Research Journal of Animal Sciences 1 (3): 102-106, 2007
ISSN: 1993-5269

SSNP Detection, Imprinting and Expression Analysis in Prenatal Skeletal Muscle of the Porcine Succinate Dehydrogenase Complex, Subunit D (SDHD) Gene

Jing Huang, Zheng-Mao Zhu and Shu-Hong Zhao
Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education and Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, Huazhong Agricultural University, Wuhan, P.R. China

Abstract: SDHD (Succinate Dehydrogenase D) is a maternally imprinted gene in human carotid body tissue. Mutations carried by the father of this gene may cause embryo growth retardation and tumor, therefore it is important to study the function of this gene. In this study, we detected the imprinting status of porcine SDHD in the 90 day prenatal muscle and placental tissues, analyzed the genotype frequencies of a non-synonymous *Bst*F5I SNP in the 4th exon of this gene and detected the expression in prenatal muscle of three embryonic developmental stages (33d, 65d, 90d). The results showed that *Mob*I PCR-RFLP from both DNA and cDNA from the same animal muscle and placental tissue are heterozygote, sequencing of both DNA and cDNA showed there is no preferential expression of any of the two alleles. Thus SDHD has no imprinting effect in porcine 90 day fetus muscle tissue and placenta tissue. The allele frequencies of *Bst*F5I PCR-RFLP in different pig breeds revealed a much higher frequency of allele A in Chinese indigenous breeds Erhualian and Yushanhei pigs whereas allele G has higher frequency in Tongcheng, Large White and Landrance pigs. The RT-PCR result of SDHD showed that this gene is expressed in prenatal muscles in all the three embryonic developmental stages in the porcine.

Key words: Pig, SDHD, imprinting, allele frequency, expression, genotype

INTRODUCTION

Succinate-ubiquinone oxidoreductase (SDHD) is an important enzyme complex in the tricarboxylic acid cycle (Saraste, 1999). It consists of 4 subunits (Baysal *et al*., 2001) and is the only respiratory chain complex completely encoded by nuclear genes. Flavoprotein and iron–sulphur protein, encoded by succinate dehydrogenase complex, subunit D (SDHA) and SDHB, constitute the hydrophilic catalytic part of the complex. The other two units, which are encoded by SDHC and SDHD called CybL and CybS, are hydrophobic integral membrane proteins which can form the cytochrome-b and link the catalytic subunits to the matrix side of the mitochondrial inner membrane (Hagerhall, 1997; Ackrell, 2000; Iverson *et al*., 2000). Deficiency of Complex II in human may present themselves clinicallies as Leigh syndrome (Birch-Machin *et al*., 1996), Kearns-Sayre syndrome (Rivner *et al*., 1989), encephalopathy (Stratilova *et al*., 1998), isolated cardiomyopathy (Haut *et al*., 2004) muscle weakness with exercise intolerance (Mancuso *et al*., 2003) and polymyositis with sarcoidosis and myopathy (Reichmann *et al*., 1995).

Imprinted genes do not rely on traditional laws of Mendelian genetics, which describe the inheritance of traits as either dominant or recessive. In Mendelian genetics, both parental copies equally contribute to the offspring. The impact of an imprinted gene copy, however, depends only on the parent from which it was inherited. For some imprinted genes, the cell only uses the copy from the mother to make proteins and for others only that from the father. Since DeChiara discovered an endogenous imprinted gene in mice, scientists began more genetic research on such special genes and made meaningful results. According to those studies, about 80% of the imprinted genes present clusters on the chromosome (Lee *et al*., 1997); in mammals, imprinted genes are highly conserved (Bartolomei *et al*., 1997); the copy of imprinted genes are often out of step (Kitsberg *et al*., 1993); some imprinted genes only transcript to

Corresponding Author: Shu-Hong Zhao, Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education and Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, Huazhong Agricultural University, Wuhan, P.R. China

mRNA but not express to protein (Pteifer and Tilghman, 1994). Generally speaking, methylation of the regulatory sequence DNA in mammalian imprinted genes is a major cause of most single allele expression, it can be eliminated or marked. Genome imprint is related to diseases, fetal development and many important qualitative and quantitative characters. SDHD was known as a maternally imprinted gene in human carotid body tissue (Roqueplo *et al*., 2001).

In this study, we detected the imprinting effect of porcine SDHD in the 90 day prenatal muscle and placental tissues, analyzed the genotype frequencies of a non-synonymous SNP in the 4th exon of this gene and detected the expression differences in three embryonic developmental stages (33d, 65d, 90d).

