High-Resolution Radiation Hybrid Mapping of Human Chromosome 17 Genes to the Pig Genome Extends Gene Order Conservation with Pig Chromosome 12 and Limited Synteny with Pig Chromosome 2

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Summary and Implications

A comparative study of human chromosome 17 (HSA17) and pig chromosome 12 (SSC12) and 2 (SSC2) was conducted using both somatic cell hybrid panel (SCHP) and radiation hybrid (RH) panel analysis. A cDNA library from an expressed sequence tag (EST) project in pig reproduction was examined and six genes and ESTs from HSA17 were selected for this study. The genes/ESTs were TATA box binding protein-associated factor (*TAF2N /RBP56*), plasmin inhibitor (*PLI*), H3 histone family 3B (*H3F3B*), aminopeptidase puromycin sensitive (*NPEPPS/PSA*), P311 protein (*P311*), and an expressed sequence tag (*ESTMI015*). SCHP analysis mapped five genes/ESTs (*TAF2N , H3F3B, PLI, NPEPPS,* and *ESTMI015*) to SSC12q11-q15 and SSC12p11-p15 with 100% concordance, and assigned *P311* to SSC2 (1/2q24)-q29 with 100% concordance. RH analysis of all six genes confirmed the SCHP mapping results, with average retention frequency of 25%. An expanded comparative SSC12 RH map integrating the five new type I markers and 23 previously mapped loci was established using a LOD score threshold of 4.8. The gene order of the five genes/ESTs on the SSC12 framework RH map (*H3F3B-ESTMI015-PSA-RBP56-PLI*) is identical to the HSA17 GB4 map but with map inversion. Although synteny conservation between HSA17 and SSC2 has not been detected by previous painting experiments, the mapping of *P311* to SSC2 suggests that some synteny conservation exists between HSA17 and SSC2.

Introduction

Comparative mapping is expected to be an efficient and powerful strategy in predicting gene location from the well-developed human genome map, and as a method for selecting candidate genes for improvement of economically important traits in livestock species. Bidirectional painting has revealed 37 synteny groups conserved between the pig and human (2). However, even within conserved synteny groups, rearrangement of gene order and distance discrepancy of adjacent loci have been documented (1, 4, 9, 13).

Accumulation of comparative mapping information between human and swine is helpful for understanding the evolutionary relationship between the two genomes and in predicting gene order and location with increased certainty. 128 linkage groups covering all porcine chromosomes (n = 19) were established in a first-generation porcine whole-genome radiation hybrid (WG-RH) analysis (3). This useful map consists almost exclusively of microsatellites, thus RH mapping of additional type I loci is required to fully use the RH panel for high-resolution comparative mapping. For example, only a single type I locus, GH, was mapped to SSC12 by using the INRA-Minnesota porcine radiation hybrid (IMpRH) panel (3). Therefore, the objective in this study was to provide additional comparative mapping references between human chromosome 17 (HSA17) and pig chromosome 12 (SSC12) using both somatic cell hybrid panel (SCHP) and radiation hybrid (RH) panel analysis.

Materials and Methods

As part of an expressed sequence tag (EST) project in pig reproduction (11), a directionally cloned cDNA library was produced from day 20 porcine embryos at University of Missouri-Columbia., The 3´-untranslated region of 220 clones was sequenced at Iowa State University. BLAST hits were evaluated for 187 clones, and six genes/ESTs on HSA17 were selected for the present study (Table 1). The six genes/ESTs were TATA box binding protein-associated factor (*TAF2N*), plasmin inhibitor (*PLI*), H3 histone family 3B (*H3F3B*), aminopeptidase puromycin sensitive (*NPEPPS/PSA*), P311 protein (*P311*), and an expressed tag sequence (*ESTMI015*).

The oligonucleotide primers for these genes/ESTs were designed using the OLIGO 5.0 program (NBI, Plymouth, MN), and they were then PCR-tested on a panel of templates, including pig, mouse, and Chinese hamster genomic DNAs. All primer sets for these six genes/ESTs showed robust and porcine specific amplification.

