Mapping of Three Genes to Pig Chromosome 7q Demonstrates the Similarity with Human Chromosomes 14q and 15q

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Summary and Implications

Previous data have suggested the presence of pork quality quantitative trait loci (QTL) on pig chromosome (SSC) 7q. Improving the resolution of the pig-human-mouse comparative map in this region can facilitate the search for candidate genes for these possible QTL. Therefore, three genes, the cytochrome P-450, subfamily I, polypeptide 1 (*CYP1A1*), somatostatin receptor 1 (*SSTR1*), and transcription termination factor 1 (*TTF1*) genes, all with expected location on pig chromosome 7 based on painting studies, were selected for mapping in the pig. Porcine sequence tagged sites (STSs) spanning 0.9 - 1.5 kb genomic DNA sequence were generated and all genes were physically assigned to SSC7q12-q23, q26 using a pig/rodent somatic cell hybrid panel. Direct sequencing of polymerase chain reaction (PCR) products from different breeds revealed single nucleotide polymorphisms (SNPs) in all genes. The *SSTR1* and *CYP1A1* single SNPs allowed simple genotyping with PCR-restriction fragment length polymorphism (RFLP) analysis, and were informative for linkage mapping with the PiGMaP reference families. This revealed that *SSTR1* is closely linked to *CYP1A1* (0.0 cM; LOD = 11.1) and *ANPEP* (0.0 cM; LOD = 9.0), which refined the localization of a breakpoint in the linkage map between regions with homology to human chromosome 15q (represented by *CYP1A1* and *ANPEP*) and proximal 14q (represented by *SSTR1*). Moreover, multipoint linkage data suggest inverse homology between human chromosome 14q11-q13 and and pig chomosome 7q. The *TTF1* SNP was confirmed by allele-specific PCR but was not informative in any of the families used for linkage analysis.

Introduction

Improving the resolution of the pig-human-mouse comparative maps is an important step in the search for candidate genes for observed or indicated quantitative trait loci (QTL) in the pig. This is due to the fact that the human and mouse gene maps are much more well-developed than the pig gene map. Thus, comparison between pig gene locations and their corresponding human and mouse locations enables prediction of pig locations for other yet unmapped genes that have been mapped in humans or mice. Data suggesting presence of quantitative trait loci (QTL) for meat firmness and color on the long arm of pig chromosome 7 (SSC7q) have previously been reported (6). The purpose of this study was to put additional genes on the SSC7q map to facilitate the use of comparative maps to search for pork quality candidate genes in this region. The cytochrome P-450, subfamily I, polypeptide 1 (*CYP1A1*), somatostatin receptor 1 (*SSTR1*), and transcription termination factor 1 (*TTF1*) genes were selected for mapping based on their locations on human chromosome (HSA) regions with previously observed synteny (similarity) with SSC7q (HSA15q22-q24 - *CYP1A1*, HSA14q13 - *SSTR1* and *TTF1*; 2, 3). We report here physical gene mapping assignments of all three genes to SSC7q as well as close linkage between *CYP1A1*, *SSTR1* and other genes with homologues on HSA15q or proximal HSA14q.

Materials and Methods

Initial gene-specific primers for the polymerase chain reaction (PCR) were designed from other mammalian sequences (available in GenBank) that were well conserved between species. The PCR products were directly sequenced with dye terminators and an ABI 377 instrument (Perkin-Elmer, Foster City, CA). Additional primers were designed from the generated pig-sequences to use for evaluation of polymorphisms, physical mapping and/or linkage mapping. All products were analyzed by agarose gel electrophoresis (regular 2% or Metaphor agarose 2-4%, FMC Bioproducts, Rockland, ME).

Results and Discussion

The desired gene specificity of the STSs generated was confirmed by their high degree of identity to the corresponding human genes (Table 1).
All three genes were physically mapped to SSC7q12-q23, q26 with PCR on a pig/rodent somatic cell hybrid panel (7).

The three STSs were searched for polymorphisms by direct sequencing of PCR products from pigs representing the Yorkshire, Meishan, Hampshire and Duroc breeds. For all three genes, a Meishan sequence allele with a single nucleotide substitution in comparison to the other sequences was identified. These were located in the *CYP1A1* intron 2, in the *SSTR1* coding sequence (with the predicted amino acid unaffected) and in the *TTF1* intron 1.

Table 1. Sequence tagged site (STS) data generated for the three genes included in this study.

Gene symbol[a]	STS information: Size (bp)[b]	Coding sequence (bp / aa)	Coding sequence identity to humans, nt / aa (%)
CYP1A1	1,497	627 / 209 exon 2 – 6	86 / 84
SSTR1	946	946 / 315	93 / 99
TTF1	1,433	527 / 175 exon 1 - 2	93 / 98

[a]Gene symbols as recommended by the HUGO/GDB nomenclature committee.
[b]The porcine STSs have been submitted to GenBank
Abreviations: bp, base pairs; aa, amino acids; nt, nucleotides.

Allele frequencies were observed among the PigMaP parental animals and a few additional pigs; 12, 12, 11, 9, 10, and 2 pigs of the Large White (LW), Meishan (Me), Hampshire (H), Duroc (D), Landrace (L), and Wild Boar (WB) breeds, respectively. The *CYP1A1* allele 1 frequencies were 0.62 (LW), 0.92 (Me), 0.55 (H), 0.50 (D), 0.15 (L), 0.25 (WB), the *SSTR1* allele 1 frequencies were 0.96 (LW), 0.08 (Me), 1.00 (H), 0.94 (D), 0.85 (L) 0.50 (WB), and the *TTF1* allele 1 was absent in all breeds except in Meishan where the frequency was 0.12.