MATERIALS AND METHODS

Tissues samples: The samples for imprinting identification were 90-day fetal muscle samples of five Large White pigs and 90-day placental tissues of 15 Large White pigs. The samples for allele frequency analysis were 5 unrelated pig breeds: Tongcheng pigs (17), Erhualian pigs (25), Yushanhei pigs (20), Large White (21) and Landrance (25). The samples for expression profile analysis were prenatal loin muscle from 3 different developmental stages (33d, 65d and 90d) of 9 Landrance pigs.

RNA extraction and reverse transcription: Total RNA was extracted using TRIzol reagent (Ambion, USA) according to the manufacturer's instructions and then purified with the TURBO DNA-free TM kit (Ambion, USA) to eliminate potential DNA contamination. The concentration of RNA was measured by the UV absorbance at 260 nm and performed under gel electrophoresis to detect its inaction.

RNA was reverse transcribed in the presence of 5X PCR Buffer (Mg^{2+}), 1 mM dNTPs, 300U Reverse Transcriptase (Promege, USA), 1U RNAsafe, 2.5 μM OligodT in a final reaction volume of 50μL. Reactions were carried out at 42°C for 30 min, followed by a 5 min step at 95°C to denature the enzyme and then by cooling to 4°C. After reverse transcription, the cDNA was amplified by PCR with primer pair 1: Sense: 5' GGG GCA TTG GAC AAG TCG 3'; Anti-Sense: 5' GAG CAG AGG CAA GGA GGT 3'. The PCR reaction was as follows: cDNA was amplified in 10 μL reaction mixture which containing 5× PCR buffer (Mg^{2+}), 3 pmol each primer, 75 μM dNTPs, 0.5U Taq DNA polymerase. And the PCR program is 94°C for 3 min; 35 cycles at 94°C for 30 s, 58 or 62°C for 30 s and 72°C for 20 s; with a final extension for 5 min at 72°C. The PCR products were detected on a 2.0% agarose gel stained with 0.5 μg mL^{-1} ethidium bromide. DNA was recovered with the Gel extraction Mini kit (Watson Biotechnolgies, Shang-hai, P.R.China). PCR products were sequenced to confirm the correct amplification.

SNP identification and allele frequency analysis: Alignment of expressed sequence tags from Unigene Cluster Ssc.2586 revealed several possible SNP sites of pig SDHD. Single Strand Conformation Polymorphism (SSCP) analysis and sequencing of PCR products were used to confirm two SNP sites. The allele frequency analysis was conducted using 108 unrelated animals from 5 breeds: Tongcheng pigs from Hubei province (17), Erhualian pigs from Jiangsu province (25), Large White pigs (21), Yushanhei pigs from Jiangxi province (20) and Landrance pigs (25).

Imprinting analysis: Genotypes of genomic DNA and cDNA from the same heterozygote muscle and placenta samples were using MboI restriction enzyme. Imprinting status was determined by comparing the genotypes and the sequencing results of genomic DNA and cDNA.

Expression profile of three developmental stages: RT-PCR was conducted to detect the expression of SDHD gene in the developmental stages. GAPDH was used as the positive control, primers were: FOR-5'-CCT TCA TTG ACC TCC ACT AC-3'; REV-5'- GTT GTC ATA CTT CTC ATG GTT C-3' (Pan *et al*., 2003).

RESULTS AND DISCUSSION

Mutation identification and allele frequency analysis: After sequencing confirmation of the PCR products, one genetic variation based on T---G was detected by PCR-restriction fragment length polymorphism using the *Mbo*I restriction enzyme and another G---A transition was detected by digestion with *Bst*F5I, resulting in allele A (220bp) and allele G (178 and 42bp) (Fig. 1). Analysis of this SNP revealed a much higher frequency of allele A in Chinese indigenous breeds Erhualian and Yushanhei pigs whereas allele G has higher frequency in Tongcheng, Large White and Landrance pigs (Table 1).

