



Molecular Characterization, Chromosomal Localizations, Expression Profile, and Association Analysis of the Porcine *PECI* Gene with Carcass Traits

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ABSTRACT : The full-length cDNA of the porcine peroxisomal Δ^3, Δ^2 -enoyl-CoA isomerase (*PECI*) gene encodes a monofunctional peroxisomal Δ^3, Δ^2 -enoyl-CoA isomerase. Cloning and sequencing of the porcine *PECI* cDNA revealed the presence of an 1185-base pair open reading frame predicted to encode a 394-amino acid protein by the 5' rapid amplification of cDNA ends (5'-RACE) and EST sequences. The porcine *PECI* gene was expressed in seven tissues (heart, liver, spleen, lung, kidney, skeletal muscle, fat) which was revealed by reverse transcriptase-polymerase chain reaction (RT-PCR). The porcine *PECI* was mapped to SSC71/2 p11-13 using the somatic cell hybrid panel (SCHP) and the radiation hybrid panel (RH) (LOD score 12.84). The data showed that *PECI* was closely linked to marker S0383. A C/T single nucleotide polymorphism in *PECI* exon 10 (3'-UTR) was detected as a *PvuII* PCR-RFLP. Association analysis in our experimental pig population showed that different genotypes of *PECI* gene were significantly associated with the Average Backfat thickness (ABF) ($p < 0.05$) and Buttock backfat thickness ($p < 0.01$). (**Key Words :** Full-length cDNA, RH Mapping, Significant Association, Backfat Thickness, *PECI* Gene, Pigs)

INTRODUCTION

Fat deposition of pigs is of economic importance because of market incentives for lean pork production and decreased feeding costs. It is crucial to investigate and characterize new candidate genes and QTL relevant to pig fat deposit traits. To date, several quantitative trait loci (QTL) significantly affecting 10th-rib, average backfat thickness and other production traits have been mapped on SSC7 (Wang et al., 1998; Nagamine et al., 2003). Peroxisomal Δ^3, Δ^2 -enoyl-CoA isomerase (*PECI*) was located near the boundary of the quantitative trait loci (QTL) region. Δ^3, Δ^2 -enoyl-CoA isomerase (*Eci1p*) is unique because its activity is necessary for β -oxidation of all unsaturated fatty acids (Geisbrecht et al., 1999). The series of enzyme-catalyzed reactions required for degradation of fatty acids are evolutionarily conserved and accomplished primarily through the β -oxidation pathway. In peroxisomes,

Eci was predicted to be a dominant enzyme for 3-*cis* 3 \rightarrow 2-*trans* and 3-*trans* 3 \rightarrow 2-*trans* isomerizations of long-chain intermediates (Zhang et al., 2002). Fatty acid β -oxidation in mammals is considerably more complicated, primarily due to the existence of overlapping but distinct fatty acid β -oxidation pathways. Mammalian peroxisomes contain at least three fatty acyl-CoA oxidases, both L-specific and D-specific 2-enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase multifunctional proteins, and at least two thiolases, all of which are encoded by different genes (Palosaari et al., 1990a, 1991; Geisbrecht et al., 1998; Gurvitz et al., 1998; Geisbrecht et al., 1999; Partanen et al., 2004). When the *Eci* was completely excised in the mouse, it extensively perturbed the metabolism of unsaturated fatty acids, especially for short interval starvation and the fatty acid pattern of complex phospholipids was strongly altered (Palosaari et al., 1990b; Janssen et al., 2002). The *PECI* gene can be encoded by *ECI1* and it is required for growth of *saccharomyces cerevisiae* on unsaturated fatty acids (Gurvitz et al., 1998). It can be concluded that the *PECI* gene may play an important role during the metabolic processing of unsaturated fatty acids. Deposition of fat by

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Received January 12, 2006; Accepted May 18, 2009

animals in their bodies is associated with the metabolism of fatty acids, and more research would contribute to understanding of porcine fat deposition.

