Assignments of GRB7, GCN5L2, COL1A1 and TBCD to pig chromosome 12 by radiation hybrid mapping

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Rationale and significance

Comparative mapping with model species like human is an important tool for livestock as it might speed up the search for candidate genes in QTL (Quantitative Trait Loci) regions through the use of information derived from mapped genes in humans. It also gives insight on mammalian genome evolution. Bidirectional chromosome painting between human and pig has shown that pig chromosome 12 (SSC12) is homologous to human chromosome 17 (HSA17) (Goureau et al., 1996). It is reported that 1,689 genes have been mapped on human chromosome 17 (GenBank human genome resources: <http://www.ncbi.nlm.nih.gov/mapview/maps.cgi>) while only 37 and 31 genes or ESTs have been located on the porcine cytogenetic and RH maps, respectively. Nevertheless, the number of porcine ESTs available in public databases is increasing rapidly, constituting a valuable resource to compare the porcine genome with the human draft genome sequences. In this study, four genes originally mapped to HSA17 were selected. The genes were growth factor receptor-bound protein 7 (GRB7), general control of amino-acid synthesis 5-like 2 (yeast) (GCN5L2), collagen type I alpha 1 (COL1A1) and tubulin-specific chaperone d

(TBCD). We isolated their porcine fragments and mapped them to porcine chromosome 12 using the porcine radiation hybrid panel IMpRH (Yerle et al., 1998).

Methods and materials

Isolation and sequencing of the porcine four gene fragments

To clone the porcine fragment corresponding to each gene, human cDNA sequences (GenBank accession numbers are NM005993, NM000088, NM021078 and NM005310, respectively) were used to identify pig ESTs through standard BLAST (www.ncbi.nlm.nih.gov/blast/) analysis of the EST-otors database. Pig ESTs that have more than 80 % similarity with human corresponding genes were used to select porcine specific primers (Table 1). The polymerase chain reaction was performed in 20-μl reactions containing 50 ng porcine genomic DNA, 1× PCR buffer, 6 pmol of each primer, 75 μM of each dNTP, 1 U Taq DNA polymerase (Promega, Madison, WI, USA). The PCR was carried out at 95 °C for 5 min, followed by 35 cycles of 94 °C for 40 s, 60–64 °C for 30 s and 72 °C for 30 s and a final extension of 72 °C for 5 min. The PCR products were purified with the Wizard prep PCR purification system (Promega, Madison, WI, USA) and were cloned into the pGEM-T easy vector and were sequenced commercially. The sequenced fragments of these four genes were then used in the BLAST searches against the “nr” databases at the NCBI to ensure they were the expected porcine sequences.

Radiation hybrid (RH) mapping

The same primer pair was used to amplify TBCD gene on IMpRH panel (Yerle et al. 1998), while other forward primers PF2, PF4 and PF6 were selected from the intron region of COL1A1, GCN5L2 and GRB7, respectively, to avoid co-amplification of hamster sequences (see also Table 1). Each hybrid clone was amplified in a 10-μl reaction containing 25 ng of hybrid DNA, 1× PCR buffer, 3 pmol of each primer, 75 μM of each dNTP, 0.5 U Taq DNA polymerase (Promega) under the same PCR profile mentioned above. Controls consisted of hamster genomic DNA, porcine genomic DNA and a negative control reaction containing no DNA. The PCR products for each gene were typed on a 2.0 % agarose gel stained with 0.5 μg/ml ethidium bromide. For each gene, the panel was tested twice and the typing results were analyzed by the tools provided by the web server (<http://imprh.toulouse.inra.fr/>; Milan et al., 2000). The primer sequences, GenBank accession numbers of the porcine genomic sequences, annealing temperatures and PCR product sizes for the four genes are shown in Table 1.

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Table 1. The primer sequences, annealing temperatures and size of PCR products of the four genes

Gene symbol	GenBank Acc. No. (pig)	Sequence similarity ^d	Primer sequence (5'→3')	Primer binding region	T _m value (°C)	Size (bp)
TBCD	AY350908	82%	PF-AGCAGGCGAGCGAGAAGATT PR-GTGCAGCCCAGTTCACGGAG	Exon 28 Exon 29	60	240
COL1A1 ^a	AY350905	93%	PF1-GCAACCTGGACGCCATCAAA PF2-CTGGGGTATCTTCAGTGGGACG PR-TCTGGGAAGCCTCAGTGGACAT	Exon 49 Intron 49 Exon 50	64	529 338
GCN5L2 ^b	AY350906	93%	PF3-CCGCTGGGCAAGGAGAAAGG PF4-CTGAAAGGCACGCCCTGTCC PR-AAGCGAAACTGGAAATCCTGCG	Exon 15 Intron 17 Exon 18	63	1034 576
GRB7 ^c	AY350907	82%	PF5-GTCTCATCTCAGCAGCTCC PF6-CTCACCGTCACTTCTGTCC PR-GAGCATCTCGCACACGTAG	Exon 2 Intron 2 Exon 4	63	626 425

^a The primer pair PF1 – PR was used to isolate the 529-bp length genomic DNA fragment of the COL1A1 gene. The primer pair PF2 – PR was used for RH mapping.

^b The primer pair PF3 – PR was used to isolate the 1034-bp length genomic DNA fragment of the GCN5L2 gene. The primer pair PF4 – PR was used for RH mapping.

^c The primer pair PF5 – PR was used to isolate the 626-bp length genomic DNA fragment of the GRB7 gene genomic DNA fragment. The primer pair PF6 – PR was used for RH mapping.

^d Similarity scores come from the predicted coding regions.

Table 2. RH mapping results of the four porcine genes

Gene symbol	GenBank Acc. No. (human)	Human Cytogenetic location ^a	Porcine RH mapping result				
			Retention (%)	Lod score	Closed Marker	Chr	Dist (cR)
TBCD	NM005993	17q25.3	26	4.96	SW2490	12p15	73
COL1A1	NM000088	17q21.31–q22	37	21.28	SWR390	12p13–p11	14
GCN5L2	NM021078	17q21	26	10.03	SW943	12p13–p11	42
GRB7	NM005310	17q21.2	23	15.25	SW943	12p13–p11	23

^a From <http://www.ncbi.nlm.nih.gov/UniGene/>

Results and discussion

As shown in Table 1, the porcine fragments of genes TBCD, COL1A1, GCN5L2 and GRB7 were all matched to the corresponding human mRNA sequences with similarities from 82 to 93%, which confirmed the expected porcine sequences. All four genes were assigned to the p arm of porcine chromosome 12 under the minimum threshold of 4.8. The cytogenetic positions of these genes were identified based on the cytogenetic positions of the closest linked markers when known or estimated using the proximal markers previously mapped (Hawken et al., 1999). The mapping results are presented in Table 2, which confirmed the previous data on the chromosomal homology between pig and human through chromosome painting. It was also evidenced by two other genes: ferredoxin reductase (FDXR) and rod cGMP-phosphodiesterase γ -subunit (PDE6G), which were mapped to the same region (Yu et al., 2002, 2003). The studies of quantitative trait loci (QTL) in pigs revealed that genetic factors controlling economic traits such as carcass and meat quality and reproduction were located on pig chromosome 12 (SSC12) (Cassady et al., 2001; Paszek et al., 2001). Our mapping of the four genes provides additional reference loci that increases the comparative data between HSA17 and SSC12 and will contribute to the construction of the future

high density RH map of SSC12 that will be essential for the fine mapping of QTL on this chromosome.

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