

Addition of Thirteen Genes to the Porcine Comparative Gene Map Reveals New Regions of Conserved Synteny

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Summary and Implications

Thirteen genes were mapped to the porcine genome by using either linkage mapping of the PiGMaP families (eight genes) or typing of a porcine somatic cell hybrid panel (12 genes). The genes were chosen from interesting locations in the human genome. The physical gene assignments to pig chromosomes (SSC) with corresponding human chromosome (HSA) locations include the following: *FGF7* (HSA15), *MADH4* (HSA18), and *MC4R* (HSA18) to SSC1, *RXRβ* (HSA6), and *SSTR1* (HSA14) to SSC7, *UCP1* (HSA4) to SSC8, *PGR* (HSA11) to SSC9, *TTN* (HSA2) and *ANTI* (HSA4) to SSC15, *GRIA1* (HSA5) to SSC16, *AR* (HSA-X), and *GRIA3* (HSA-X) to SSC-X. Additionally, *CD59* (HSA11) was linkage mapped to SSC2. The majority of the assignments confirm results from bidirectional chromosome painting (4). A rearrangement in gene order was detected within the region of correspondence between SSC1 and HSA15. Two assignments were made that were not expected from the painting results (*MC4R* and *GRIA1*) and one assignment of a gene from a region where the painting study was not informative (*ANTI*).

Introduction

The porcine linkage maps now include approximately 1,550 anonymous markers and 250 genes. This density of genes is usually too low to enable the positional candidate gene approach for identification of quantitative trait loci (QTL [2]). Instead, the choice of candidate genes currently relies on information from the human and mouse genome projects, which requires well-developed comparative maps.

Between the pig and human genomes, bidirectional painting (Zoo-FISH) has produced valuable information including 37 chromosomal regions of homology (3, 4). Mapping of single genes, selected for their human genome location, is necessary to define the correspondence of unpainted regions and reveal possibly rearranged gene orders within regions of conserved synteny. In this study we have improved the resolution of the pig-human comparative map by adding 13 genes to the porcine map.

Materials and Methods

The genes investigated are in Table 1. Physical gene mapping was carried out using amplification by the polymerase chain reaction (PCR) on a pig-rodent somatic cell hybrid panel (12). In a few cases restriction fragment analysis was necessary to distinguish between pig and mouse-hamster panel products of equal or similar lengths.

Porcine polymorphisms were revealed using direct sequencing and/or restriction fragment length analysis of PCR products, or both, from individuals representing different breeds. Single nucleotide polymorphisms were genotyped as PCR-restriction fragment length polymorphisms (RFLPs) on agarose gels whereas microsatellites were analyzed using native acrylamide gels. The PiGMaP reference families (1), the USDA-MARC reference families (10) and CRI-MAP (v. 2.4 [5]) were used for linkage analyses.

Results and Discussion

Twelve genes were assigned to pig chromosome regions by using the SCHP. Seven of these assignments were confirmed by linkage analysis by using polymorphisms identified in this study. Additionally, *CD59* was mapped by linkage to the middle region of pig chromosome 2 (SSC2). The types of polymorphisms found are given in Table 1, whereas mapping results are shown in Table 2. The distribution of the mapped genes over eight pig chromosomes also are illustrated in Figure 1a-g. Most results were expected from chromosome painting data (3, 4), but the locations of *MC4R* and *GRIA1* were unexpected.

A rearrangement within the region of SSC1 - HSA15 correspondence was indicated by the close proximity of *FGF7*, *IGF1R* (6, 7) and *MEF2A* (8) in the middle of the SSC1 region painted by human chromosome (HSA)15 (Figure 1a). This may be a pig-specific organization as further indicated by the mouse map where *FGF7* and *IGF1R/MEF2A* are located on MMU7 and 2, respectively.

The HSA18q11-q12 should correspond to SSC6q27-q31, and the remainder of HSA18q should correspond to SSC1q12-q14 although SSC1 did not produce a painting signal anywhere in the human genome (4). The *MC4R* mapping in this study, however, indicates that the distal half of HSA18 also shares homology with middle region of SSC1q (Figure 1a).

The mapping of *GRI1* to SSC16 was unexpected. The *GRI1* is a distal marker on HSA5q. Painting results show correspondence between HSA5q and SSC2 and also between the complete SSC16 and HSA5p plus proximal HSA5q. Thus, *GRI1* may be a part of a rearrangement in the HSA5 - SSC16 homology that has not been resolved by painting studies (Figure 1f).

