An integrated comparative map of the porcine X chromosome

S. A. McCoard*, S. C. Fahrenkrug*, L. J. Alexander†, B. A. Freking*, G. A. Rohrer*, T. H. Wise* and J. J. Ford*

*USDA-ARS US Meat Animal Research Center, Clay Center, NE, USA. †Department of Veterinary PathoBiology, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA.

Summary

The objectives of this study were to assign both microsatellite and gene-based markers on porcine chromosome X to two radiation hybrid (RH) panels and to develop a more extensive integrated map of SSC-X. Thirty-five microsatellite and 20 gene-based markers were assigned to T43RH, and 16 previously unreported microsatellite and 15 gene-based markers were added to IMpRH map. Of these, 30 microsatellite and 12 gene-based markers were common to both RH maps. Twenty-two gene-based markers were submitted to BLASTN analysis for identification of orthologues of genes on HSA-X. Single nucleotide polymorphisms (SNPs) were detected for 12 gene-based markers, and nine of these were placed on the genetic map. A total of 92 known loci are present on at least one porcine chromosome X map. Thirty-seven loci are present on all three maps; 31 loci are found on only one map. Location of 33 gene-based markers on the comprehensive map translates into an integrated comparative map that supports conservation of gene order between SSC-X and HSA-X. This integrated map will be valuable for selection of candidate genes for porcine quantitative trait loci (QTLs) that map to SSC-X.

Keywords chromosome X, comparative map, follicle-stimulating hormone, radiation hybrid, swine.

Introduction

Comparative gene mapping is a valuable tool to investigate evolutionary history of chromosomes allowing for identification of regions of conserved synteny, thereby facilitating extrapolation of information from one genome to another. Construction of a high-resolution porcine–human comparative map provides an opportunity to exploit information from the human genome project and facilitate comparative positional candidate gene cloning. Specifically a genomic address for a QTL in a map poor species can be translated to a corresponding human chromosomal region and its respective host of potential candidate genes.

Radiation hybrid (RH) mapping was utilized in this study as it offers the most rapid method to link both microsatellite and gene-based markers as a means to construct a high-resolution physical map. This provides opportunity to integrate existing linkage and cytogenetic maps of a species. Thus, the objective of this study was to employ both RH (two RH panels differing in resolution) and linkage mapping, utilizing both microsatellite and gene-based markers, to increase the resolution of SSC-X relative to human chromosome X (HSA-X).

Address for correspondence

J. Joe Ford, USDA, ARS, RLH US Meat Animal Research Center, PO Box 166, State Spur 18D, Clay Center, NE 68933-0166, USA.
E-mail: ford@email.marc.usda.gov
Present address: S.A. McCoard, Nutrition and Metabolism Team, AgResearch Limited, Grasslands Research Centre, Private Bag 11008, Tennent Drive, Palmerston North, New Zealand.
Present address: S.C. Fahrenkrug, Department of Animal Science, College of Agricultural, Food, and Environmental Sciences, University of Minnesota, St Paul, MN, USA.

Accepted for publication 15 November 2001

Methods

PCR primers

The expressed sequence tag (EST)-associated markers were developed from porcine ESTs generated from normalized cDNA libraries (Fahrenkrug *et al.* 2002). The EST sequences were selected based on their potential orthology, as predicted by BLASTN (score > 300), with genes on HSA-X. Clone identity was confirmed by re-arraying, 5′ and 3′-end sequencing, and comparison (BLASTN) with public sequence respositories (Table 1). Polymerase chain reaction (PCR) primer pairs (Table 1) for ESTs were designed against the 3′-end sequence of EST clones using Oligo 5.0 software (MBI, Cascade, CO, USA) and purchased from Integrated DNA Technologies Inc. (Coralville, IA, USA). Primer information for *DAX1*, *F9* and *HCFC1* were obtained from Parma *et al.* (1998), Hu *et al.* (1997) and Hisamatsu *et al.* (1997), respectively. The PCR amplification products for all loci were sequenced and compared with original sequences in GenBank to confirm amplification of sequence tagged sites (STS) corresponding to target ESTs. Primers for microsatellite markers previously assigned to SSC-X were designed as described by Rohrer *et al.* (1994); information can be obtained from http://www.marc.usda.gov/. Primer pairs that amplified porcine-specific products or those that amplified hamster products that could be distinguished from porcine products were selected for RH analysis.