PCR was performed on an MJ Research PTC-200 thermal cycler in a 15-µl reaction volume containing 1 × PCR buffer (Promega, Madison, WI), 66 µM dNTP, 0.5 µM of each primer, 1.5 mM $MgCl_2$, 0.25 U *Taq* polymerase (Promega), and 25 ng of hybrid DNA. PCR reactions were preheated at 94ºC for 2 min and then followed by 35 cycles at 94ºC for 30 s, 60-64ºC for 30 s, and 72ºC for 45 s and completed at 72ºC for 5 min. The PCR products were size-separated on a 3% agarose gel. Detailed information on primer sequences, annealing temperature and product size for each locus was submitted to http://imprh.toulouse.inra.fr/.

A somatic cell hybrid panel (SCHP) comprised of 27 clones was used for regional assignments of the six genes/ESTs on pig chromosomes (12; also see http://www,toulouse.inra.fr/lgc/pig/pcr/pcr.htm).

A WG-RH (whole-genome radiation hybrids) panel included 118 hybrid clones was used for determining the gene order. The panel was tested at least twice for each gene/EST. If a discrepancy occurred between the first two tests, then a third confirmation test was performed.

Results and Discussion

For the six genes/ESTs examined, five (*TAF2N, PLI, H3F3B, NPEPPS*, and *ESTMI015*) were located on pig chromosome 12 with 100% concordance. These genes map to one of two subchromosomal regions, the first is SSC12q11-q15 for *TAF2N* and *PLI*, and the second is SSC12p11-p15 for *H3F3B*, *NPEPPS* and *ESTMI015*. To our surprise, gene *P311* mapped to pig chromosome 2 with 100% concordance. Previous painting experiments have not found synteny conservation between HSA17 and SSC2. However, a single HSA17 gene, *CTLC*, has been mapped to SSC2 (10). A summary of the somatic cell hybrid panel (SCHP) mapping data is presented in Table 2.

To determine the gene order and precise location of these loci within the pig genome, high-resolution mapping on a porcine whole-genome radiation hybrid panel (WG-RH) was used. The average retention frequency for all six genes/ESTs was calculated to be 25%. The six genes/ESTs mapped to SSC12 or SSC2, in all cases confirming the SCHP results (Table 2).

A radiation hybrid map of SSC12 was built using the RHMAP3.0 statistical package (5). Analyses were performed under the equal retention probability model. Using RH2PT program, two point distances were calculated between all markers. Linkage groups were defined using an LOD score threshold of 4.8. Multipoint analyses were performed using RHMAXLIK. For each linkage group, a framework map was built with a threshold likelihood ratio >1000:1 (minimum log 10 likelihood difference of 3), except for the *SWC23-PLI* group, which used a ratio of 690:1. Independent linkage groups were ordered according to the genetic map of Rohrer *et al*. (7). The final map was checked again by removing one marker at a time from the framework, then analyzing the likelihood of all its possible locations on the map of the other markers. A comprehensive map was established by adding the remaining markers at their most likely location (data not shown). The predicted gene order is *H3F3B-ESTMI015-NPEPPS-TAF2N-PLI* on pig chromosome 12 (Fig. 1). The SSC12 RH framework map was consistent with the recently published first-generation porcine whole-genome radiation hybrid map (3). However, one discrepancy was observed between the two maps, involving *Sw957*, *GH* and *Sw943*. In the published map (3), we notice that *Sw957* and *GH* were inadvertently assigned to the second linkage group. The mapping of *ESTMI015* allows us to localize *GH* and *Sw957* to the first linkage group. The results of our analysis are in agreement with the two point analysis of the first generation map available from the Web site at http://fabctr.umn.edu/RHMaps/2ptdata/chr12-2pt.html

