Comparative mapping of human chromosome 3 genes in the pig shows different gene order

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Summary and Implications

A comparative map of human chromosome 3 (HSA3) and pig chromosome 13 (SSC13) was constructed using physically assigned pig sequence tagged sites (STSs). Pig STS representing 11 HSA3 genes were developed and 10 pig STS were regionally mapped using a somatic cell hybrid panel (SCHP) to SSC13 with 80–100% concordance. Large-insert probes were obtained by screening a YAC library with primers for each STS. YACs were identified for DRD3, GAP43, PIT1, SI, and SST for fluorescent *in situ* hybridization (FISH) mapping. Single gene and bicolor FISH with each pairwise combination was used to further define gene order on SSC13. These data confrim chromosome painting results that showed HSA3 probes hybridize to a major portion of pig chromosome 13 and demonstrate extensive gene rearrangements within this conserved synteny group.

Introduction

Pig chromosome 13 (SSC13) has attracted attention because several important genes or effects have been recently localized on SSC13. For example, the locus controlling susceptibility to *Escherichia coli* K88 was mapped to SSC13 (4) and significant QTL effects for growth and fat deposition traits also were found on SSC13 (1,12). From earlier work in our group, the SSC13 locus PIT1 has been suggested as a candidate gene for controlling growth and carcass traits in pigs (12). The SSC13 linkage map is well developed (8) but is mostly constructed by using anonymous markers. As there are less than 10 Type I genes mapped on SSC13, it is of interest to place additional Type I loci on the SSC13 map to increase the comparative information and to increase the integration of the cytogenetic and linkage maps.

Previous knowledge of comparative mapping between the human and pig suggested that a large proportion of pig chromosome 13 shares homology with human chromosome 3 (HSA3) (5). The objectives of our study were to apply available information from human and mouse databases to develop pig sequence tagged sites (STSs) of HSA3 homologs and to examine gene order within this conserved synteny group by physical assignment of each locus by using somatic cell hybrids and fluorescent *in situ* hybridization (FISH). Eleven genes including v-Raf-1 murine leukemia viral oncogene homolog 1 (RAF1), Retinoic Acid Beta Receptor (RARB), Cholecystokinin (CCK), Pituitary transcription factor 1 (PIT1), Ceruloplasmin (CP), Guanine Nucleotide binding protein, Alpha-Inhibiting polypepetide 2 (GNAI2), Sucrase-Isomaltase (SI), Rhodopsin (RHO), Dopamine Receptor D3 (DRD3), Growth-Associated Protein 43 (GAP43), and Somatostatin (SST) from HSA3 were chosen for this study. These loci were selected for covering the entire HSA3 and as part of a joint effort on comparative anchored tagged sequences (CATS) (6).

Materials and Methods

Primer design and pig sequence tagged sites (STSs) development. Heterologous primers were designed from regions that were highly conserved between the human and a second species and the resulting primers were used to amplify pig DNA fragments by the polymerase chain reaction (PCR). The PCR products were sequenced with the original primers and the sequences were aligned with their human targeted loci to verify identity.

Somatic cell hybrid panel typing and regional assignments. Nineteen pig x Chinese hamster and eight pig x mouse somatic cell hybrids were prepared and characterized as previously described (11). Ten nanograms of genomic DNA from 27 hybrids were typed for all loci using PCR primers. Regional assignments were analyzed for concordant segregation of PCR results and chromosome fragments retained in the hybrid cells.

Pig yeast artificial chromosome (YAC) library screening and fluorescent in situ hybridization (FISH) mapping. A pig YAC library constructed and characterized by Rogel-Gaillard et al. (7) was used in this study to screen gene-containing YACs for FISH mapping. FISH was performed with modified procedure described previously (10) and the bicolor FISH was done by hybridizing slides to both biotinylated and digoxigenin- labelled probes.

Results and Discussion

Ten pig genomic fragments amplified with heterologous primers were sequenced to confirm the homology. An overall 85 to 100% nucleotide similarity was found in sequence comparisons between human and pig exon regions ranging in length from 45–190 bp. Information of all pig STSs have been submitted to GenBank database.

The new pig STSs were used to map these loci by using a somatic cell hybrid panel. All loci mapped were assigned to SSC13. The RHO locus failed to be mapped using the panel due to indistinguishable patterns of the PCR products among the pig and the rodent background. Subchromosomal assignments of these loci on SSC13 are given in Table 1.

To improve the map resolution and further investigate gene order within the same subchromosomal region, a YAC library was screened to identify large insert probes for use in a second physical mapping method, the FISH technique. One or more YAC clones were obtained for five genes (DRD3, GAP43, PIT1, SI, and SST).

In Figure 1 the physical assignments of ordered loci on HSA3 and SSC13 are illustrated. The relative locations for several HSA3/SSC13 genes are quite different in these two species. Clearly, if the mapping data are all correct, the different orders of these loci in the pig and human cannot be accounted for by a single, simple rearrangement. Multiple break-and-join points and complicated evolutionary events would be required to produce the current gene arrangements seen in humans and pigs. For example, PIT1 is apparently the most distal HSA3 homolog on SSC13 even though several genes on both sides of PIT1 on HSA3 were mapped. And it appears that a rearrangement involving the HSA3 region encompassing PIT1 to SST (HSA3p11 -q28) has occurred to invert the general order of these genes on SSC13. However, additional rearrangements are required. For example, the HSA3 order of PIT1 - DRD3 - CP - SI - SST is clearly CP - SI - SST - DRD3 - PIT1 in pigs based on our's and others' data (2, 9). Further, RAF1 is within the same subchromosomal region with SI in pigs whereas RAF1 is found quite distant to SI in humans on distal HSA3p.

In summary, 10 genes selected from the human chromosome 3 were physically assigned to pig chromosome 13 by using FISH and/or SCHP mapping. Our results confirm the extensive synteny between HSA3 and SSC13, and reveal significant differences in gene order between HSA3 and SSC13.

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Table 1. Regional assignments and the corresponding concordance score of HSA3 homologs in the pig.

Loci Symbols	Regional Assignment by SCHP mapping	Regional Assignment by FISH
RARB	13q11-14	NA
CCK	13q21-36	NA
GNAI2	13q21-36	NA
RAF1	13q23-36	NA
CP	13q23-36	NA
SI	13q23-36	13q36-41
SST	13q41 or q46-49	13q36-41
PIT1	13q42-46	13q46
DRD3	13q42-46	13q42-44
GAP43	13q41 or q46-49	13q42-43

Figure 1. Comparative maps of human chromosome 3 (HSA3) and pig chromosome 13 (SSC13). Defined cytogenetic regions were denoted A to F on SSC13. The physical map of HSA3 was obtained from Debry and Seldin (3) and the assignments of the homologs on SSC13 were obtained from this study. Loci with an asterisk were also linkage mapped.