RH panel analysis

A 3000-rad whole genome porcine RH panel of 100 hybrids (T43RH, Research Genetics; www.ri.bbbsrc.ac.uk/radhyb) and a 7000-rad panel of 118 hybrids (IMpRH; Yerle *et al.* 1998; Hawken *et al.* 1999; http://fabctr.umn.edu/RHmaps/chromosome/chromosomex.html) were screened by PCR amplification for the presence of microsatellite and gene-based markers. The PCR products for each marker were size-fractioned by 2.5% agarose gel electrophoresis. Controls consisted of hamster genomic DNA, porcine genomic DNA and a reaction containing no DNA. Each marker was genotyped independently in duplicate. Each hybrid was scored as either present (1) or absent (0) for each locus. Each ambiguous hybrid was re-amplified and any remaining discrepancies scored as ambiguous. Typing of markers using the IMpRH panel was performed as previously described (Hawken *et al.* 1999). Marker linkage and order were determined by analysing co-retention of markers with the haploid model of RHMAP 3.0 (Lange *et al.* 1995). Markers were assigned to linkage groups requiring a minimum two-point LOD score of 6.0 with the RH2PT program of RHMAP3.0. Multipoint analysis and ordering of markers were performed with the RHMAXLIK program of RHMAP3.0 using an equal retention frequency model. Marker order with the greatest likelihood was accepted. No attempt was made to differentially calculate distance within the pseudoautosomal region. Therefore, distances might be altered because of diploid representation for those markers. Marker order is presented relative to that predicted by genetic linkage analysis (http://map.marc.usda.gov/genome/swine/swine.html). The RH maps were constructed using Genetic Map Creator (http://www.webarris.com).

SNP discovery and genetic mapping

Primers designed for EST-associated markers were used to amplify genomic DNA of 16 individuals including nine parents of the US Meat Animal Research Center (MARC) reference population followed by sequencing of PCR products. Sequences were analysed with polyphred software (Nickerson *et al.* 1997) in conjunction with phred/phrap/consed software (Ewing & Green 1998; Ewing *et al.* 1998; Gordon *et al.* 1998). Single nucleotide polymorphisms (SNPs) were manually identified by interacting with consed software. Tagged SNPs were then transferred into an interactive relational database (Keele *et al.* 1994). Selected SNPs which provided the maximum number of meioses for an amplicon were genotyped by MALDI-TOF-coupled microsequencing using primer oligonucleotide base extension (PROBE), nanolitre dispensing of extension products onto silicon chips (Sequenom, Inc., San Diego, CA, USA) (Little *et al.* 1997), and mass spectrometric analysis as described (Heaton *et al.* 2001). The MALDI-TOF microsequencing was performed with an array mass spectrometer and genotypes were scored automatically with Genolyzer software (Sequenom, Inc.).

The gene-based markers that were mapped using SNPs were incorporated into the existing porcine linkage map with Cri-map version 2.4 (Green *et al.* 1990) as described by Rohrer *et al.* (1996).

Comparative mapping

Map data were extracted from various human genome databases including http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/maps.cgi?org = hum&chr = X; http://www.sanger.ac.uk/cgi-bin/humace/X_genes; and http://www.ensembl.org/perl/mapview?chr = X.

Results

An integrated mapping approach that utilized both RH and genetic mapping was employed to increase the resolution of the SSC-X map. The 55 markers assigned to the T43RH panel included 35 microsatellite, 17 EST-associated and

Table 1 Clone identity, BLASTN results, human homologies and primer information for EST-associated markers used in this study.