Eighteen type I genes or ESTs have been physically mapped on SSC12 by SCHP and FISH analysis (http://www.toulouse.inra.fr/lgc/pig/cyto/gene/chromo/SSCG12.htm). However, SSC12 gene order is unclear due to overlapping localizations for many of these loci. Radiation hybrid mapping can provide higher resolution, yet of these loci only *GH* has been mapped using the IMpRH panel (3). Assignment of these five genes/ESTs in the present study thus significantly increases the density of type I markers on the SSC12 RH map. Because many of the microsatellite-based markers on the current RH map have been placed on the porcine linkage map (1, 7), the data reported here also can help integrate the comparative and linkage maps. By comparing this new SSC12 RH map and the HSA17 GB4 map (8), an inverted gene order was revealed (Fig. 1). This conclusion agrees with recently published results obtained through cross-species fluorescent in situ hybridization (FISH) of goat bacterial artificial chromosome (BAC) clones onto pig chromosomes (6). Data from FISH analysis of six goat BACs containing specific HSA17 genes also demonstrated gene order conservation and map inversion.

Finally, that *P311* was unambiguously mapped on SSC2 suggests an evolutionary relationship between HSA17 and SSC2, even though conserved synteny between the two chromosomes has not been detected through chromosome painting experiments. The HSA17 gene *CTLC* has been mapped to SSC2 (10). Thus, if mapping results for *P311* and *CTLC* are correct, small HSA17 regions are apparently conserved on porcine chromosome 2.

Conclusions

- Assignment of these five genes/ESTs in the present study significantly increases the density of type I markers on the SSC12 RH map.
- By comparing this new SSC12 RH map and the HSA17 GB4 map, an inverted gene order was revealed between SSC12 and HSA 17.
- Gene P311 was unambiguously mapped on SSC2 suggesting an evolutionary relationship between HSA17 and SSC2.

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Table 1. Genes/ESTs and location on human chromosome 17.

Genes/ ESTs	UniGene accession[a]	Human Cytogenetic location[a]	Human GB4 position (in cR_{3000}[b])
H3F3B	Hs.180877	17q25	482.47/ 483.02
ESTMI015[c]	Hs.3402	17q23	458.13
NPEPPS	Hs.132243	17q12	302.19
TAF2N	Hs.66772	17q11.1-q11.2	300.39
PLI	Hs.159509	17pter-p12	10.91
P311	Hs.142827	17	N.A.

[a] http://www.ncbi.nlm.nih.gov/UniGene
[b] Schuler *et al.* (1996), also http://www.ncbi.nlm.nih.gov/genemap99
N.A. not available.

Table 2. SCHP and RH mapping results for six human chromosome 17 (HSA17) genes/ESTs.

Genes/ ESTs	Pig SCHP			Pig RH		
	Location	Concordance	Probability	Highest LOD Score [a]	Retention %	Position (in cR_{7000})
H3F3B	12p11-p15	1.00	0.99	25.65 (S0143)	27	0
ESTMI015[b]	12p11-p15	1.00	0.99	5.23 (Sw943)	29	80.4
NPEPPS	12p11-p15	1.00	0.99	17.45 (Sw874)	27	275.9
TAF2N	12q11-q15	1.00	0.99	19.85 (Sw62)	29	594.6
PLI	12q11-15	1.00	0.99	11.01 (Sw1936)	14	780.8
P311	2(1/2q24)-q29	1.00	0.87	11.41 (S0010)	25	N.A.

[a] LOD Score obtained from two point analysis with locus shown.
[b] Human EST name: sts-D20379.
N.A. not available.

