

# Comparative mapping of human chromosome 13 genes in the pig shows a similar gene arrangement

H. S. Sun, research associate,  
M. F. Rothschild, professor,  
and C. K. Tuggle, associate professor,  
Department of Animal Science

M. Yerle and P. Pinton,  
Laboratoire de Genetique Cellulaire, INRA,  
Castanet-Tolosan, France

P. Chardon and C. Rogel-Gaillard,  
Laboratoire de Radiobiologie Applique'e, INRA,  
France

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### Summary and Implications

Previous comparative mapping between the human and pig genomes suggested complete conservation of human chromosome 13 (HSA13) to pig chromosome 11 (SSC11). The objectives of this study were comparative gene mapping of pig homologs of HSA13 genes and an examination of gene order within this conserved synteny group by physical assignment of each locus. A detailed HSA13 to SSC11 comparison was chosen since the comparative gene map is not well developed for these chromosomes and a rearranged gene order within conserved synteny groups was observed from the comparison between human chromosome 13 and bovine chromosome 12. Pig sequence tagged sites (STSs) for six HSA13 genes were developed and physically mapped using a somatic cell hybrid panel (SCHP) to SSC11 with 85–100% concordance. Fluorescent *in situ* hybridization (FISH) mapping also was applied to determine the gene order within each subchromosomal region. Results from this study increase the comparative information available on SSC11 and suggest the same gene order among examined loci on SSC11 and HSA13.

### Introduction

The previous chromosome painting results (3) and the mapping of human homologs of erythrocyte antigen M (EAM), tripeptidylpeptidase (TPP2), and tyrosinase-related protein 2 (TYRP2) in pigs (2) suggested a complete conservation of human chromosome 13 (HSA13) to pig chromosome 11 (SSC11). However, the pig homolog of HSA13 locus esterase D (ESD) was mapped to SSC13 by *in situ* hybridization (4) and mapped to SSC11 by linkage analysis (2). The discrepancy raises the interests to reexamine the position of ESD as well as to map additional loci in pigs. The synteny comparison between the human and bovine chromosomes has shown an extensive conservation of HSA13 and BTA12 but one rearranged gene order also was indicated (8). Because bovine and swine are both in the artiodactyls lineage, it is of interest to examine if the same rearranged gene order exists in the pig genome.

The objectives of this study were comparative gene mapping of pig homologs of HSA13 genes and an examination of gene order within this conserved synteny group by physical and/or genetic assignment of each locus. Genes selected for this study are fms-related tyrosine kinase 1 (FLT1), retinoblastoma 1 (RB1), serotonin receptor subtype 2, alpha chain (HTR2A), esterase D (ESD), endothelin receptor B (EDNRB), and coagulation factor 10 (F10). These loci were selected for covering the entire q arm of HSA13 and as part of a joint effort on comparative anchored tagged sequences (CATS) project (6).

### Materials and Methods

**Primer design and pig sequence tagged sites (STSs) development.** The heterologous primers were designed from regions that were highly conserved between the human and a second species, and the CATS primers were designed as described earlier by Lyons et al., (6). The primers were used to amplify pig DNA fragments by the polymerase chain reaction (PCR) and the products were sequenced with the original primers 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 (10). Ten ng 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. The DNA from gene-containing YACs was prepared and the FISH was performed with the modified procedure described previously (9) and bicolor FISH was done by hybridizing slides to both biotinylated and digoxigenin-labelled probes.

### Results and Discussion

Three out of six CATS distributed primers (ESD, EDNRB, and F10) amplified well in the pig genome. However, CATS primers for the other selected loci needed to be redesigned. One gene, GBJ, was replaced by HTR2A in this study for a direct comparison with the result from the human/bovine work. Six pig genomic fragments amplified with heterologous primers were sequenced to confirm the homology. An overall 81 to 100% nucleotide similarity was found in sequence comparisons between human and pig exon regions.

The developed pig STSs were used to synteny map these loci by using a somatic cell hybrid panel (SCHP). All loci were assigned to SSC11 with 85 to 100% concordance. Subchromosomal assignments of these loci on SSC11 are

given in Table 1. The assignment of ESD has 100% probability to SSC11 but only has 0.71E-10 probability to be assigned to SSC13.

To improve the map resolution and further investigate gene order within the same subchromosomal region, a second physical mapping method using the FISH technique also was applied to map these SSC11 loci. Results from the FISH mapping assign FLT1 to SSC11p14-15, RB1 to 11p12, ESD to 11p12-13, and F10 to 11q17 (Table 1).

Results from this study assigned ESD to SSC11 by using two independent physical mapping methods thus confirming the previous linkage mapping result by Ellergren et al. (2) but refuting the physical assignment by Graphodatsky et al. (4). Five additional HSA13 homologs mapped to SSC11 further support the hypothesis that a major portion of the HSA13 is conserved in SSC11.

The gene order on the SSC11 based on the physical mapping results is given in Figure 1. Even though both HTR2A and EDNRB have no FISH data due to the failure of screening the YAC library, the linkage analysis of these two loci are available (Sun et al., unpublished). The most likely gene order of this six loci on SSC11 is FLT1 - (ESD - RB1 - HTR2A) - EDNRB - F10. The alternative orders of (ESD - HTR2A - RB1) and (RB1 - ESD - HTR2A) are also possible according to the present study.

The extensive conservation of this synteny group also was identified in cattle (8) and sheep (1) species. However, an interrupted linkage group has observed in mouse and canine (11) genomes. Previous studies on the comparative mapping have shown a high frequency of rearranged gene order within the conserved synteny groups across species (5). The comparative mapping of HSA13 and BTA12 has suggested an extensive conservation with one alternated gene order in bovine relative to the human genome. Results from this study indicating the same gene order for these six examined loci were observed on the SSC11 and HSA13 and suggest the rearranged FLT1 position on BTA12 relative to HSA13 may have happened recently. The divergence time between the common ancestor of primates and artiodactyls is estimated to be around 65-80 million years ago; thus, this event should occur after the bovine and pig diverged from each other within the artiodactyls lineage and the proximate time should be less than 65 million years ago.

In summary, six genes selected from the human chromosome 13 were physically mapped to pig chromosome 11 by using FISH and/or SSCP mapping. Our results confirm the assignment of the ESD locus to SSC11 and show extensive synteny conservation between HSA13, BTA12, OAR10, and SSC11.

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**Table 1. Regional assignments and the corresponding concordance score of HSA13 homologs in the pig.**

Loci Symbols	Regional Assignment by SChP mapping	Regional Assignment by FISH
FLT1	11p11-15	11p14-15
ESD	11p11-15	11p12-13
RB1	11p11-15	11p12
HTR2A	11p11-15	NA
EDNRB	11q11-17	NA
F10	11q11-17	11q17

**Figure 1. Comparative maps of human chromosome 13 (HSA13) and pig chromosome 11 (SSC11). The physical map of HSA13 is obtained from online GDB database and the assignments of the homologs on SSC11 are obtained from this study. Defined cytogenetic regions were denoted A and B on SSC11 and loci with the asterisk also were linkage mapped.**

