Radiation hybrid mapping of the pig *SMNDC1*, *ARPP-19* and *PRO2730* genes to SSC14, SSC1 and SSC13 respectively

H.Y. Ren^{a, b} Z.M. Zhu^b H.L. Wang^{a, b} H. Wang^{a, b} S.L. Yang^b K. Li^b

^aLaboratory of Molecular Biology and Animal Breeding, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan; ^bDepartment of Gene and Cell Engineering, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing (PR China)

Received 8 July 2005; accepted in revised form for publication by T. Shows, 1 September 2005.

Rationale and significance

Pig is an important economic animal; it supplies food for our daily life, so pork quality and quantity are of interest to people. Several genes are related to pork quality, especially those expressed in early stages of muscle development. In this study, three genes *SMNDC1* (Survival motor neuron domain containing 1), *ARPP-19* (19-kDa cAMP-regulated phosphoprotein) and *PRO2730* (hypothetical protein PRO2730) were screened from the cDNA library constructed from porcine 55-day fetus skeletal muscle. We isolated these three gene EST fragments and mapped them to SSC14, SSC1 and SSC13 respectively, using the porcine radiation hybrid panel IMpRH (Yerle et al., 1998).

SMNDC1 is necessary for spliceosome assembly, and may play an important role in the metabolism of specific subclasses of mRNA. Recent studies suggest that this gene has a close

relation to regulating programmed cell death in the nervous system (Iwahashi et al., 1997). In humans, mutations in this gene are responsible for autosomal recessive proximal spinal muscular atrophy (SMA) (Talbot et al., 1998). *ARPP-19* protein is a substrate for cAMP-dependent protein kinase of *Mr* = 19,000. Its homologue *ARPP-16*, which differs from *ARPP-19* only by a shortage of 16 N-terminal amino acids may play an important role in regulating postnatal skeletal muscle growth in pig (Janzen et al., 2000). *PRO2730* encodes a hypothetical protein PRO2730 whose biological function has not yet been reported.

Materials and methods

cDNA clones isolated from the cDNA library were employed to screen the porcine expressed sequence tags (ESTs) in EST-others database through standard BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). The porcine ESTs showing at least 85% sequence identity with the cDNA sequence were selected and assembled into an EST-contig used for primer design (Table 1).

Radiation hybrid mapping was performed using the INRA-University of Minnesota radiation panel (IMpRH) (Yerle et al., 1998). The panel of 118 hybrid clones was screened by PCR analysis with a set of primers. Polymerase chain reaction using primers was performed in 10- μ l reactions consisting of 25 ng hybrid DNA, 1 $\times$ PCR buffer, 3 pmol of each primer, 75 μ M of each dNTP, 1.5 mM MgCl₂ and 0.5 U Taq DNA polymerase. Amplification conditions were 95°C for 5 min, followed by 35 cycles of 94°C (20 s), 58–60°C (20 s) and 72°C (20 s), with a final extension for 5 min at 72°C. The amplified PCR products were detected in 2.0% agarose gels stained with 0.5 μ g/ml ethidium bromide. The PCR results were analyzed with the online IMpRH mapping tool (Milan et al., 2000) (<http://imprh.toulouse.inra.fr/>).

Supported by the Key Project of National Natural Science Foundation of China (30330440), the Key Project of National Basic Research and Developmental Plan of China (G2000016103), National High Science and Technology Foundation of China (2004AA222170) and National Natural Science Foundation of China (30400311). We are grateful to Dr. Martine Yerle for providing the RH panel.

Corresponding author: Kui Li, Department of Gene and Cell Engineering Institute of Animal Science, Chinese Academy of Agricultural Sciences 2 Yuanmingyuan West Road, Beijing, 100094 (PR China)
telephone: +86 10 62833312; fax: +86 10 62899982
e-mail: kuili@iascaas.net.cn and likuihau@yahoo.com

Table 1. Primer sequences and binding region of the three porcine genes

Gene symbol	GenBank Acc. No. (pig)	Primer name	Primer sequence (5'→3')	Binding region	Tm	Size (bp)
<i>SMNDC1</i> ^a	DQ098952	PL1	ACATTCGTGGAAAGTAGGA	Exon 3	58	1,098
		PR1	ATAGCCAGCAAAGGTGATT	Exon 4		
		PL2	TGTATGGCAATCTGGAGT	Exon 3		
		PR2	GCTGTAAGCAGGTGAGTAA	Intron 3		
<i>PRO2730</i>	DQ098953	PL	GCAGGGAAGGTATCAGTGTGTA	5'UTR	58	233
<i>ARPP-19</i>	DQ098954	PR	TGAAAAGGAGGGAGGAGAG	3'UTR	60	367
		PL	TGCAGGTAGCGGTAGTGTGT			
		PR	GCCCCATCTATTTGTGTTTG			

^a The primer pair PL1–PR1 spanning exon 3 and exon 4 was used to isolate the *SMNDC1* genomic DNA fragment. The primers PL2–PR2 spanning exon 3 and intron 3 were used for physical mapping.

Table 2. Physical assignment of the three porcine genes

Gene	GenBank Acc. No. (human)	Sequence similarity to human	Location in human	Location in pig	Retention (%)	Closest marker	Distance (cR)	LOD score
<i>SMNDC1</i>	NM_005871	96%	10q23	14q	23	S0072	68	5.6
<i>PRO2730</i>	NM_025222	86%	3p21.2	13q21→q22	21	SSC24F05	41	9.5
<i>ARPP-19</i>	NM_006628	85%	15q21.2	1q21→q27	33	SW1846	61	6.66

Results

The mapping results are as follows: the porcine *SMNDC1* gene was localized to SSC14 with a LOD score of 5.6, closely linked to S0072; *ARPP-19* was localized to SSC1 with a LOD score of 6.66, closely linked to SW1846; *PRO2730* was localized to SSC13 with a LOD score of 9.5, closely linked to SSC24F05 (Table 2). Comparing the human mRNA sequences with the coding sequences of the three genes using BLAST2 software provided by the NCBI server (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>), we found that the three assembled contigs shared a high percentage of homology with those of the human genes. The results revealed that the expected porcine gene sequences were isolated.

References

- Iwahashi H, Eguchi Y, Yasuhara N, Hanafusa T, Matsuzawa Y, Tsujimoto Y: Synergistic anti-apoptotic activity between Bcl-2 and SMN implicated in spinal muscular atrophy. *Nature* 390:413–417 (1997).
- Janzen MA, Kuhlers DL, Jungst SB, Louis CF: ARPP-16 mRNA is up-regulated in the longissimus muscle of pig possessing an elevated growth rate. *Anim Sci* 78:1475–1484 (2000).
- Milan D, Hawken R, Cabau C, Leroux S, Genet C, Lahbib Y, Tosser G, Robic A, Hatey F, Alexander L, Beattie C, Schook L, Yerle M, Gellin J: IMpRH server: an RH panel mapping server available on the web. *Bioinformatics* 16:558–559 (2000).
- Talbot K, Miguel-Aliaga I, Mohaghegh P, Ponting CP, Davies KE: Characterization of a gene encoding Survival Motor Neuron (SMN)-related protein, a constituent of the spliceosome complex. *Hum Mol Genet* 7:2149–2156 (1998).
- Yerle M, Pinton P, Robic A, Alfonso A, Palvadeau Y, Delcros C, Hawken R, Alexander L, Beattie C, Schook L, Milan D, Gellin J: Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenet Cell Genet* 82:182–188 (1998).