						BLASTN Results							
Locus Symbol*	Clone Address	Read	Accession No.	5' and 3' seq. Overlap[1]	BLAST Match	Best P-value	Best bit score	Identities	Gene Name	UniGene ID	HSA-X Map Position	PCR Primers for STS amplification	SNP
ATP6S1 (a, b, c)	1PIG 12L5	3'-end 5'-end	BE749699 AW346508	N	U10039 U10039	4e19 9e-84	101 315	100/115 213/231	*ATPase, H+ transporting, lysosomal subunit 1*	Hs.6551	Xq28	TATTAGGAAGGAAACCACGAA ACCCCCTCTCTGTACCAAT	Y^2
ZNF261 (a)	1PIG 145B20	3'-end 5'-end	BE749712 AW360022	N	XM_010140 XM_010140	e-106 e-159	391 565	215/221 370/403	*H. sapiens zinc finger protein 261*	Hs.9568	Xq13.1	CCCGGTCTTGGTGTAGATATA CCAGCCCATTCGTGAG	N
KIAA0443 (a, b, c)	1PIG 19B8	3'-end 5'-end	BE749713 AW360224	Y	AL035427 AL035427	e-155 e-104	553 383	458/515 265/289	*clone 769N13 - Gene for KIAA0443 protein*	Hs.113082	Xq24.3-q24.1	TATTGAAAACACAGACTCGAA GTGGGACAACAAAGCTAAG	Y^2
GATA1 (a, b)	1PIG 23G3	3'-end 5'-end	BE749716 AW414967	N	XM_010214 XM_010214	e-119 4e-89	434 333	377/427 284/322	*GATA-binding protein 1*	Hs.765	Xp11.23	TTTTTCAGGACTCCGGTAGTT TGTGTGCAAACGCCTGT	Y^3
SAT (b)	1PIG 24K3	3'-end 5'-end	BE749717 AW435778	Y	BC002503 BC002503	1e-64 1e-64	252 252	191/213 191/213	*Spermidine/spermine N1-acetyltransferase*	Hs.28491	Xp22.1	AATCAAAACAGAAACTCTAAA GCTTCAAGATCGACAA	N
BMX (a)	1PIG 31H5	3'-end 5'-end	BE749703 AW430887	N	XM_010319 XM_010319	2e-76 2e-82	291 311	210/231 202/217	*BMX non-receptor tyrosine kinase*	Hs.27372	Xp22.2	TTTTTCCATACTTGTTAATAA ATTTCAGCAACTCCTATC	N
MECP2 (a, b, c)	1PIG I36C3	3'-end 5'-end	BE749707 AW312479	N	NM_004992 NM_004992	e-163 e-100	579 371	406/444 244/263	*H. sapiens mRNA for Methyl CpG binding protein 2*	Hs. 3239	Xq28	AAGGGAGTGGCACCACGAGAC TTGCACGGACCGGATG	Y^2
PIM2 (a, b)	2PIG 14P20	3'-end 5'-end	BE749740 lowQuality[4]	N	XM_010208 XM_010208	2e-23 e-106	115 391	130/151 308/344	*pim-2 oncogene*	Hs. 80205	Xp11.3	TCCCCTCCCCCAATACA CTACCTCATCCCAGGGTTGTT	N
ARAF1 (a, c)	2PIG 15020	3'-end 5'-end	BE749743 AW480731	Y	D88385 D88385	0.0 0.0	825 779	416/416 393/393	*V-raf murine sarcoma 3611 viral oncogene homolog 1*	Hs. 77183	Xp11.4-p11.2	AGGAGCCCCTGAATTTATAAG ACCGAACCCAGGTTGAT	N
PLP1 (a, b, c)	2PIG 24N21	3'-end 5'-end	BE749753 AW416665	Y	AJ009912 AJ009912	0.0 e-178	654 630	339/342 318/318	*proteolipid protein*	Hs.1787	Xq22	GAAGGGTTGGTTATTTGTTAT CAGCTATTCAGTTGGGTAAG	Y^2
NADE (b)	2PIG 28G5	3'-end 5'-end	BE749758 AW359320	N	AF187064 AF187064	0.0 7e-26	638 123	439/476 86/93	*nerve growth factor receptor associated protein 1*	Hs.17775	Xq22.1	GCAAATGGGTGAAACTCTACT CCAGCAGCAAATAGACG	Y^3
HADH2 (a)	1PIG 133B21	3'-end 5'-end	BE749795 AW311920	N	AB002156 NM_000167	0.0 e-110	781 402	542/591 282/307	*Hydroxyacyl-CoA Dehydrogenase, Type II*	Hs.171280	Xp11.2	GTGACGTGGGCAGAGGTTAC TGGCCAGCAAGACATACAACT	N