Imprinting identification: PCR-RFLP was used to detect the genotypes of DNA and cDNA, the result showed that MobI PCR-RFLP from both DNA and cDNA from same animal muscle tissue are heterozygotes (Fig. 2 and 3),

Table 1: Genotype and allele frequencies in different breeds of the SDHD *Bst*F5I SNP\

Breeds	No. of animals	Genotype			Allele frequency	
		AA	AG	GG	G	A
Tongcheng	17	0	4	13	0.8824	0.1176
Erhualian	25	17	7	1	0.18	0.82
Yushanhei	20	6	12	2	0.4	0.6
Large white	21	0	0	21	1	0
Landrance	25	0	3	22	0.94	0.06

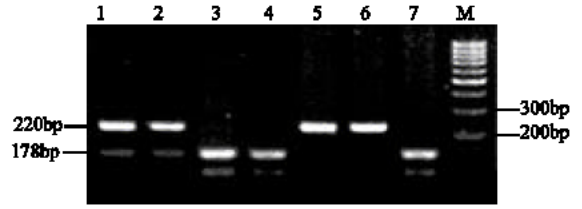

Fig. 1: SDHD *Bst*F5I SNP genotyping in different pig breeds. Lanes 1, 2: GA genotypes; Lanes 3, 4, 7: GG genotypes; Lanes 5, 6: AA genotypes. Lane M: Marker 100bp ladder

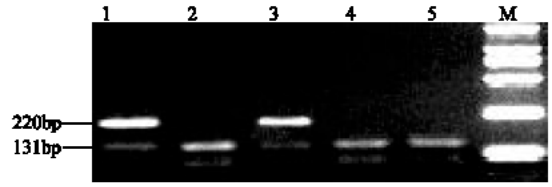

Fig. 2: Identification of heterozygote individuals. Lanes 1, 3: TG genotypes; Lanes 2, 4, 5: GG genotypes. Lane M: Marker DL2000

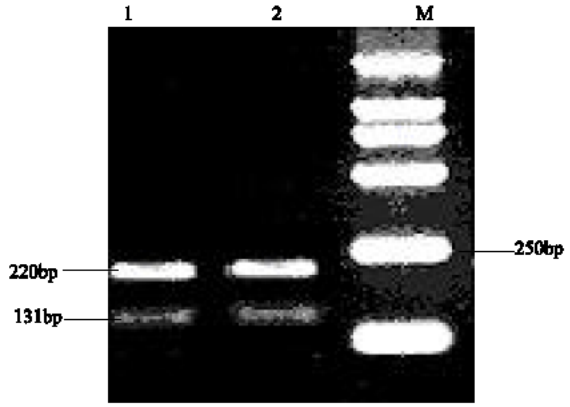

Fig. 3: Genotype of cDNA from 90 day fetus muscle tissue of heterozygote individuals. Lanes 1, 2: the two heterozygous genotype of TG. Lane M: Marker DL2000

indicating no imprinting effect in porcine 90 days fetus muscle tissue. Similar method was used to analyze the imprinting effect of SDHD in placental tissue, the PCR-RFLP result also showed that it has no imprinting effect in 90 days placental tissue (Fig. 4).

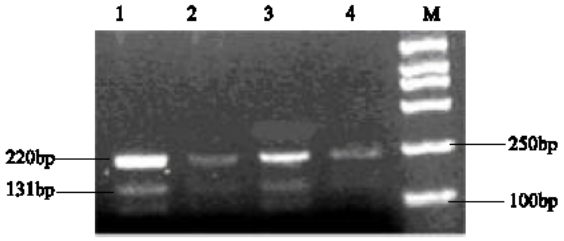

Fig 4: Genotype of genomic DNA and cDNA from placental tissue. Lanes 1, 2: DNA and cDNA from the same individual. Lanes 3, 4: DNA and cDNA from the same individual. Lane M: Marker DL2000

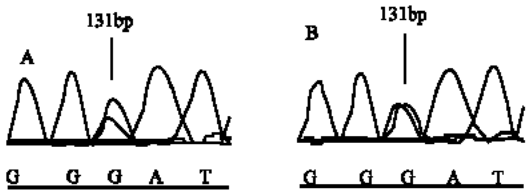

Fig 5: Sequencing results of DNA and fetal muscle cDNA from one heterozygous individual. A is DNA sequence. B is cDNA sequence

In order to make our result more convictive, we sequenced the DNA and cDNA from muscle of one heterozygote animal (Fig. 5), indicating no preferential expression of any of the two alleles.

Expression profile: The RT-PCR result of SDHD in different developmental stages showed that this gene is expressed in all the three developmental stages in porcine prenatal muscle (Fig. 6). Intensity of the bands were quantified and F tests were conducted to compare the differential expression among these stages, but no significant differences in expression were detected.

