC35D3* sequence is similar to that described in other animals. Based on predicted physicochemical properties, porcine *SLC35D3* is a hydrophobic, multi-transmembrane

protein. Further sequence analysis revealed the stability and conservation of *SLC35D3* gene structures.

We showed that *SLC35D3* can inhibit the adipogenic process in pig intramuscular adipocytes. Our results uncover a previously unknown role of *SLC35D3* in porcine





adipogenesis, and suggest a fruitful area of research for improving the quality of porcine meat quality as well as its potential role in human cardiovascular diseases.

Essential in the study of mechanisms involved in fat deposition, is the study of IMF. IMF is a primary factor of meat quality, it is affected by fatty acid transport, fat anabolism, and catabolism. To date there are no studies examining expression of *SLC35D3* in the various tissues of pigs based on age or breed. Our results showed that the expression levels of *SLC35D3* were high in fat and LDM tissue. In LDM, expression is higher in 7-day old piglets than in 180-day old pigs. In addition, the level of gene expression is significantly different in Yorkshire and Yimeng Black pigs. The Yorkshire pig is a typical lean meat breed, it has less subcutaneous and intramuscular fat than the Yimeng Black pig. It is still unknown whether the expression level of *SLC35D3* is related to the fat deposition between the two pig breeds. During differentiation of porcine intramuscular preadipocytes, *SLC35D3* expression increased from day 0 to day 4 and then declined until day 8. This trend was also seen with the expression of the adipogenic marker genes. We therefore speculate that *SLC35D3* has important functions during adipocyte differentiation, however the underlying mechanism is still unknown. Adipogenesis is a complex programmed process, during which the early adipogenic transcription factors *C/EBP α* and *PPAR γ* are activated, inducing the expression of adipogenic genes. These factors also play a critical role in the terminal differentiation of adipocytes in vitro, ultimately leading to the formation of mature adipocytes [25, 26]. *ATGL* initiates the process of triglyceride metabolism by hydrolyzing triglycerides into diacylglycerol and fatty acids [27, 28]. We found that reducing expression of *SLC35D3* in porcine intramuscular preadipocytes resulted in

significantly higher expression of adipogenic marker genes such as *PPAR γ* and *aP2*, while inhibiting the expression of lipid hydrolytic gene *ATGL*. However, the observed trends of *C/EBP α* were not significant. Consistent with these results, FFA levels increased in the culture medium of intramuscular preadipocytes during differentiation. These results together suggest that *SLC35D3* may be a specific transcription regulatory factor during porcine intramuscular preadipocyte differentiation.

Conclusions

In summary, this is the first report describing the cDNA sequence of *SLC35D3* from the Yimeng Black pig. The gene contains a 1272 bp CDS that encodes 423 amino acids, and a 966 bp 3' UTR. The deduced amino acid sequence of *SLC35D3* is highly similar (92–95%) to homologous proteins in other mammalian species. Phylogenetic analysis shows that pig *SLC35D3* has a close evolutionary relationship with the *Ovis aries* version of the gene. Gene expression analysis suggests that *SLC35D3* inhibits adipogenesis in pig intramuscular preadipocytes. Our results provide a basis for further studies on the function and regulation of *SLC35D3*.

Methods

Experimental animals and sampling

Three 180-day old Yorkshire pigs (average live weight 100 kg; range, 99–105 kg), three 7-day old Yorkshire pigs (average live weight, 1 kg; range, 0.8–1.3 kg), and three 180-day old Yimeng Black pigs (average live weight 100 kg; range, 90–110 kg) were purchased from the experiment farm at the Chinese Academy of Agricultural Sciences; all animals were female. Animals had been maintained under the same management conditions, fed the same food three times a day, and had access to water

ad libitum. Animals selected for tissue collection were humanely euthanized by electrical stunning followed by exsanguination. Tissue samples from the liver, kidney, lung, heart, spleen, LDM, LF, and SAT were dissected from each pig, immediately frozen in liquid nitrogen, transported to the laboratory, and stored at -80°C until RNA extraction. Intramuscular preadipocytes were collected from the 7-day old piglets and cultured. Finally, stored at -80°C until use.

RNA isolation and cDNA synthesis

Total RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA concentrations were determined by absorption spectrophotometry at 260 nm. First-strand cDNA synthesis was conducted with $2\mu\text{g}$ of purified total RNA using a RevertAid First-Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA). RNA and cDNA were stored at -80°C and -20°C , respectively.