Table 1 (Contd.)

Locus Symbol*	Clone Address	Read	Accession No.	5' and 3' seq. Overlap[1]	BLAST Match	BLASTN Results: Best P-value	BLASTN Results: Best bit score	BLASTN Results: Identities	Gene Name	UniGene ID	HSA-X Map Position	PCR Primers for STS amplification	SNP
KIAA0189 (a, b)	1PIG 11N4	3'-end 5'-end	BE749796 AW344842	N	XM_010387 XM_010387	1e-06 e-100	60 371	43/46 250/271	*KIAA0189 gene product*	Hs.95140	Xq11.2	TAGTTGGTGTCTGGCGTTTGA CCGCTCTTGTTGCCTATGG	N
LAMP2 (b)	1PIG 14F8	3'-end 5'-end	BE749798 AW480102	Y	XM_010337 XM_010337	3e-34 3e-11	151 74	113/124 55/61	*Lysosomal-associated membrane protein 2*	Hs.8262	Xq24	AATGGACAGGCAAATATCTAA CGTCCGCTCTATTTGGT	N
TIMP1 (a)	1PIG 23N21	3'-end 5'-end	BE749800 AW415094	Y	S96211 S96211	0.0 e-127	1015 460	521/524 235/236	*Tissue inhibitor of metalloproteinase 1*	Hs.5831	Xp11.3-p11.23	GCAGCATAGATCTCGGTGA AGATGTTCAAAGGGTTCAATG	N
GJB1 (a, b)	2PIG 24A18	3'-end 5'-end	BE749806 AW416676	N	X95311 X95311	2e-81 e-167	307 593	269/304 362/383	*Gap junction protein, beta 1*	Hs.2679	Xq13.1	CTGCTTTTCCCTCCCTAGAT CCTGTGCTGTCAGATACCCTA	Y[3]
FGD1 (a, b, c)	2PIG 36L5	3'-end 5'-end	BE749762 AW431649	N	XM_010215 XM_010215	e-120 0.0	438 684	425/490 387/401	*Faciogenital dysplasia (Aarskog-Scott syndrome)*	Hs.1572	Xp11.21	GGTCACCACCCCGCTTAG ATCATGGGCACTTTCAATCCT	Y[2]
APT6M8-9 (a, c)	2PIG 19024	3'-end 5'-end	BE749749 AW352739	N	AF248966 AF248966	1e-13 e-141	84 507	96/114 340/368	*ATPase, H+ transporting, lysosomal associated protein M8-9*	Hs.183434	Xp21.1-p11.4	CCGCAAACCATAGGAC AGTGATATTTGGGCTGTTATA	Y[2]
GS1 (a, b, c)	2PIG 28MI7	3'-end 5'-end	BE749759 AW482560	N	XM_010289 XM_010289	2e-15 1e-49	90 202	75/85 309/378	*GS1 gene*	Hs.78991	Xp22.32	TTAAAAACCCCTCCACAATGT CCGCCCTACGAGTGAGA	Y[2]
EBP (a, b)	2PIG 29A20	3'-end 5'-end	BE749760 AW359658	Y	Z37986 Z37986	8e-57 e-107	226 394	210/242 301/335	*Emopamil-binding protein (sterol isomerase)*	Hs.75105	Xp11.23-p11.22	CCATCTTGGACTTCCTAGGT ATATACGGGGATGTGCTCTAT	N
CLCN4 (c)	2PIG 11N18	3'-end 5'-end	BE749732 AW313850	N	AC00366 NM_001830	3e-75 e-128	287 462	259/297 321/349	*Chloride Channel 4*	Hs.199250	Xp22.3	CATGCCGAAGGTAAATATGGT TTGGTGCAGGAGGCGTAAA	Y[2]

*Parenthesis identifies map assignment; a = T43RH, b = IMpRH, c = genetic map.