MATERIALS AND METHODS

Materials and reagents

Genomic DNA was isolated from blood of mature Tongcheng pigs (Hubei province, China) by phenol/chloroform extraction. RNA was extracted from muscle tissue of adult Tongcheng pigs and adult Swedish Landrace with TRIzol reagent kit (Life Technologies, Grand Island, NE, USA). RACE (the rapid amplification of cDNA ends) was performed according to the instructions of the SMARTTM RACE cDNA Amplification Kit (Clontech Inc, Palo Alto, CA, USA). The PCR products of RACE were purified with the Wizard PCR Preps DNA Purification System (Promega, Madison, WI, USA). ORF were found by the program SeqMan (DNA star, Madison, WI, USA) and the amino acid sequences were deduced with Primer5.0 (Primer Premier5.0, Premier, Canada). Using the pGEM T-easy vector, DNase I (RNase-free) and M-MLV reverse transcriptase from TaKaRa Dalian (Dalian, China), primers were synthesized (Table 1) and PCR products were sequenced by AuGCT Biotechnology (Beijing, China).

Isolation of full-length cDNA of porcine *PECI* gene

Full-length cDNA sequence of porcine *PECI* was obtained using the RACE method and EST contigs. Gene-specific primers were designed from pig EST sequences (Table 1). The PCR products of RACE were purified and then cloned into the pGEM T-easy vector, and were sequenced using a commercial service. ORF were found using the program Seqman (DNA star, Madison, WI, USA) and the amino acid sequences were deduced with Primer5.0 (Primer Premier5.0, Premier, Canada).

Spatio-temporal expression pattern of *PECI* gene

Gene expression patterns were determined using RT-PCR. Total RNA of tissue expression was extracted from

adult porcine heart, liver, spleen, lung, kidney, skeletal muscle, and fat. PCR conditions were as follows: 4 min at 94°C followed by 29 cycles of 40 s at 94°C, 40 s at 57°C, 30 s at 72°C, and a final extension of 5 min at 72°C. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA was used as internal standard and the relative levels of specific mRNAs were determined by comparing the ratio of PCR products. The cycle numbers were selected to avoid the polymerase chain reactions (PCRs) entering plateau stages. PCR products (10 µl) were used to detect the expression profile.

Genetic variation identification and association analysis

SNP sites were discovered by direct sequencing of individuals. The SNP was validated using Single-Strand Conformation Polymorphism (SSCP) and polymorphic sites were detected as PCR-RFLP. DNA samples of 126 unrelated animals from four Chinese indigenous pig breeds (Meishan, Erhualian, Dahuabai, Qingping) and the commercial Duroc pig were genotyped. PCR fragments showing different genotypes were cloned and sequenced. A chi-squared test on the allele frequencies distribution for five pig breeds was performed using SAS (version 8.0). Pigs (n = 120) used for association studies were from a crossbred population. The PCR products were digested with *PvuII*.

An experimental population (198 pigs) including two cross-bred groups and three pure-bred groups, Large White×(Landrace×Tongcheng) (46 individuals), Landrace×(Large White×Tongcheng) (50 individuals), Tongcheng breed (53 individuals), Landrace (25 individuals) and Large White (24 individuals), were selected for association analysis. Firstly, the interaction effect between population and the genotypes of the gene was detected by linear model and no significant interaction effects were found. Therefore, it could not be incorporated into the model of association analysis. The association between genotype and traits (carcass traits and meat quality) was performed with the least square method (GLM procedure, SAS version 8.1).

Table 1. Primer pairs designed for *PECI* gene

Gene	Primer	Primer sequence (5'-3')	Binding region	PCR (Tm)	Size (bp)
PECI	5'-RACE	CCACCAGGCTGTCACTTCCGCTTGTT		74.0	487
	primer1F	AATCTGCCCAAGGAAACTGC	Exon3, 4	59.2	1,215
	primer1R	CTGTGACTCTGTGTGGACGG	Exon 11	56.3	
	expression-F	ATGCGGAGGAAAGCAGAGTC	Exon 10	59.2	267
	expression-R	CACCTGCCTCCCTTCTCAA	Exon 10	60.0	
	Mapping-F	CAGAGCAGCGCCGTCTTACT	Exon 6	60.7	402
	Mapping-R	CTCCGAGTCCGCCTTGCTAC	Intron 6	62.2	
GAPDH	Primer-F	CCTTCATTGACCTCCACTAC			320
	Primer-R	GTTGTCATACTTCTCATGGTTC			

The model used to analyze the data was assumed to be: $Y_{ijk} = \mu + B_i + P_j + G_k + e_{ijk}$, where, Y_{ijk} is the observation value of the trait; μ is the population mean; B_i is breed effect, P_j is carcass batch effect ($j = 1$ to 8); G_k is genotype effect ($k = TT, CT, CC$) and e_{ijk} is the random residual. The analysis was processed with the GLM procedure of SAS (version 8.1).

