Full-length coding sequences and mapping of porcine *ATP6VOE* and *ATP5G1* genes

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Rationale and significance

ATPase, H⁺ transporting, lysosomal 9-kDa, V0 subunit e (*ATP6VOE*) and ATP synthase, H⁺ transporting, mitochondrial F0 complex, subunit c, isoform 1 (*ATP5G1*) are two subunits of the ATP synthase family. Four genes coding for ATPase have been previously mapped in our laboratory (Wang et al., 2003). They play important roles in ATP synthesis, hydrolysis, proton transporting and oxidative phosphorylation (Lu et al., 2002). In this study, we first got the full-length coding sequences and then mapped the two genes to porcine chromosomes using porcine somatic cell hybrid panel (SCHP) and radiation hybrid panel (IMpRH).

Methods and materials

Full-length coding sequences of porcine *ATP6VOE* and *ATP5G1* genes

The cDNA sequences were obtained by 5' and 3' sequencing of a skeletal muscle cDNA library established in our laboratory from porcine embryos. 5' and 3' sequences were assembled into a contig.

Primer design, PCR conditions and sequencing

The porcine cDNA sequences were used to design primers using primer premier 5.0 software. After an initial denaturation step, 5 min at 95°C, PCR was performed for 35 cycles at 94°C for 20 s, 58°C for 20 s and 72°C for 20 s with a final extension at 72°C for 10 min. The products were purified with the Wizard prep PCR purification system (Promega, Madison, WI, USA), and then sequenced. All the primer sequences and their corresponding binding regions, annealing temperature and PCR product sizes are listed in Table 1.

Somatic cell hybrid (SCHP) and radiation hybrid (RH) mapping

PCR reactions were carried out using the primers listed in Table 1 using SCHP or IMpRH DNA samples as templates. DNA from each hybrid was amplified in duplicate in 10-μl reactions containing 25 ng of hybrid DNA, 1× PCR buffer, 3 pmol each primer, 75 μM each dNTP, 0.5 U Taq DNA polymerase. The controls consisted of parental DNA: porcine, hamster and mouse genomic DNA for SCHP and porcine and hamster genomic DNA for IMpRH. A negative control (no DNA) was also added in all cases. The PCR conditions were the same as described above. The PCR products were detected on a 2.0% agarose gel stained with 0.5 μg/ml ethidium bromide. The results were statistically analyzed by the tools provided at <http://www.toulouse.inra.fr/lgc/pig/hybrid.htm> for SCHP (Yerle et al., 1996) and <http://imprh.toulouse.inra.fr/> for IMpRH panel (Yerle et al., 1998).

Results and discussion

We found that the two assembled contigs shared a high percentage of homology with human *ATP6VOE* and *ATP5G1* sequences (82 and 88% respectively) through standard BLAST search of the blastn database (www.ncbi.nlm.nih.gov/blastn/). The cDNA sequences corresponding to full-length coding sequences of porcine *ATP6VOE* (854 bp) and *ATP5G1* (604 bp) genes (GenBank accession numbers: AY633618 and AY633617) were respectively gained by comparing to human *ATP6VOE* and *ATP5G1* mRNA (NM_003945 and GI: NM_005175) in the bl2seq databases using the BLAST algo-

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1  ggt tga cac ttc cgg gtg gca cct aag tga ggc ggc ggg gca ggg
46  gct ggc agc tcc gac ggc aac c  atg gcg tat acc ggc ctc acg
                                     M  A  Y  T  G  L  T
89  gtg cct ctc atc gtg atg agc gtg ttc tgg ggc ttc gtc ggc ttc
    V  P  L  I  V  M  S  V  F  W  G  F  V  G  F
134 ctg gtt ccc tgg ttc atc cct aag ggt ccc aac cgg gga gtc atc
    L  V  P  W  F  I  P  K  G  P  N  R  G  V  I
179 atc acc atg ttg gtg acc tgt tgc gtt tgc tgc tat ctc ttt tgg
    I  T  M  L  V  T  C  S  V  C  C  Y  L  F  W
224 ctg att gca att ctg gcc caa ctc aat cct ctg ttt gga cca cag
    L  I  A  I  L  A  Q  L  N  P  L  F  G  P  Q
69  ttg aaa aat gaa acc atc tgg tac ctc aag tat cat tgg cct tga
    L  K  N  E  T  I  W  Y  L  K  Y  H  W  P  *
314 gga aga aga cct gct cgc cag tgt tca gtc att gag gtc acg aag
359 aga gtt cct cta gat gca aaa tca cct cca tac cca gac ctc ctt
404 cct tga cct gcc tct gct ggc cgt cag atg cct taa acg ttc acg
449 cca cag ttg aat atc tta ttc ctc aac gca gta ttt ttt tcc tgt
494 cgc ctt tct tac act ttg atg aat gat gtg cct ctt tta ctt aaa
539 cgc tct ccg taa aag gtt cat gtg ctc ttc tga gat aga cag tgc
584 tct ttc ttc ggg gaa ata aca tta ctc cct ccg tgg aac ctg tgg
629 att gga aga tgg ctc ctg cct gct cac agc gtg gga atc cgc aga
674 gcg ttt aaa cac tgt aac agg aca gtg aga ccc cag tgg ggc tgt
719 cag tag gag atc ata ttc aga ggg aga atc tgt ctc gcc aga acc
764 ata cca aag tac att ttt agg atg aat tct tat ttc tgc cgt ctc

809 tgg gaa taa att att ttt ctg cat tct ttg gaa aaa aaa aaa aaa

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Fig. 1. cDNA sequences and deduced amino acid sequences of the porcine *ATP6VOE* gene.