PCR amplification of the internal fragment of *SLC35D3*

Forward (F) and reverse (R) primers (Table 1) were designed with the Primer Premier 5.0 application, using porcine *SLC35D3* (XM_013986971.1) as the reference gene sequence. The PCR reaction contained $1\mu\text{L}$ of cDNA, $12.5\mu\text{L}$ of $2\times$ Es Taq Master Mix, $0.5\mu\text{L}$ of each primer, and $10.5\mu\text{L}$ of RNase-free water. PCR cycling conditions were 94°C for 5 min, then 34 cycles of 94°C for 30 s, 57°C for 5 s, 72°C for 26 s, followed by 72°C for 10 min.

5' RACE and 3' RACE

First-strand cDNA synthesis was accomplished using the SMARTer RACE 5'/3' Kit (Takara, Dalian, China) according to the manufacturer's protocol. 5' RACE and 3' RACE reactions were performed by nested PCR, using the *SLC35D3*-specific primers GSP5/3 and NGSP5 and the universal primers UPM long and UPM short (Table 1).

All PCR products, including the internal fragment and fragments generated by 5' RACE and 3' RACE, were subjected to agarose gel electrophoresis, then recovered using an agarose gel DNA Purification Kit (Tiangen, Beijing, China). The products were cloned into the pEASY-T1 vector (Trans, Beijing, China). Clones were submitted to Sangon Biotech Co., Ltd. (Shanghai, China) for nucleotide sequencing.

Sequence analysis

Molecular weight and isoelectric point were predicted using Compute pI/Mw (http://us.expasy.org/tools/pi_tool.html). The secondary structure of the deduced amino acid sequence was predicted by SOPMA ([**Table 1** Primer sequences and their use in this study](http://</p>
</div>
<div data-bbox=)

Primer name	Primer sequence (5' → 3')
F	TGCACCTACATCAACTCGG
R	TCATTTCTTCAGGGCTGTCT
UPM long	CTAATACGACTCACTATAGGGCAAGCAG TGGTATCAACGCAGAGT
UPM short	CTAATACGACTCACTATAGGGC
GSP3	GGTGAAGAGCATCGCCACCATCACGG
GSP5	CCCCTCGCCTCTTGGCCACCCTGCT
NGSP5	GCCTTCCACCTCTGACCCGCC
<i>SLC35D3</i> -F	CCTCAGCCTGCCTATGTACG
<i>SLC35D3</i> -R	CAGCGCTTGCTTTCTGGATG
<i>ATGL</i> -F	TTGCTGTCAACCAACCACCTC
<i>ATGL</i> -R	TAATAGTGCTCTGAGGGCCG
<i>PPARγ</i> -F	AGGACTACCAAAGTGCCATCAAA
<i>PPARγ</i> -R	GAGGCTTTATCCCCACAGACAC
<i>Sirt1</i> -F	AACCGATGGAGAGTCCAGGT
<i>Sirt1</i> -R	TACCTCAGCGCCATGGAAAA
<i>aP2</i> -F	GAGCACCATAACCTTAGATGGA
<i>aP2</i> -R	AAATTCTGGTAGCCGTGACA
<i>C/EBPα</i> -F	CGATGCTCTTAGCTGAGTGT
<i>C/EBPα</i> -R	GGTCCAAGAATTCACCTCT
<i>GAPDH</i> -F	AGGGCATCCTGGGCTACACT
<i>GAPDH</i> -R	TCCACCACCCTGTTGCTGTA
<i>18S</i> -F	CGTCTGCCTATCAACTTT
<i>18S</i> -R	TTTCTCAGGCTCCCTCTC

npsa-pbil.ibcp.fr/) [29]. Phylogenetic analyses were performed using MEGA 5.1, applying the neighbor-joining method [30]. Amino acid sequences from different species were aligned using DNAMAN V6 (LynnonBiosoft, Los Angeles, CA, USA) [31]. SWISS-MODEL was used to model 3D protein structure [32, 33]. TMHMM Server v2.0 program (<http://www.cbs.dtu.dk/services/TMHMM/>) was used for protein transmembrane Structure analysis. ExPASy ProtScal (<http://web.expasy.org/protscale/>) was used for hydrophobic analysis. The signal peptide was predicted using SignalP (<http://www.cbs.dtu.dk/services/SignalP/>) [34]. N-glycosylation and O-glycosylation sites were predicted using NetNGlycears 1.0 (<http://www.cbs.dtu.dk/services/NetNGlyc/>) and NetOGlycubles 3.1 (<http://www.cbs.dtu.dk/services/NetOGlyc/>), respectively [35].