Somatic cell hybrid and radiation hybrid mapping

A somatic cell hybrid panel (SCHP) (Yerle et al., 1996) was used for chromosomal region assignments and the radiation hybrid (IMpRH) panel was used for precise locations (Yerle et al., 1998). PCR reactions were performed in a volume of 10 μ l of 1 \times PCR Buffer (TakaRa), containing 20 ng of cell hybrid line DNA, 0.2 μ M of each primer, 100 μ M of each dNTPs, 1.5 mM MgCl₂ and 2.0 units Taq DNA Polymerase (TakaRa). The PCR profile was 5 min at 95°C followed by 35 cycles of 40 s at 94°C, 40 s at 62°C, 30 s at 72°C, and a final extension of 5 min at 72°C. Analysis of PCR results was performed with the software available on <http://www.toulouse.inra.fr/lgc/pig/hybrid.htm> (Chevalet et al., 1997) and the IMpRH mapping tool (<http://imprh.toulouse.inra.fr/>) (Milan et al., 2000) for SCHP and RH mapping. Two-point RH analysis was used for identification of linkage groups with LOD score threshold of 5.0. Multipoint locations were obtained by the minimum break analysis.

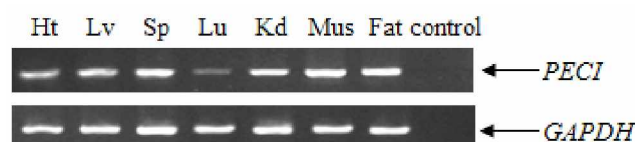
Phylogenetic tree of *PECI* gene

In order to realize the different species evolution in the *PECI* gene, a phylogenetic tree of the *PECI* gene based on CDS sequence was completed in MEGA3.1 software and contained bootstrap values computed from 1,000 replicates.

RESULTS

Sequence characteristics of porcine *PECI*

The cDNA sequence of the human *PECI* gene (GenBank: NM_006117) was used to search the pig EST databases by BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). Porcine EST (28 for *PECI*), which shared at least 80% identity with the corresponding human cDNAs, was assembled into contigs for primer design. The 5'-RACE procedures were used to obtain a complete porcine *PECI* CDS of 1,185 nucleotides (GenBank: DQ291159). Porcine *PECI* was 81% and 83% identical to human (NM_006117, NM_206836), and 80% identical to mouse homologues (NM_011868). The *PECI* gene was predicted to encode 394 amino acids with a molecular mass of 43.33 kDa and isoelectric point of 5.77. The predicted coding sequences (CDS) showed 75% identity to the corresponding human sequences (accession number NM_006117). For the porcine



Expression profile in Tongcheng pig

Figure 1. RT-PCR tissue expression analysis of *PECI* and *GAPDH* in Heart (Ht), Liver (Lv), Spleen (Sp), Lung (Lu), Kidney (Kd), Skeletal Muscle (Mus), and Fat. The PCR products were examined by electrophoresis on 2% agarose gel in 1 \times TAE buffer. The gels were stained with ethidium bromide and photographed.

PECI gene, the 5'-RACE PCR gave rise to 487 bp fragments and the 3'-terminate obtained through *in silico* cloning. Computer analysis of the combined nucleotide sequence revealed an 1,185 bp ORF flanked by a 61-bp 5'-UTR and a 60-bp 3'-UTR. The comparison of cDNA and DNA sequences revealed that the *PECI* gene spanned 1.695 kb and was made up of 11 exons.

Spatio-temporal expression pattern of *PECI* gene

RT-PCR analysis of total RNA showed that *PECI* expressed in all seven tissues examined. The template was selected from a famous indigenous Tongcheng breed of pig. By the expression profile, it could be concluded that there was little difference between heart and other tissues, the gene expressed in every tissue which testified to its broad expression pattern. The *PECI* gene was expressed comparatively higher in fat tissue (Figure 1).