Large parts of HSA4 correspond to SSC8; *UCP1* was mapped to SSC8q21 as one additional gene assignment in this region of conserved synteny (Figure 1c). HSA4q32-q35, however, was not painted by any porcine chromosome (4). One gene from this region, *MTNR1A* from HSA4q35, was mapped to SSC17 (9), but in the current study *ANTI*, a second gene from HSA4q35, mapped to SSC15. This is consistent with a recent mapping of the *IRF2* (interferon regulatory factor 2) gene to SSC15 (HSA4q35 [11]), which shows that sequences present on HSA4q35 are divided onto at least two different pig chromosomes. The split between *ANTI* and *MTNR1A* to different pig chromosomes contrasts to the situation in cattle and mouse where *ANTI* and *MTNR1A* are syntenic.

Conclusions

The porcine gene map was expanded with 13 genes; 12 genes were mapped physically using a somatic cell hybrid panel and eight genes were mapped by linkage.

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Ten assignments corroborate chromosome painting studies between the human and porcine genome.

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Three gene mapping results indicate new regions of conserved synteny or rearranged correspondence between human and pig chromosomes, which have not been predicted by published Zoo-FISH painting studies.

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The unpainted telomeric region HSA4q35 contains segments of correspondence to at least two pig chromosomes (SSC15 and SSC17).

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References

1. Archibald, A.L., Haley, C. S., Brown, J.F., Couperwhite, S., McQueen, H.A., Nicholson, D., , Coppieters, W., van de Weghe, A., Sratil, A., Winterø, A.K., Fredholm, M., Larsen, N.J., Nielsen, V.H., Milan, D., Woloszyn, N., Robic, A., Dalens, M., Riquet, J., Gellin, J., Caritez, J.-C.C., Burgaud, G., Ollivier, L., Bidanel, J.-P., Vaiman, M., Renard, C., Geldermann, H., Davoli, R., Ruyter, D., Verstege, E.J.M., Groenen, M.A.M., Davies, W., Høyheim, B., Keiserud, A., Andersson, L., Ellegren, H., Johansson, M., Marklund, L., Miller, J.R., Anderson Dear, D.V., Signer, E., Jeffreys, A.J., Moran, C., Le Tissier, P., Muladno, Rothschild, M.F., Tuggle, C.K., Vaske, D., Helm, J., Liu, H.-C., Rahman, A., Yu, T.-P., Larson, R.G., and Schmitz, C.B.. (1995). The PiGMap consortium linkage map of the domestic pig (*Sus scrofa domestica*). *Mammalian Genome* 6; 157–175.
2. Ballabio, A. (1993). The rise and fall of positional cloning. *Nature Genetics* 3; 277–279.
3. Frönicke, L. Chowdhary, B.P., Scherthan, H., and Gustavson, I. (1996). A comparative map of the porcine and human genomes demonstrates ZOO-FISH and gene mapping-based chromosomal homologies. *Mammalian Genome* 7; 285–290.
4. Goureau, A. Yerle, M., Schmitz, A., Riquet, J., Milan, D., Pinton, P., Frelat, G., and Gellin, J. (1996). Human and porcine correspondence of chromosome segments using bidirectional painting. *Genomics* 36; 252–262.
5. Green, P., Falls, K., and Crooks, S. (1990). Documentation for CRI-MAP, version 2.4. (St. Louis, MO: Washington University School of Medicine).
6. Hu, Z., Rohrer, G.A., Stone, R.T., Rutherford, M., Osinski, M.A., Pampusch, M.S., Murtaugh, M.P., Brown, D.R., and Beattie, C.W. (1997). Linkage assignment of eleven genes to the porcine genome. *Mammalian Genome* 8; 559–563.
7. Lahbib-Mansais, Y., Yerle, M., and Gellin, J (1995) Localization of the *IGF1R* and *EDN* genes to pig

chromosomes 1 and 7 by in situ hybridization. *Cytogenet. Cell Genet.* 71; 225–227.

8. Larsen, N.J., Kenealy, S., Tuggle C.K., Rothschild, M. F., and Yerle, M. (1997) Mapping five new candidate genes in the pig. Iowa State University 1997 Swine Research Report, ASL-R1484

9. Messer, L.A., Wang, L., Tuggle, C.K., Yerle, M., Chardon, P., Pomp, D., Womack, J.E., Barendse, W., Crawford, A., Notter, D.R., and Rothschild, M.F. (1997). Mapping the melatonin receptor 1a (*MTNR1A*) gene in pigs, sheep, and cattle. *Mammalian Genome* 8; 368–370.

Rohrer, G.A., Alexander LJ, Hu Z, Smith TP, Keele JW, and Beattie CW (1996). A comprehensive map of the porcine genome. *Genome Research* 6; 371–391.

10. Winterø, A.K., Jørgensen, C.B., Robic, A., Yerle, M., and Fredholm, M. (1998). Improvement of the porcine transcription map: localization of 33 genes, of which 24 are orthologous. *Mammalian Genome* 9; 366–372.