RT-qPCR for expression profile analysis

Primers for *SLC35D3*, *Sirt1* (NM_001145750.2), *ATGL* (EF583921.1), *PPAR γ* (NM_214379), *C/EBP α* (XM_003127015), and *aP2* (AJ555153.1) were designed using Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA). Relative mRNA levels were normalized

against *GAPDH* and *18S* expression. The PCR reaction contained 7.2 μ L of 2 \times SYBR Premix Ex Taq (Takara, Dalian, China), 0.3 μ L of each primer, 1 μ L of cDNA, 0.3 μ L of Dye II, and sterile water to a final volume 15 μ L. PCR cycling conditions were: 95 °C for 5 min, followed by 40 cycles at 95 °C for 5 s and 60 °C for 34 s. Finally, a dissociation step was performed at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. All samples were amplified in triplicate, and the mean was used for further analysis. Amplification of target genes was determined using the $2^{-\Delta\Delta Ct}$ method.

Isolation and culture of intramuscular preadipocytes

LDM was collected from 7-day old piglets under aseptic conditions. Tissue samples were washed 3 times in PBS containing 1% penicillin and streptomycin, and cut into small pieces (approximately 1 mm³). Tissue pieces were digested in 0.1% type I collagenase (Invitrogen, Carlsbad, CA, USA) for 1 h at 37 °C, then filtered through 400 mesh filters. The filtrates were centrifuged for 5 min at 1500 rpm/min. Cell pellets were washed with PBS then centrifuged again for 5 min at 1500 rpm/min, repeat 3 times. The preadipocytes were resuspended in DMEM/F12 containing 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO, USA) and 100 U/mL penicillin and streptomycin, then seeded into 6-well plates at a density of 5×10^4 cells/cm², and incubated at 37 °C in a humidified 5% CO₂ atmosphere. Culture medium was changed every two days.

siRNA design and transfection

Gene-specific siRNAs (Table 2) for *Sus scrofa SLC35D3* was synthesized based on our cDNA sequence; these were designed and synthesized by Gene Pharma Co., Ltd. Preadipocytes at 70–80% confluence were transfected with a negative control siRNA (20 nM) or *SLC35D3*-siRNA (20 nM) using Lipofectamine 2000 in OPTI-MEM, according to the manufacturer's protocol. 48 h after transfection, culture medium was replaced with DMEM/F12 supplemented with 10% FBS and IBMX-DEX-insulin (0.5 mmol/L IBMX, 1 mol/L DEX, 5

mg/mL insulin) to induce differentiation. Cells were incubated in this medium for 48 h, then the culture medium was changed to DMEM/F12 with 10% FBS and 5 mg/mL insulin, this medium was changed every 2 days. Total RNA was extracted from cells on days 0, 4, and 8.

Oil red O staining

Oil Red O staining was conducted as described previously [36].

Measurement of cellular lipid metabolites

Preadipocytes were induced, as described above, for 8 days, then culture medium was collected and submitted to North Life Science Co., Ltd. (Beijing, China) for free fatty acid analysis. The FFA measurements were conducted according to the manufacturer's protocol provided in the free fatty acid analysis kit (Njjcbio, Nanjing, China).

Statistical analysis

Data was analyzed using the SPSS v22.0 (SPSS, Chicago, IL), and one-way analysis of variance was used to assess the significance of experimental results. All data are presented as the means \pm standard error of the mean. Differences were considered significant at a *P* value of < 0.05 (*), < 0.01 (**), or < 0.001 (***).

Abbreviations

CDS: Coding sequence; FBS: Fetal bovine serum.; FFA: Free fatty acid; IMF: Intramuscular fat; LDM: Longissimus dorsi muscle; LF: Leaf fat; MetS: Metabolic syndrome; SAT: Subcutaneous adipose tissue; SLC35: Solute carrier family 35; UTR: Untranslated region

Acknowledgements

We thank the farmers at the Experiment Farm at the Chinese Academy of Agricultural Sciences for allowing us to collect the samples.

Authors' contributions

HT and RZ conceived the project and designed the experiments; WL and YY analyzed the data. XL and WW collected samples; YL, WL and YZ performed the experiments; WL wrote the manuscript; HT, QZ and KW revised the paper. All authors read and approved the final manuscript.