[1]N = no overlap between 5′ and 3′ sequence, Y = overlap.

[2]Informative meioses for development of mass spectrometric analysis.

[3]Inadequate meioses to be informative.

[4]Sequence sufficient to identify blast match to Pim2 but sequence quality was insufficient to submit to the public database.

three additional gene-based markers (Fig. 1). Sixteen previously unassigned microsatellite markers and 14 EST-associated markers were added to the IMpRH panel (Fig. 1). Four gene-based markers were added to the genetic map, three that were assigned to the T43RH panel, and one of these three was assigned to IMpRH. In total, 44 unique microsatellite and 24 gene-based markers have been assigned to RH maps (Fig. 1). Similarity of porcine EST sequences to their predicted human orthologue is presented in Table 1.

Mean retention frequency of the markers was 28% for the T43RH and 41% for the IMpRH panels. Mean retention frequencies for gene-based markers were 31% (range 16–53%) for T43RH and 39% (range 27–80%) for IMpRH panels. For microsatellite markers, mean retention frequencies were 25% (range 9–48%) for the T43RH and 42% (range 17–95%) for the IMpRH panels. Each marker was ordered by analysis of the minimum number of breaks, maximum two-point LOD score and minimum distance between markers. Two-point analyses of the 3000-rad T43RH panel data supported five linkage groups with a two-point LOD of 6.0, with 32 of 55 markers comprising a large linkage group in the centromeric region. Localization of 31

