MOLECULAR CHARACTERIZATION OF PORCINE PGC-1 cDNA

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INTRODUCTION

From a thermodynamic perspective, obesity is the result of energy imbalance over time. Due to the cumulative nature of energy imbalance effects, obesity can develop, when energy intake exceeds energy expenditure by only a small margin (Lowell and Spiegelman, 2000). In contrast to thermodynamic and some physiological approaches, which frequently treat the organism as a black box, recent developments in molecular biology enabled identification of a number of factors, which are involved in energy metabolism. Mitochondria occupy the central role in energy metabolism. Several transcription/replication factors which directly regulate expression of mitochondrial genes have been identified. However, the co-ordination of these factors in the programme, responsive to the environmental changes is still poorly understood. In eukaryotes the mitochondrial function and copy-number can be increased in response to external stimuli. The important player in this process is PPARγ coactivator 1 (PGC-1), the cold inducible coactivator of nuclear receptors (Wu et al., 1999). PGC-1 stimulates mitochondrial biogenesis and respiration in muscle cells through an induction of uncoupling protein 2 (UCP-2) and through regulation of nuclear respiratory factors (NRFs). In the brown adipose tissue, which is the most responsive tissue in adaptive thermogenesis in small rodents, PGC-1 increases transcriptional activities of PPARγ and thyroid hormone receptor on the uncoupling protein 1 (UCP-1) promoter (Boss et al., 1999). Mouse PGC-1 also promotes expression of several mitochondrial genes in brown adipose tissue, heart, kidney and brain (Puigserver et al., 1998).

Comparison between obese and lean persons showed no significant difference in PGC-1 expression in adipose tissue in man, although lean persons expressed more PGC-1 mRNA than obese persons in skeletal muscle (Larrouy et al., 1999).

Until recently the transcriptional coactivators were regarded as constitutively active components, using transcription factors for localisation of their function. In the case of PGC-1 it has been shown that this coactivator promotes transcription through the assembly of a complex that includes the histone acetyltransferases steroid receptor coactivator 1 (SRC-1) and CREB binding protein (CBP)/p300 (Puigserver et al., 1999). The transcriptional activity of PGC-1 depends on conformational changes in PGC-1, which take place upon binding of PGC-1 to peroxisome proliferator-activated receptor γ (PPARγ). Docking of PGC-1 to PPARγ and subsequent change of conformation allow binding of SRC-1 and CBP/p300 to PGC-1, which in turn increases transcriptional activity considerably. In addition, PGC-1 binds and co-activates the action of NRF-1 on the mitochondrial transcription factor A (mtTFA) promoter and stimulates mitochondrial biogenesis (Lowell and Spiegelman, 2000).

Since differences in energy metabolism are of striking importance in pig production, the aim of this study was to characterise at molecular level PGC-1 which is involved in the regulation of
energy metabolism and biogenesis of mitochondria. We cloned the porcine PGC-1 cDNA and sequenced the entire coding region. In order to find informative polymorphisms in PGC-1 gene among different breeds, the genomic sequences of the largest coding exon (exon 8) from eight breeds were compared.

MATERIAL AND METHODS

RNA isolation, cDNA synthesis and identification of BAC clones. The total RNA was extracted from skeletal muscle tissue from Duroc piglet using Trizol reagent (Gibco-BRL). Reverse transcription was performed using PGC-1 specific PPARGC1.R primer (5’-TTACCTGCCAAGCTTCTCT-3’) and AMV reverse transcriptase (Promega). The cDNA was amplified using several sets of heterologous oligonucleotides and most of the 5’-end cDNA sequence was derived using 5’/3’-RACE kit (Roche Diagnostics).

Sequence analysis. PCR amplificates of either cDNA fragments or genomic DNA were sequenced using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit and ABI Prism 310 DNA sequencer (PE Applied Biosystems). The entire PGC-1 cDNA sequence was obtained using a battery of oligonucleotides situated in the conserved parts of the PGC-1 coding sequence. Multiple sequence alignment was performed using Clustal W (Thompson et al., 1994).

Identification of the porcine PGC-1 polymorphism. Screening for polymorphic sites within the PGC-1 gene was carried out by sequencing amplified genomic DNA fragments representing the exon 8. Genomic DNA from animals from eight breeds (Yorkshire, Mangalica, Goettingen Mini Pig, Duroc, Krsko Polje Pig, Pietrain, German Landrace and Swedish Landrace) was extracted from white blood cells by phenol extraction procedure.

RESULTS AND DISCUSSION

The porcine PGC-1 cDNA sequence containing 13 exons in a total length of 2388 bp coding region and 150 bp of the 5’-UTR was obtained (data not shown). The interspecies comparison of the coding region for PGC-1 revealed 94, 91 and 90% sequence identity with human, rat and mouse cDNA sequences, respectively. The deduced amino acid sequence showed the same level of conservation among species: the porcine amino acid sequence is 94, 92 and 91% identical with human, rat and mouse amino acid sequence, respectively. Due to the conserved exon/intron boundary sites within the porcine PGC-1 gene we assume that porcine PGC-1 has the same gene structure as its human, murine and rat counterparts. Porcine amino acid sequence of the exon 8 of PGC-1, aligned with sequences from human, rat and mouse is presented in figure 1. The high degree of inter species sequence identity of PGC-1 supports the evolutionary importance of this gene. This could also be assumed from its central in cold-inducible regulation of thermogenesis and its involvement in the biogenesis of mitochondria.