The PiGMaP reference families (1) and the CRI-MAP software (v. 2.4; 4) were used for linkage analyses. The results confirmed the physical mapping of *CYP1A1* and *SSTR1*, whereas the *TTF1* SNP was not informative for mapping with the PiGMaP families. Twopoint linkage analysis revealed that *CYP1A1* and *SSTR1* are closely linked to each other and to several other markers, including genes with comparative locations on the q-arms of HSA14 or 15. A multipoint map including both these genes and neighboring markers on SSC7q are shown in Figure 1. Chromosome painting studies show alternating HSA14 and HSA15 homology on the q-arm of SSC7. The short recombination distance between *SSTR1* and *ANPEP*/*CYP1A1* observed in this study shows that these genes are located very close to one of the HSA14 and HSA15 homology breakpoints. Moreover, these genes are closely linked to the S0047 and S0078 microsatellites, both of which have been physically assigned to SSC7q12-q14. This provides evidence for that these genes are located at the proximal painting breakpoint between HSA15q and HSA14q homology. Our multipoint linkage analysis also shows that *SSTR1* is closer than *MYH7* to this breakpoint. Because the corresponding human locations of these two genes are HSA14q13 and HSA14q11.2-q12, respectively, this suggests inversed homology between SSC7q and proximal HSA14q (Figure 1).

Looking at the HSA15 map, *CYP1A1* is very close to a breakpoint between regions with mouse chromosome (MMU) 2 and MMU9 homology (*CYP1A1* on MMU9). Even though a few other genes with homologes on HSA15q and MMU9 also have been linkage mapped to SSC7q (e.g. *PKM2* and *CHRNB4*), none of these are as close as *CYP1A1* to this breakpoint (see http://www.ncbi.nlm.nih.gov/Homology/). Moreover, this breakpoint seems to correspond well with a breakpoint between HSA15 regions with SSC1 and SSC7 homology (5). Thus, the linkage mapping of *CYP1A1* expands the SSC7q segment with observed homology to HSA15q/MMU9 and moves its border closer to a HSA15 region with SSC1 homology.

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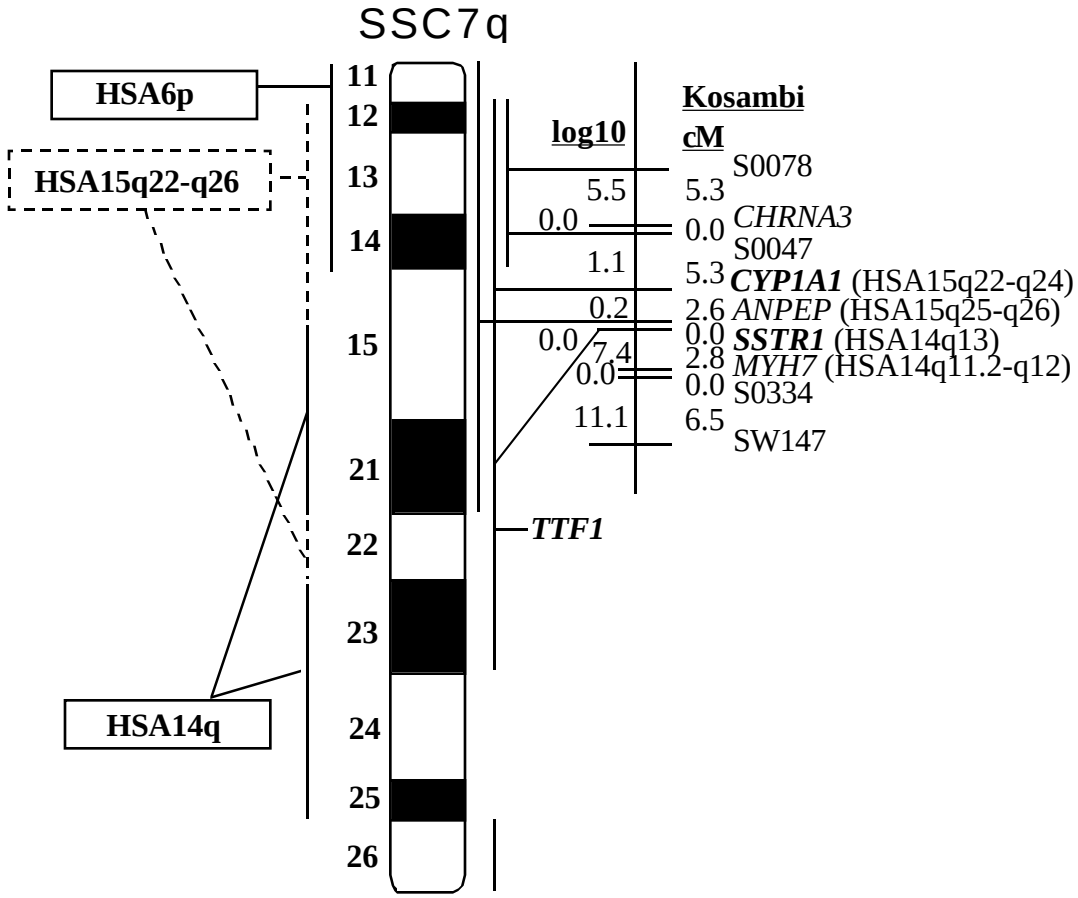

Figure 1. A multipoint linkage map from SSC7q with recombination distances in Kosabi centiMorgan (cM) and the $\log^{10}$ odds against the inversed order of loci. Corresponding human regions according to painting studies (2, 3) are shown to the left whereas vertical bars to the right indicate physical assignments. The three genes mapped in this study are in bold whereas information and references for the previous human and porcine assignments indicated can be found through the Genome Database (http://www.gdb.org/) and the PIGBASE (http://www.genome.iastate.edu/maps/pigbase.html), respectively.