Funding

The study was supported by the Shandong "Double Tops" Program (SYL2017YSTD12) (study design, travel and sampling costs, manuscript preparation); the National Natural Science Foundation of China (31501931 and 31330074) (travel and sampling costs, and costs incurred analyzing and interpreting the data); the Program of New Breed Development via Transgenic Technology (2016ZX080011-006) (travel and sampling costs); and the National Nonprofit Institute Research Grant (2018-YWF-YB-7 and Y2016JC07) (costs incurred analyzing and interpreting data and writing the manuscript). The data interpretation was also supported by the Foshan University Initiative Scientific Research Program.

Availability of data and materials

All gene sequences in this study were deposited in NCBI. *SLC35D3* (KY631756.1), *Sirt1* (NM_001145750.2), *ATGL* (EF583921.1), *PPAR γ* (NM_214379), *C/EPBa* (XM_003127015), and *aP2* (AJ555153.1).

Ethics approval and consent to participate

All animals were treated humanely according to criteria outlined in the "Guide for the Care and Use of Laboratory Animals" published by the

Table 2 siRNA sequences

name	sequence(5' \rightarrow 3')
negative control	Sense 5'-UUCUCCGAACGUGUCACGUTT-3' Antisense 5'-ACGUGACACGUUCCGAGAATT-3'
<i>SLC35D3</i> -siRNA1	Sense 5'-UCAUCAGCCGCUACCAAGUUTT-3' Antisense 5'-AACUGGUAGCGGCGAUGATT-3'
<i>SLC35D3</i> -siRNA2	Sense 5'-GCAUCUUCGUGGCCUGUAUTT-3' Antisense 5'-AUACAGGCCACGAAGAUGCTT-3'
<i>SLC35D3</i> -siRNA3	Sense 5'-GCCACCCUCUCUAUUCUUTT-3' Antisense 5'-AAUGAAUAGAGGUGGGCTT-3'
<i>SLC35D3</i> -siRNA4	Sense 5'-GGAAGUGUGGCGGUUAGUUTT-3' Antisense 5'-AACUAACCGCCACACUUCCTT-3'

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China). Procedures were approved by the Animal Care and Use Committee. Pigs were slaughtered following the Animal Care Guidelines of the Ethics committee of Chinese Academy of Agricultural Sciences.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, College of Animal Science and Technology, Shandong Agricultural University, 61 Daizong Street, Tai'an 271018, People's Republic of China. ²The State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, People's Republic of China. ³College of Animal Science and Technology, China Agricultural University, Beijing 100193, People's Republic of China. ⁴School of Life Science and Engineering, Foshan University, Foshan 528231, Guangdong, China.