Sequence analysis of the genomic DNA, coding for exon 8 of the PGC-1 gene in eight different breeds of pigs revealed two single nucleotide polymorphisms at nucleotide positions 1107 and 1290 of the coding sequence. The later one, causing amino acid substitution at position 430 is presented in figure 2. The transversion at nucleotide position 1290 (A → T)
abolishes AluI restriction site in the Mangalica and Goettingen Mini Pig breeds. In all other breeds studied, the AluI site was present.

Human           LTPPTTPHKANNPPRRASKLKSCKTVPPPPKPYRSESTQGNNKSTKKGPEQSE
Pig             LTPPTTPPHKANNPPRRASKLKSCKTVPPPPKPYRSESTQGNNKSTKKGPEQSE
Rat             LTPPTPPPHKANNPPRRASKLKSCKTVPPPPKPYRSESTQGNNKSTKKGPEQSE
Mouse           LTPPTPPPPHKNPFPKASPLKPSCKTVPPPPKPYRSESTQGNNKSTKKGPEQSE

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Human           LYAQLSKSSVLTGHEERKTKRPSRLFGDDYQCSINSKTEILINISQELQDSRQLENK
Pig             LYAQLSKSSVLTGHEERKTKRPSRLFGDDYQCSINSKTEILINISQELHDSRQLENK
Rat             LYAQLSKSSVLTGHEERKTKRPSRLFGDDYQCSINSKTEILINISQELQDSRQLENK
Mouse           LYAQLSKSSVLTGHEERKTKRPSRLFGDDYQCSINSKTEILINISQELQDSRQLENK

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Human           DVSSDQQQIQCSSTSQDCYLEETLEASKVSPCSTRKQLQERIALNKHGPQSAQV
Pig             DAASDQQQIQCSSTSQDCYLEETLEASKVSPCSTRKQLQERIALNKHGPQSAQV
Rat             DASCDQQQIQCSSTSQDCYLEETLEASKVSPCSTRKQLQERIALNKHGPQSAQV
Mouse           DASCDQQQIQCSSTSQDCYLEETLEASKVSPCSTRKQLQERIALNKHGPQSAQV

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Human           FDDKVDKTEGARKDFDQCSSKLPFLNSGALSMLDGLDQFQSDKLYPDQSYSL
Pig             FDDKVDKTEGARKDFDQCSSKLPFLNSGALSMLDGLDQFQSDKLYPDQSYSL
Rat             FDDKVDKTEGARKDFDQCSSKLPFLNSGALSMLDGLDQFQSDKLYPDQSYSL
Mouse           FDDKVDKTEGARKDFDQCSSKLPFLNSGALSMLDGLDQFQSDKLYPDQSYSL

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Human           FNVSPSSFSNFPCSQRRSISPQMRSSRSHCRSGSPYSRSRSPS
Pig             FDVSPSSFSNFPCSQRRSISPQMRSSRSHCRSGSPYSRSRSPS
Rat             FDVSPSSFSNFPCSQRRSISPQMRSSRSHCRSGSPYSRSRSPS
Mouse           FDVSPSSFSNFPCSQRRSISPQMRSSRSHCRSGSPYSRSRSPS

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Human           RSSS
Pig             RSSS
Rat             RSSS
Mouse           RSSS

Figure 1: Alignment of deduced amino acid sequence of the largest exon (exon 8) of the PGC-1 gene from man, pig, rat and mouse. Sequence identity is marked by asterisks and polymorphic sites by semi colons or dots

Studies in mouse showed positive effect of PGC-1 on the transcriptional activities of PPARγ and thyroid hormone receptor on the UCP1 promoter and on expression of several mitochondrial proteins in brown adipose tissue (Spiegelman et al., 1998). In addition, murine PGC-1 is also expressed in heart, kidney and brain and upon cold exposure in brown adipose tissue and skeletal muscle. Due to the very limited amount of brown adipose tissue in pig, the major function of PGC-1 may be related with regulation of gene expression in skeletal muscle in cold induced thermogenesis.
Figure 2: Sequence polymorphism at nucleotide position 1290 (exon 8) of the *PGC-1* gene in eight pig breeds. The nucleotides at polymorphic site are printed in bold. Transversion A→T causes amino acid substitution (Ser → Cys).

**CONCLUSION**

Due to its crucial role in cold inducible thermogenesis PGC-1 is one of the most important regulators of energy metabolism. The porcine PGC-1 cDNA revealed high degree of nucleotide and amino acid sequence identity with its human, murine and rat counterparts. Also the exon/intron organisation of the genomic sequence seems to be identical with PGC-1 genes of other mammalian species. In spite of the fact that PGC-1 is highly conserved among species, two single nucleotide mutations, from which one is causing amino acid exchange at position 430 were found in Goettingen Mini Pig and Mangalica breed. Further functional studies of PGC-1 co-activating potential in pig are needed.

**REFERENCES**