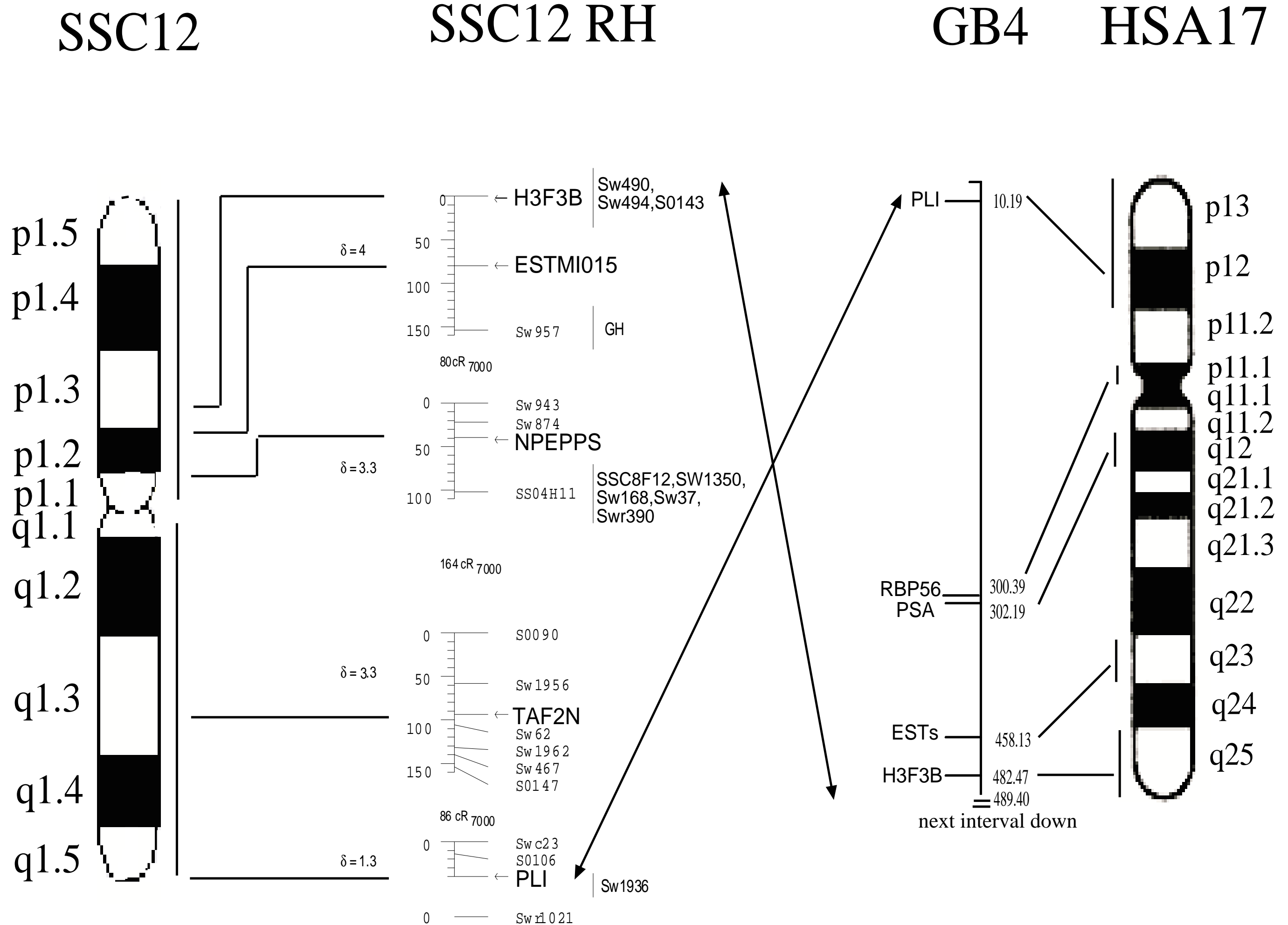

Figure 1. Improved RH comparative maps of human chromosome 17 (HSA 17) and pig chromosome 12 (SSC12). In this figure, SSC12 represents the pig chromosome 12 physical map, SSC12 RH is the framework radiation hybrid map of swine chromosome 12, GB4 stands for the human chromosome 17 GB4 radiation hybrid map, and HSA17 for the human chromosome 17 physical map. The SSC12 RH map consists of five new type I loci (bold) and 22 markers previously mapped by Hawken *et al.* (3). Distances between linkage groups were determined from multipoint analyses. δ is the difference of log 10-likelihood between the most likely and the reverse order of the linkage group. The distance of these five loci on GB4 map is from Gene Map 99 at http://www.ncbi.nlm.nih.gov/genemap99 and Schuler *et al.* (8). Placement of the five loci on HSA17 can be found in UniGene at http://www.ncbi.nlm.nih.gov/UniGene/