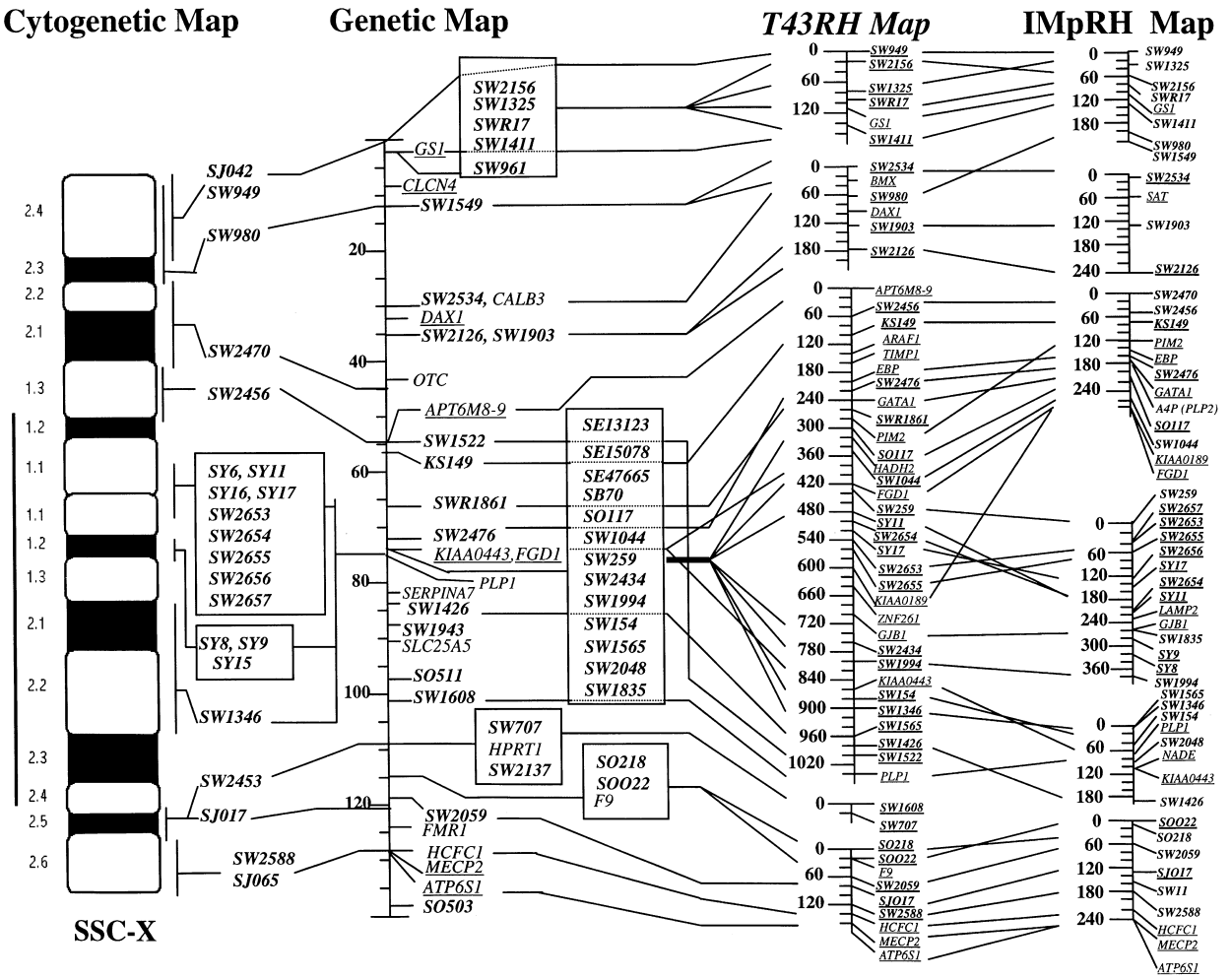

Figure 1 An integrated map of porcine chromosome X (SSC-X) incorporating microsatellite, EST- and other gene-associated markers. *T43RH ($cR_{3,000}$) and IMpRH ($cR_{7,000}$) maps*: RH map positions were determined with RHMAP 3.0. Markers were assigned to linkage groups requiring a minimum two-point LOD score of 6.0. The gene-based markers are indicated as non-bold text; microsatellite markers are displayed as bold, and all newly assigned markers are underlined. The order of the markers is presented relative to that predicted by genetic linkage analysis. Both RH maps are integrated with the genetic map but for ease of representation, the connections emanate only through T43RH. *Boxed markers:* Regions of SSC-X where marker order was resolved by RH analysis. *Genetic map:* Gene-based markers are indicated as non-bold text; microsatellite markers are displayed as bold, and all newly assigned markers are underlined. Meiotic map positions as predicted by Cri-Map version 2.4 are presented in conjunction with the existing SSC-X genetic map: (http://map.marc.usda.gov/genome/swine/swine.html); *Cytogenetic map:* Assignment of markers based on those reported at http://www.toulouse.inra.fr/lgc/pig/cyto/gene/chromo/SSCGX.htm. *QTL for testicular size and plasma FSH:* The vertical line to the left of the cytogenetic map delineates location of QTL reported by Rohrer *et al*. (2001).

previously unmapped markers improved the IMpRH map of SSC-X. Two-point analyses of the 7000-rad IMpRH panel data supports six linkage groups with a two-point LOD of 6.0. Markers were distributed relatively evenly across linkage groups (Fig. 1). All markers within the pseudoautosomal region (lower boundary begins between *SW961* and *SW980*) were in the same linkage group. Using minimum breakage criteria, a previously unlinked microsatellite marker, *SW1903* (Hawken *et al.* 1999), was incorporated into a linkage group containing two other microsatellite (*SW2534* and *SW2126*) and one EST-associated marker (*SAT*). The order on the original IMpRH SSC-X map of linkage groups 4 (*SW1426, SW2048, SW154, SW1346, SW1565*) and 5 (*SW259, SW1994, SW1835*) has been reversed, and marker order for linkage group 4 (previous group 5) has been refined with addition of 11 new markers. Comparison of locus order on these two RH maps revealed minor discrepancies, predominant in the centromeric region, likely resulting from different resolutions.

