Assignment¹ of *UCK2*, *ATF3* and *RGS18* from human chromosome 1 to porcine chromosomes 4, 9 and 10 with somatic and radiation hybrid panels

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¹ To our knowledge this is the first time these genes have been mapped in swine.

Received 15 July 2005; accepted for publication by M. Schmid, 25 July 2005.

Rationale and significance

Comparative maps are useful tools in the discovery of positional candidate genes for Quantitative Trait Loci (QTL). The comparative maps document the evolutionary breaks in chromosomes occurring between the pig and the human from a common ancestor and therefore permit recognition of conserved blocks of genes. Increasing the density of markers on the comparative map between pigs and humans improves our ability to find positional candidate genes and refine the boundaries of evolutionary break points. Human chromosome 1 has regions of conserved synteny relative to porcine chromosomes 4, 6, 9, 10 and 14 (Rettenberger et al., 1995; Fronicke et al., 1996; Goureau et al., 1996). Here we report our mapping results of *ATF3* (activating transcription factor 3), *RGS18* (regulator of G-protein signalling 18) and *UCK2* (uridine-cytidine kinase 2) from human chromosome 1 to porcine chromosomes.

Materials and methods

Primer design

Human mRNA sequences from *UCK2*, *ATF3* and *RGS18* were used in a nucleotide Basic Local Alignment Search Tool (BLASTn, Version 2, 2.3) (<http://www.ncbi.nlm.nih.gov/BLAST/>) (Altschul et al., 1990) of the GenBank (<http://www.ncbi.nlm.nih.gov>) EST database to identify matching ESTs from pigs. Human genome sequence was used to predict the position and approximate size of the introns. Primers were designed on exons flanking introns. The primers were designed using the Primer3 program (Rozen and Skaletsky, 2000) in ANGIS's Biomanager (www.angis.org.au).

Physical mapping

The INRA-SCHP (Yerle et al., 1996) and the 90 clone IMPRH panel (Yerle et al., 1998) were used to regionally assign specific genes or markers to one of the 18 porcine autosomes or the X chromosome, and to more accurately physically map the genes. The PCR reactions were carried out in a 25- μ l volume containing 20 ng (2 μ l) of DNA from the hybrid clones, 10 $\times$ Taq DNA polymerase buffer, 0.8 μ M of each primer, 1.5 mM MgCl₂ and 100 μ M dNTPs covered with two drops of paraffin oil. After denaturation at 95°C for 10 min and then holding at 80°C, 1 U of Taq polymerase was added. A touchdown program was used (44 cycles 95°C for 40 s, 63–55°C for 60 s, 72°C for 60 s, 1 cycle 72°C 20 min). Products were separated using a 2% agarose gel, and were visualised by ethidium bromide fluorescence under ultraviolet light. PCR product for each hybrid was scored either as present or absent. Ambiguous data points were re-amplified. The INRA-SCHP chromosomal and regional assignments were made using software available on the WWW INRA server (<http://www.toulouse.inra.fr/lgc/pig/hybrid.htm>). For the IMPRH results were analysed using the mapping tool developed by Milan et al. (2000) (<http://www.toulouse.inra.fr/lgc/pig/RH/Menuchr.htm>).

When a rodent product of identical size to the porcine product was amplified, restriction enzyme digestion was tested to attempt to discriminate between the products. The restriction enzyme mix of 30 μ l consisted of 7 μ l of PCR product, 0.5 μ l of enzyme, 3 μ l of the appropriate buffer and 19.5 μ l of water. This mix was incubated at 37°C for 2 h. The reaction was ended when the samples were placed at 4°C and/or 4 μ l of loading buffer was added.

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Table 1. Primers designed, and distinguishing enzymes

Locus	Porcine EST (Human mRNA) GenBank Acc. No.	Primer sequences (5'–3') (Forward and Reverse)	Estimated size of PCR product
<i>ATF3</i> (activating transcription factor 3)	BP144750 (NM_001674)	CCGATGAAGGTTGAGCATGTA GCCCCTGAAGAAGATGAAAG	900 ^a
<i>RGS18</i> (regulator of G-protein signalling 18)	CF181629 (NM_130782)	CTCAGGTTTCTGCACAAGAAGA TTTTCAAGTTAATACATGGTTCAGG	500
<i>UCK2</i> (uridine-cytidine kinase 2)	CK464222 (NM_012474)	TCCTTCGGTGATTTCTTTGAG CCTGGGACAGAATGAGGTAGA	900 ^b

^a *Nci*I digestion required to distinguish porcine and rodent PCR products.
^b *Hae*III digestion required to distinguish porcine and rodent PCR products.

Table 2. Gene mapping locations from INRA-SCHP and IMpRH

Locus	Human location	Localisation in pigs on INRA-SCHP			Localisation on ImPRH			
		Porcine location	Regional probability	Correlation coefficient	2pt nearest marker	Distance (cR)	2pt LOD score	Retention frequency
<i>ATF3</i>	1q32.2	9q12→(1/3q21)	0.9475	0.6614	SW1651	66	4.63	0.27
<i>RGS18</i>	1q31.2	10p16→p11	0.9999	0.779	S0038	32	9.69	0.26
<i>UCK2</i>	1p23	4q15→q16	0.8782	0.922	SW1364	71	4.1	0.24

Results

The three primers designed produced a porcine PCR product, but only one was porcine specific. Primer sequences and distinguishing restriction enzymes are presented in Table 1.

The vector results for *ATF3* was + + + + + + + + + +
+ - + + + + - + + - - - - for the INRA-SCHP and 00
0110111010000111001001001100000000010000000000
0001000110000001010001 000000010100010001 for the
IMpRH. The result for *RGS18* was + - + - + - + + + - - -
- - + - + + + + - - - + - - for the INRA-SCHP and 0001
001010000100000101000100000010110100100000100
00000000000010000100000110100010010111100 for the
IMpRH. The result for *UCKK* was + - - - + - + - - - + -
- - - + - - - - - + + for the INRA-SCHP and 00000000
000000000100010000001100100 100010010010000000101
000101000000100011010001010010110 for the IMpRH.

Our results from both the INRA-SCHP and ImpRH indicated that *ATF3* was located on SSC9, *UCK2* on SSC4 and *RGS18* on chromosome 10 (Table 2). These results are in accordance with comparative mapping data (Rettenberger et al., 1995; Fronicke et al., 1996; Goureau et al., 1996).

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