In this study, we identified a new non-synonymous SNP in the porcine SDHD gene and detected allele frequencies of this SNP in Chinese and foreign breeds. Zhu *et al.* (2005) reported that another polymorphism in the SDHD gene on chromosome 9 is associated with loin-muscle area. Malek and Thomas Identified QTLs for reproduction and carcass traits on porcine chromosome 9, such as average daily gain to weaning, average daily gain on test, early growth, 16-d weight and loin muscle area etc. In this study, the high allele frequencies of allele G in Yorkshire and Large White indicating these two breeds were almost fixed for this allele. Since the alleles were not segregated well in our population, we didn't analyze the association between this SNP and economic traits.

There was evidence that SDHD has maternal imprinting effect in human carotid body tissue, e.g. the mutation from paternal may cause later fetal growth

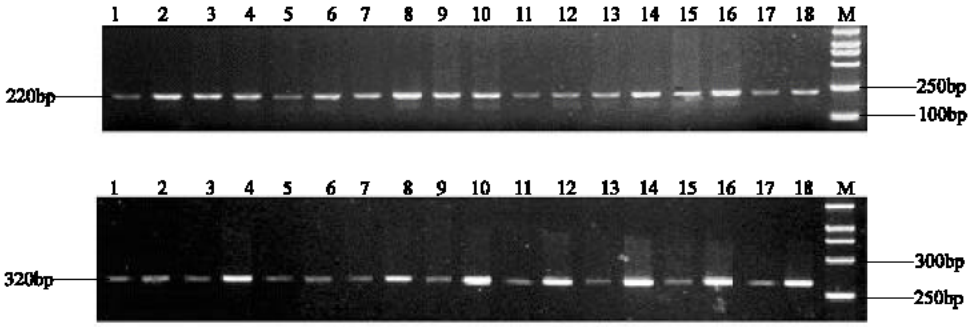

Fig 6: Expression profile of SDHD in fetal muscle of different developmental stages. a) Indicates SDHD; Lanes 1-6: 33d, 7-12: 65d, 13-18: 90d; the odd number: 30 cycles, even number: 35 cycles. b) Indicates GAPDH; Lanes 1-6: 33d, 7-12: 65d, 13-18: 90d; the odd number: 20 cycles, even number: 25 cycles

and tumor (Hitchins and Moore, 2002; Renard *et al*., 2003). Since Thomsen suggested that there are genomic imprinting regions for some production and carcass traits on pig chromosome 9, which also contains SDHD, it may be interesting to detect the imprinting status of the SDHD in the pig by genotyping genomic and cDNA from the sample heterozygous sample, however, our results showed biallelic expression of SDHD gene in the porcine 90 day fetus muscle and placenta. Some imprinted genes can not be identified only by PCR-RFLP when the gene is not completely imprinted, for example, Clark *et al*. (2002) proved that *Tnfrsf23* gene was weakly imprinted in several organs in mouse. In order to detect the unequal expression of the parental allele, we sequenced the DNA and cDNA from the same heterozygote, the result also showed that there is no preferential expression of any of the alleles. Imprinted genes were frequently conserved among different species of mammals (Jinno *et al*., 1996). Khatib's study in the sheep also showed that SDHD has no imprinting effect in sheep muscle tissue (Khatib *et al*., 2005) which is in agreement with our results in the pig. However, it was in disagreement with the result of human, which demonstrated that SDHD gene in extant mammals has divergent imprinted status.

RT-PCR by samples from muscle samples from three different prenatal stages showed that the SDHD gene is expressed in all stages and the different amplification cycles did not show there is differential expression among these stages. This result indicates that SDHD may be an important gene in all three developmental stages.

ACKNOWLEDGEMENT

This research was supported by the National Natural Science Foundation of China (30671138), the Key Project of National Basic Research and Developmental Plan (2006CB102105) of China, the Program for New Century Excellent Talents in University (NCET-05-0669) and the Hubei province natural science creative team project (2006ABC008).

REFERENCES

Ackrell, B.A., 2000. Progress in understanding structure-function relationships in respiratory chain complex II. FEBS. Lett., 466: 1-5.

Birch-Machin, M.A., C. Marsac, G. Ponsot, B. Parfait, R.W. Taylor, P. Rustin and A. Munnich, 1996. Biochemical investigations and immunoblot analyses of two unrelated patients with an isolated deficiency in complex II of the mitochondrial respiratory chain. Biochem. Biophys. Res. Commun., 220: 57-62.

Bartolomei, M.S. and S.M. Tilghman, 1997. Genomic imprinting in mammals. Annu. Rev. Genet., 31: 493-525.

Baysal, B.E., W.S. Rubinstein and P.E. Taschner, 2001. Phenotypic dichotomy in mitochondrial complex II genetic disorders. J. Mol. Med., 79: 495-503.