12. Yerle, M., Echard, G., Robic, A., Mairal, A., Dubut-Fontana, C., Riquet, J., Pinton, P., Milan, D., Lahbib-Mansais, Y., and Gellin, J. (1996). A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. *Cytogenet. and Cell Genet.* 73; 194–202.

Table 1. Genes and mapping methods included in this study.

Gene abbreviation	Gene name	Mapping methods ^a	Type of polymorphism ^b
<i>ANK1</i>	Adenine Nucleotide Translocase 1	Linkage/SCHP	STR
<i>AR</i>	Androgen Receptor	Linkage/SCHP	STR
<i>CD59</i>	Complement Regulatory Protein 59	Linkage	<i>HincII</i> ^c
<i>FGF7</i>	Fibroblast Growth Factor 7	Linkage/SCHP	<i>AluI</i> ^c
<i>GRIA1</i>	Glutamate Receptor, Ionotropic, AMPA 1	SCHP	—
<i>GRIA3</i>	Glutamate Receptor, Ionotropic, AMPA 3	SCHP	—
<i>MADH4</i>	Pancreatic Carcinoma Gene (DPC4)	SCHP	—
<i>MC4R</i>	Melanocortin-4 Receptor	Linkage/SCHP	<i>TaqI</i> ^c
<i>PGR</i>	Progesterone Receptor	SCHP	—
<i>RXRβ</i>	Retinoid X Receptor Beta	Linkage/SCHP	<i>BglI</i> ^c
<i>SSTR1</i>	Somatostatin Receptor 1	SCHP	—
<i>TTN</i>	Titin	Linkage/SCHP	<i>NlaIV</i> ^c
<i>UCP1</i>	Uncoupling Protein 1	Linkage/SCHP	STR

^a SCHP - somatic cell hybrid panel;

^b STR - short tandem repeat;

^c Restriction endonuclease used for PCR-RFLP analysis;

Table 2. Summary of chromosomal assignments for the genes mapped in this study and genes for which assignments have been published. Genes are ordered by their chromosomal locations in the pig genome.

Gene	Pig: expected by painting ^a	Pig: physical	Pig: by linkage	Linked to published loci ^b	Distance (cM)	LOD Scores	Human chromosome ^c	Telomeric in humans
FGF7	1	1q11-q17	1	S0122	9	14.12	15q	No
MC4R	17	1q22-q27	1	S0082	5	17.76	18q21-q22	No
MADH4	1	1q11-q17	—	—	—	—	18q21.1	No
CD59	27	—	2	FSHB	3	11.53	11p13	No
RXRβ	7	7-cen	7	TWFB	2	28.49	6p21.3	No
SSTR1	7	7q12-q23, q26	—	—	—	—	14q13	No
UCP1	8	8q21	8	S0089	1	22.73	4q31	No
FGR	9	9p13-p11	—	—	—	—	11q14-qter	No
ANT1	87	15q15-q22	15	S0149	10	13.89	4q35	Yes
TTN	15	15q23-q26	15	DPP4	0	6.02	2q31	Yes
GRIA1	2	16q14, q22-23	—	—	—	—	5q33	No
GRIA3	X	Xq22	—	—	—	—	Xq25-q26	No
AR	X	X	X	SW1861	8	28.08	Xq11.1-q11.3	No

^a Bidirectional painting of human and porcine chromosomes (4). Question-mark means that this region was not painted in chromosomes from one or both species.

^b The map positions of the loci are from published data (1).

^c Human chromosome locations are from genome database (gdb).

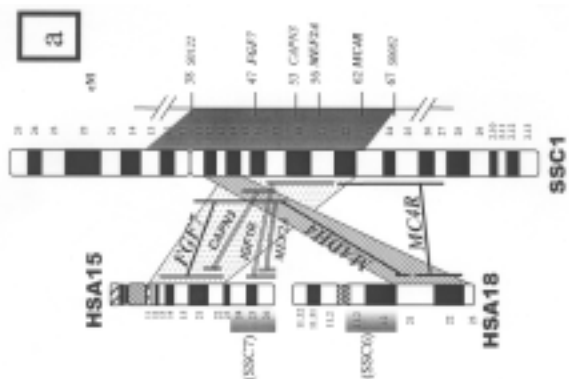


Figure 1 (a-g)
 Schematic diagram showing gene assignments distributed over eight porcine chromosomes. Dotted shades represent pig-human homology region based on chromosome painting. Gradient shades represent approximate correspondence between the porcine physical gene map and the porcine linkage map. Solid vertical bars indicate physical map localizations obtained in this study. Double lines and smaller font has been used for the previously mapped *IGFR1* (6, 7), *IRF2* (11), *CAPN3*, *MEF2A* and *TSHR* (8) genes, which has been included for clarity.