Received: 18 October 2018 Accepted: 4 February 2020

Published online: 22 February 2020

References

- Lunney JK. Advances in swine biomedical model genomics. *Int J Biol Sci*. 2007;3(3):179–84.
- Ross JW, et al. Generation of an inbred miniature pig model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2012;53(1):501–7.
- Xing K, et al. Integration of transcriptome and whole genomic resequencing data to identify key genes affecting swine fat deposition. *PLoS One*. 2015;10(4):e0122396.
- Stachowiak M, Szczerbal I, Switonski M. Genetics of adiposity in large animal models for human obesity—studies on pigs and dogs. *Prog Mol Biol Transl Sci*. 2016;140:233–70.
- Li C, et al. Molecular microevolution and epigenetic patterns of the long non-coding gene H19 show its potential function in pig domestication and breed divergence. *BMC Evol Biol*. 2016;16:87.
- Fang M, Andersson L. Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. *Proc Biol Sci*. 2006;273(1595):1803–10.
- Huang W, et al. Differential regulation of mRNAs and lncRNAs related to lipid metabolism in two pig breeds. *Oncotarget*. 2017;8(50):87539–53.
- Bohan AE, et al. The proliferation and differentiation of primary pig preadipocytes is suppressed when cultures are incubated at 37 degrees Celsius compared to euthermic conditions in pigs. *Adipocyte*. 2014;3(4):322–32.
- Spurlock ME, Gabler NK. The development of porcine models of obesity and the metabolic syndrome. *J Nutr*. 2008;138(2):397–402.
- Speakman JR. Obesity: the integrated roles of environment and genetics. *J Nutr*. 2004;134(8 Suppl):2090S–1055S.
- Zhang H, Wu J, Yu L. Association of Gln27Glu and Arg16Gly polymorphisms in Beta2-adrenergic receptor gene with obesity susceptibility: a meta-analysis. *PLoS One*. 2014;9(6):e100489.
- de Oliveira CM, et al. Heritability of cardiovascular risk factors in a Brazilian population: Baependi heart study. *BMC Med Genet*. 2008;9:32.
- Vieira-Potter VJ, et al. Disconnect between adipose tissue inflammation and cardiometabolic dysfunction in Ossabaw pigs. *Obesity (Silver Spring)*. 2015;23:2421–9.
- Comuzzie AG, et al. Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. *PLoS One*. 2012;7(12):e51954.
- Bai X, Moraes TF, Reithmeier RAF. Structural biology of solute carrier (SLC) membrane transport proteins. *Mol Membr Biol*. 2017;34(1–2):1–32.
- Zhang Z, et al. Mutation of SLC35D3 causes metabolic syndrome by impairing dopamine signaling in striatal D1 neurons. *PLoS Genet*. 2014;10(2):e1004124.
- Chintala S, et al. The Slc35d3 gene, encoding an orphan nucleotide sugar transporter, regulates platelet-dense granules. *Blood*. 2007;109(4):1533–40.
- Meng R, et al. SLC35D3 delivery from megakaryocyte early endosomes is required for platelet dense granule biogenesis and is differentially defective in Hermansky-Pudlak syndrome models. *Blood*. 2012;120(2):404–14.
- Wei ZB, et al. SLC35D3 increases autophagic activity in midbrain dopaminergic neurons by enhancing BECN1-ATG14-PIK3C3 complex formation. *Autophagy*. 2016;12(7):1168–79.
- Picard F, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature*. 2004;429(6993):771–6.
- Schoiswohl G, et al. Impact of reduced ATGL-mediated adipocyte lipolysis on obesity-associated insulin resistance and inflammation in male mice. *Endocrinology*. 2015;156(10):3610–24.
- Janani C, Ranjitha Kumari BD. PPAR gamma gene—a review. *Diabetes Metab Syndr*. 2015;9(1):46–50.
- Ji S, Doumit ME, Hill RA. Regulation of Adipogenesis and key Adipogenic gene expression by 1, 25-Dihydroxyvitamin D in 3T3-L1 cells. *PLoS One*. 2015;10(6):e0126142.
- Cao H, et al. Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab*. 2013;17(5):768–78.
- Nagai S, et al. Suppression of Fatty Acid and Triglyceride Synthesis by the Flavonoid Orientin through Decrease of C/EBPdelta Expression and Inhibition of PI3K/Akt-FOXO1 Signaling in Adipocytes. *Nutrients*. 2018;10(2).
- Bouraoui L, Gutierrez J, Navarro I. Regulation of proliferation and differentiation of adipocyte precursor cells in rainbow trout (*Oncorhynchus mykiss*). *J Endocrinol*. 2008;198(3):459–69.
- Zimmermann R, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*. 2004;306(5700):1383–6.
- Liu X, et al. Long non-coding RNA NEAT1-modulated abnormal lipolysis via ATGL drives hepatocellular carcinoma proliferation. *Mol Cancer*. 2018;17(1):90.
- Geourjon C, Deleage G. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Comput Appl Biosci*. 1995;11(6):681–4.
- Biradar SS, et al. Genetic characterization of toxoplasma gondii isolates from chickens in India by GRA6 gene sequence analysis. *Acta Parasitol*. 2014;59(4):666–74.
- Wan L, et al. Molecular cloning, structural analysis and tissue expression of protein phosphatase 3 catalytic subunit alpha isoform (PPP3CA) gene in Tianfu goat muscle. *Int J Mol Sci*. 2014;15(2):2346–58.
- Kiefer F, et al. The SWISS-MODEL repository and associated resources. *Nucleic Acids Res*. 2009;37(Database issue):D387–92.
- Arnold K, et al. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*. 2006;22(2):195–201.
- Li Z, et al. Characterization of the visfatin gene and its expression pattern and effect on 3T3-L1 adipocyte differentiation in chickens. *Gene*. 2017;632:16–24.
- Bashir U, et al. Molecular detection and characterization of respiratory syncytial virus B genotypes circulating in Pakistani children. *Infect Genet Evol*. 2017;47:125–31.
- Zhao C, et al. MAT2B promotes adipogenesis by modulating SAME levels and activating AKT/ERK pathway during porcine intramuscular preadipocyte differentiation. *Exp Cell Res*. 2016;344(1):11–21.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