Genetic variation identification and association analysis

The PCR-RFLP assay was used to examine the 267 bp fragment for the presence and location of two *Pvu*II restriction sites by comparing the sizes of restriction fragments. The restriction fragments were detected by PAGE gel electrophoresis with 1 \times TBE buffer. The gels were stained with silver and photographed, and the band patterns were screened for polymorphism. The PCR product amplified using the primer expression-F and expression-R, was 267 bp from exon 10. Restriction enzyme analysis revealed a polymorphic *Pvu*II site in the 3'-UTR. The two allele-specific patterns obtained after *Pvu*II digestion were two uncut fragments of 142-bp and 125-bp fragments for allele T and two fragments of 125 and 17 bp for allele C. SNPs and association studies were used for *PECI*. PCR fragments representing different genotypes were sequenced and revealed a C to T mutation at position 143. Allele frequencies of *PECI* genotypes were significantly different beside Chinese indigenous Meishan (Table 2). By genetic variation analysis, allele frequency of T appeared preponderant in Duroc, Dahuabai and Erhualian pigs, while

Table 2. Allele frequency and genotype frequency of *PECI* in different pig breeds

Breed	Genotype frequency			Allele frequency	
	CC	CT	TT	C	T
Duroc	0.1111(2/18)	0.2778(5/18)	0.6111(11/18)	0.2500	0.7500
Qingping	0.3750(12/32)	0.4375(14/32)	0.1875(6/32)	0.5938	0.4062
Dahuabai	0.0556(1/18)	0.3889(7/18)	0.5556(10/18)	0.2500	0.7500
Erhualian	0.1765(3/17)	0.2941(5/17)	0.5294(9/17)	0.3235	0.6765
Yushan Black	0.7308(19/26)	0.2692(7/26)	0	0.8654	0.1346
Meishan	0.4667(7/15)	0.2667(4/15)	0.2667(4/15)	0.6000	0.4000

the C allele was preponderant in all other breeds, especially the Yushan Black pig in which no TT genotype presented. There was an imbalance in genotype between the indigenous and exotic pigs (Table 2). The genotype and allele frequencies of the SNPs of the *PECI* gene were detected in different breeds for association analysis (Table 3). Statistical analysis revealed that the CC-TT genotype had a significant association with average backfat thickness and muscle color ($p < 0.05$) and especially with the trait of Buttock backfat thickness, this genotype expressed an highly significant association ($p < 0.01$) (Table 4).

SCHP and RH mapping of the porcine *PECI*

PECI was mapped to SSC71/2 p11-13 (probability of localization to region equals 0.4703 and error risk $< 0.1\%$) by SCHP analyses. RH mapping allowed the locations of *PECI* to be defined more precisely. *PECI* showed close

linkage to S0383 (*PECI*: 22 cR, LOD = 12.84), which was already mapped to porcine chromosome 7. Thus the most probable chromosomal localization for *PECI* was SSC71/2 p11-13.

Phylogenetic analysis of *PECI* gene in diverse species

A phylogenetic tree of the *PECI* gene was constructed by use of the UPGMA method. The analysis included the following species: *Homo sapiens* (NM_006117); *Sus scrofa* (DQ291159); *Mus musculus* (NM_011868); *Rattus norvegicus* (NM_001006966); and *Gallus gallus* (XM_418965). The data clearly showed distinct clusters with high bootstrap support (Figure 2). From the figure, *Homo sapiens* and *Sus scrofa* could be clustered as one (the bootstrap value was 77%). A very high bootstrap value was observed for the *Mus musculus* cluster and *Rattus norvegicus* cluster (100%).

Table 3. Genotype and allele frequencies of SNPs of the *PECI* gene in different pig breeds used for association analysis

Breeds	Genotype frequency			Allele frequency	
	CC	CT	TT	C	T
Landrace	0.4000(10/25)	0.4400(11/25)	0.1600(4/25)	0.6200	0.3800
Large White	1.0000(24/24)	0	0	1.0000	
Tongcheng	0.3019(16/53)	0.3962(21/53)	0.3019(16/53)	0.5000	0.5000
Landrace×(Large white×Tongcheng)	0.9000(45/50)	0.0800(4/50)	0.0200(1/50)	0.9400	0.0600
Large White×(Landrace×Tongcheng)	0.7391(34/46)	0.1957(9/46)	0.0652(3/46)	0.8370	0.1630
Total	0.6515(129/198)	0.2273(45/198)	0.1212(24/198)		

Table 4. Association analyses of *PvuII*-RFLP genotypes with production traits

Genotypes	Number of animals	Buttock backfat thickness	Average backfat thickness (in 3 sites)	Muscle color
<i>PECI</i> - <i>PvuII</i>				
CC	129	2.49±0.07	3.13±0.06	3.09±0.03
TT	24	2.95±0.15	3.47±0.13	2.92±0.08
CT	45	2.71±0.11	3.36±0.09	3.15±0.06
p value				
CC-CT		0.0905	0.0411*	0.3649
CC-TT		0.0065**	0.0158*	0.0428*
CT-TT		0.1644	0.4210	0.0073**

* $p < 0.05$, ** $p < 0.01$.