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1  aca gct aaa gcc ggg agg tga gtc agt cac ctt gag ccg ggc gag
46  cgc tgt gtt cca agc cgg ggc agc ggg gca gtg aga gtg cag aca
91  gaa aaa atg caa acc acc ggg gcg cta ctc att tct cct gct ctg
    M  Q  T  T  G  A  L  L  I  S  P  A  L
136 ctc cgc tct tgt acc agg ggc ctg atc agg cct gtg tct gcc tcc
    L  R  S  C  T  R  G  L  I  R  P  V  S  A  S
181 ttc ctg agt agg cca gaa atc cca tct gaa cag ctg ccc tgc agc
    F  L  S  R  P  E  I  P  S  E  Q  L  P  C  S
226 agt gtc cca ctc cag gtg gcc agg cgg gag ttc cag gcc agt gtt
    S  V  P  L  Q  V  A  R  R  E  F  Q  A  S  V
271 gtc tcc cgg gac att gac aca gcg gcc aag ttt att ggt gct ggg
    V  S  R  D  I  D  T  A  A  K  F  I  G  A  G
316 gcc gcc aca gtt ggt gtg gct ggt tcc ggg gct ggc att gga aca
    A  A  T  V  G  V  A  G  S  G  A  G  I  G  T
61  gtg ttt ggc agc atg atc att ggc tat gcc agg aac cca tct ctg
    V  F  G  S  M  I  I  G  Y  A  R  N  P  S  L
406 aag cag cag ctc ttc tcc tat gcc att ctg ggc ttt gcc ctg tct
    K  Q  Q  L  F  S  Y  A  I  L  G  F  A  L  S
51  gag gcc atg ggg ctc ttt tgt ttg atg gtc gcc ttc ctt atc ctc
    E  A  M  G  L  F  C  L  M  V  A  F  L  I  L
96  ttc gcc atg tga ggc ttc gtg gag gtc acc tac ccg tcc ctg ctg
    F  A  M  *
541 ctt cga ctc caa ggc cat gcc cgg tgc tgg att gtg ctg agc ttg
586 acc att aaa cac aac gtt t

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Fig. 2. cDNA sequences and deduced amino acid sequences of the porcine *ATP5G1* gene.

Table 1. The primers used for isolating and mapping the porcine *ATP6VOE* and *ATP5G1* genes

Gene	GeneBank Porcine Acc No.	Sequence similarity	Primer sequences (5'-3')	Binding region	Size (bp)	PCR (T _m)
<i>ATP6VOE</i>	AY633618	75%	PL :CCATACCCAGACCTCCTT PR:AGCCATCTTCCAATCCAC	3'UTR 3'UTR	257	60
<i>ATP5G1</i>	AY633619	96%	PL:ACAGTTGGTGTGGCTGGTTC PR:AAGAGCTGCTGCTTCAGAGA	Exon 4 Exon 5	417	58

Table 2. Chromosomal assignments of the two porcine genes

Gene symbol	Human Acc.No.	Human cytogenetic location	Porcine RH mapping results					SCHP assignment result (error risk <0.5)		
			Retention (%)	Closest markers	Dist (cR)	Lod score	Porcine cytogenetic position*	Regional assignment	Probability	Correlation
<i>ATP6VOE</i>	NM_004932	5q35.2	67	SW977	85	3.49	16q	16q21	0.8180	0.9282
<i>ATP5G1</i>	NM_000215	17q21.33	17	SS04H11	40	9.55	12p13→p11	12p(2/3 p13)→p11	0.8901	1.0000

* This position is deduced from the closest marker available on the cytogenetic map.

rithm (<http://www.ncbi.nlm.nih.gov/blast/>). We found ORFs and used ORF finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) to deduce the amino acid sequences. The cDNA sequences and the primary protein structures deduced for *ATP6VOE* and *ATP5G1* genes are shown in Figs. 1 and 2. Computer analysis indicated that the porcine *ATP6VOE* gene encoded 81 amino acids with a calculated molecular mass of 9.34 kDa and theoretical pI of 8.96. The *ATP5G1* gene encoded 136 amino acids with a calculated molecular mass of 14.22 kDa and theoretical pI of 9.41.

We localized *ATP6VOE* to SSC16q21 using SCHP (statistical scores in Table 2). We then mapped it on the porcine RH map using IMpRH. The two-point analysis revealed close linkage to SW977 but the LOD score value was weak (LOD score 3.49). The SCHP results indicate that *ATP5G1* is located at SSC12p13→p11 (statistical scores in the Table 2). The IMpRH panel was then employed to refine this mapping. We found that the nearest marker linked to *ATP5G1* gene is SS04H11 (distance 40 cR; LOD = 9.55), which is in agreement with the cytogenetic position defined with SCHP.

In mouse (Kandil et al., 1997) this gene is tightly linked to *Psmel* and *Psme2* which have been shown to be highly expressed in lung, spleen and liver tissue in pig (Wang et al., 2004) suggesting that *ATP5G1* might be related to animal immunity.

The mapping results of *ATP6VOE* (16q21) and *ATP5G1* (12p13→p11) are in agreement with previous comparative mapping results as human chromosomes 5 and 17 share syntenic groups with porcine chromosomes 16 and 12 respectively (seen in Table 2).

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