Clark, L., M. Wei, G. Cattoretti, C. Mendelsohn and B. Tycko, 2002. The *Tnfrh1* (*Tnfrsf23*) gene is weakly imprinted in several organs and expressed at the trophoblast-decidua interface. BMC. Genet., 3: 1-11.

Hagerhall, C., 1997. Succinate: Quinone oxidoreductases Variations on a conserved theme. Biochim. Biophys. Acta, 1320: 107-141.

Haut, S., T.B. Villemeyur, M. Brivet, A. Guiochon-Mantel, A. Boutron, P. Rustin, A. Legrand and A. Slama, 2004. The deleterious G-A mutation in mitochondrial DNA-encoded cytochrome b may remain clinically silent in homoplasmic carriers. Eur. J. Hum. Genet., 12: 220-224.

Hitchins, M.P. and G.E. Moore, 2002. Genomic imprinting in fetal growth and development. Expert Rev. Mol. Med., 9: 1-19.

Iverson, T.M., C. Luna-Chavez, I. Schroder, G. Cecchini and D.C. Rees, 2000. Analyzing your complexes: Structure of the quinol-fumarate reductase respiratory complex. Curr. Opin. Struct. Biol., 10: 448-455.

Jinno, Y., K. Sengoku, M. Nakao, K. Tamate, T. Miyamoto and T. Matsuzaka *et al*., 1996. Mouse/human sequence divergence in a region with a paternal-specific methylation imprint at the human *H19* locus. Hum. Mol. Genet., 5: 1155-1161.

Kitsberg, D., S. Selig, M. Brardels, I. Simon, I. Keshet, D.J. Driscoll, R.D. Nicholls and H. Cedar, 1993. Allele-specific replication timing of imprinted gene regions. Nature, 364: 459-463.

Khatib, H., 2005. Characterization and analysis of the imprinting status of the ovine SDHD and COPG2 genes. Anim. Genet., 36: 186-187.

Lee, M.P., R.J. Hu, L.A. Johnson and A.P. Feinberg, 1997. Human *KvLQT1* gene shows tissue specific imprinting and encompasses Beckwith-Wiedemann syndrome chromosomal rearrangements. Nat. Genet., 15: 181-l85.

Mancuso, M., M. Filosto, E. Bonilla, M. Hirano, S. Shanske, T.H. Vu and S. DiMauro, 2003. Mitochondrial myopathy of childhood associated with mitochondrial DNA depletion and a homozygous mutation (T77M) in the TK2 gene. Arch. Neurol., 60: 1007-1009.

Pfeifer, K. and S.M. Tilghman, 1994. Allele-specific gene expression in mammals: The curious case of the imprinted RNAs. Genes Dev., 8: 1867-1874.

Pan, P.W., K. Li, C.K. Tuggle, M. Yu, B. Liu and S.H. Zhao, 2003. Sequencing, tissue distribution and physical mapping of the porcine homologue of cardiomyopathy associated 3 (CMYA3). Anim. Genet., 34: 473-474.

Rivner, M.H., M. Shamsnia, T.R. Swift, J. Trefz, R.A. Roesel, A.L. Carter, W. Yanamura and F.A. Hommes, 1989. Kearns-Sayre syndrome and complex II deficiency. Neurologist, 39: 693-696.

Reichmann, H., B. Schalke, P. Seibel, M. Naumann and K. Toyka, 1995. Sarcoid myopathy and mitochondrial respiratory chain defects: clinicopathological, biochemical and molecular biological analyses. Neuromuscul. Disord., 5: 277-283.

Roqueplo, A.P., J. Favier, P. Rustin, J.J. Mourad, P.F. Plouin, P. Corvol, A. Rotig and X. Jeunemaitre, 2001. The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. Am. J. Hum. Genet., 69: 1186-1197.

Saraste, M., 1999. Oxidative phosphorylation at the findesiecle. Science, 283: 1488-1493.

Stratilova, L., J. Zeman, H. Hansikova, J. Houstek, J. Hermanska, Z. Dudkova, V. Konradova, H. Hulkova and M. Elleder, 1998. Mitochondrial myopathy, deafness and type 2 diabetes mellitus with tRNALeu (UUR) point mutation in mitochondrial DNA. Cas. Lek. Cesk, 137: 430-433.

Zhu, Z.M., J.B. Zhang, K. Li and S.H. Zhao, 2005. Cloning, mapping and association study with carcass traits of the porcine SDHD gene. Anim. Genet., 36: 191-195.