Average backfat thickness (in 3 sites): backfat thickness at the shoulder, backfat thickness depth between 6th and 7th ribs, 10th rib backfat thickness.

Table 5. Chi square testing for genotype distribution of *PECI* gene in pig breeds

Breed	Qingping	Dahuabai	Erhualian	Yushan Black	Xiao Meishan
Duroc	9.7185	0.7143	0.3717	24.4489**	5.9319
Qingping		9.4629	6.2577	9.3938	1.2914
Dahuabai			1.3585	25.5915**	7.6804
Erhualian				19.9604*	3.5230
Yushan Black					7.9798

The Chi square of all breeds is: 42.8744 $df = 10$ $\chi^2_{0.05(10)} = 18.3000$; $\chi^2_{0.01(10)} = 23.2000$.

DISCUSSION

Tissue distribution of porcine *PECI* gene

PECI gene is extensively expressed in the tissue of Tongcheng pigs. It appeared that there was a little difference between tissues in the indigenous breed. As far as the temporal expression profile was concerned, it may participate in the metabolism of fatty acids because of its comparatively high expression in fat tissue.

Mapping of porcine *PECI* gene

The *PECI* gene was mapped to porcine chromosome SSC7/2 p11-13 by SCHP panel and the most significant association was with S0383 (LOD = 12.84) analyzed by radiation hybrid (IMpRH) panels, which also characterised to SSC7. The *PECI* gene has been mapped to chromosome 6p24.3 in the human while to chromosome 13 A4 in the mouse (<http://www.ncbi.nlm.nih.gov/entrez/>). The information was also consistent with comparative mapping data as porcine chromosome 7/2 p11-13 has been shown to share homology with human chromosome 6p24.3 (update 22th May 2002 by INRA). The *PECI* gene was mapped to porcine Chr7. The largest effects were obtained for the SLA (the swine leukocyte antigens) region on SSC 7 (Quintanilla et al., 2003). Many QTL affecting growth, carcass composition, reproduction, and meat quality traits have been detected in this region (Bidanel et al., 2001a, 2001b, 2002; Milan et al., 2002). So, it could be a candidate gene for research on porcine fat deposition.

Polymorphism and association analysis

A C/T transition was found at 3'-UTR of the porcine *PECI* gene. In this study, a significant difference could be

seen among the different indigenous pig breeds. By Chi-square testing, Yushan Black pigs were significantly different from Duroc and Dahuabai pigs (Table 5) which may be because results in these pigs presented a different evolution in genetic selection. It was evident that polymorphism of the *PECI* gene was significantly associated with Average backfat thickness ($p < 0.05$) and Buttock backfat ($p < 0.01$) traits. SNP in the 3'-UTR was about 47 bp from coding sequences (CDs). Generally speaking, the 3'-UTR was a particular section of messenger RNA. A long chain of AMP residues seems to enhance its translatability by helping recruit mRNA to polysomes, thereby promoting initiation of translation. So, we deduced that the SNP site might influence porcine adipose tissue mass by way of polyadenylation. It is well known that many QTL on chromosome 7 influence fatness traits. Therefore, the *PECI* polymorphism could potentially be acting as a genetic marker for a linked QTL with effect on Buttock Backfat and Average backfat thickness. Research on fatty acid metabolism became rather significant for us to understand different deposition of adipose tissue between pig breeds. It would be convenient for us to develop porcine early breeding projects based on these results.

ACKNOWLEDGMENTS

We are grateful to Dr. Martine Yerle for providing the RH panel. This research was supported by National Natural Science Foundation of China (30771536), National High Science and Technology Foundation of China (2007AA10Z168).

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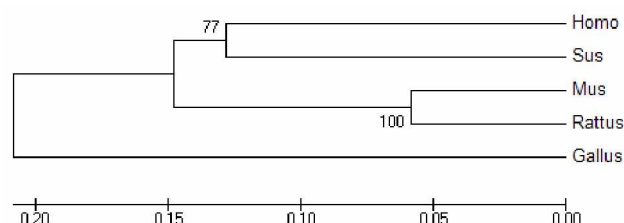


Figure 2. Phylogenetic tree of *PECI* gene in diverse species.

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