A total of 22 gene-based markers were amplified from the MARC reference population and sequenced. The SNPs were detected for 12 of these markers, and nine of these were successfully used for linkage mapping (eight listed in Table 1 plus *DAX1*). Previous placement of *PLP1* was confirmed. Collectively, 92 known markers have been assigned to at least one map, and each RH map has 45 markers in common with the genetic map (Table 2). Alignment of RH and genetic maps supported similar gene order on SSC-X (Fig. 1). The RH analysis information was useful for resolving marker order within several intervals where insufficient meioses had previously prevented definitive marker order (Fig. 1). Discrepancy of gene order between linkage and RH maps were restricted to segments corresponding to a short genetic distance and likely reflect limits in genetic map resolution or very localized rearrangements. Notably, marker order predicted from integrated RH maps revealed a discrepancy in the order of markers *SW1426* and *SW2476* on the original genetic map (Rohrer *et al.* 1996). In this case, a re-analysis of genetic linkage data supported reversal in marker order.

Table 2 Distribution of genetic markers across three maps of SSC-X.

Map title	MARC genetic map	T43RH	IMpRH
MARC genetic map	75 (9)	45	45
T43RH		55 (55)	42
IMpRH			55 (31)

Diagonal: Number of known markers assigned to each map.
Parentheses: Number of new markers assigned to each map.
Above the diagonal: Number of markers common to the two maps.

Discussion

This study represents the largest single assignment of both gene-based and microsatellite markers to SSC-X. The RH and linkage mapping in this study allowed rapid ordering of a large number of markers facilitating simultaneous integration with existing genetic, cytogenetic and RH maps. Localization of 21 previously unmapped gene-based markers, for which human homologies could be identified, allowed construction of a human-porcine comparative map that can be used for a positional candidate gene cloning approach in the hunt for genes responsible for economically important QTL. Overall, a complete syntenic relationship was observed between SSC-X and HSA-X. However, minor rearrangements in gene order cannot be discounted at this level of resolution. Expansion and integration of RH and linkage maps in this study facilitated generation of the densest map of SSC-X described to date.

A detailed genetic map of SSC-X is available (http://www.marc.usda.gov). In addition to being constrained by recombination rate, a genetic map that consists predominantly of microsatellite markers is of limited value for comparative mapping. Radiation hybrid mapping overcomes these limitations by facilitating rapid mapping of both gene-based and microsatellite markers enabling resolution of marker order and distance between closely linked markers. Because RH mapping does not require polymorphic markers, ability to associate genetic variability with potential QTL is not possible. Thus, an integrated RH and linkage mapping approach is valuable to accurately order gene loci and to identify potential candidate genes within QTL regions. The current RH maps identified an error in the original linkage map (Rohrer *et al.* 1996) that was subsequently revised (Rohrer *et al.* 2001).

There are substantially more genes mapped to HSA-X (~424 known genes) than SSC-X (~33; Fig. 2; Cepica *et al.* 2001; http://www.toulouse.inra.fr/lgc/pig/cyto/gene/chromo/SSCEX. htm). Alignment of these two maps provided strong evidence of conservation of gene order between porcine and human species (Fig. 2). A number of these genes reside in the QTL region that was identified for testicular size and differential pituitary gonadotropin secretion (Rohrer *et al.* 2001; Ford *et al.* 2001). Mutation of the androgen receptor gene (AR) has been associated with reduced testicular size, low sperm production and elevated gonadotropin secretion in humans (Bhasin *et al.* 2000; Yong *et al.* 2000). Nerve growth factor through its receptors (NADE) impacts testicular development (Levine *et al.* 2000). Thyroid hormone binding globulin (TBG or *SERPINA7*), a major transport protein for thyroid hormones in the circulation (Refetoff *et al.* 1970), regulates

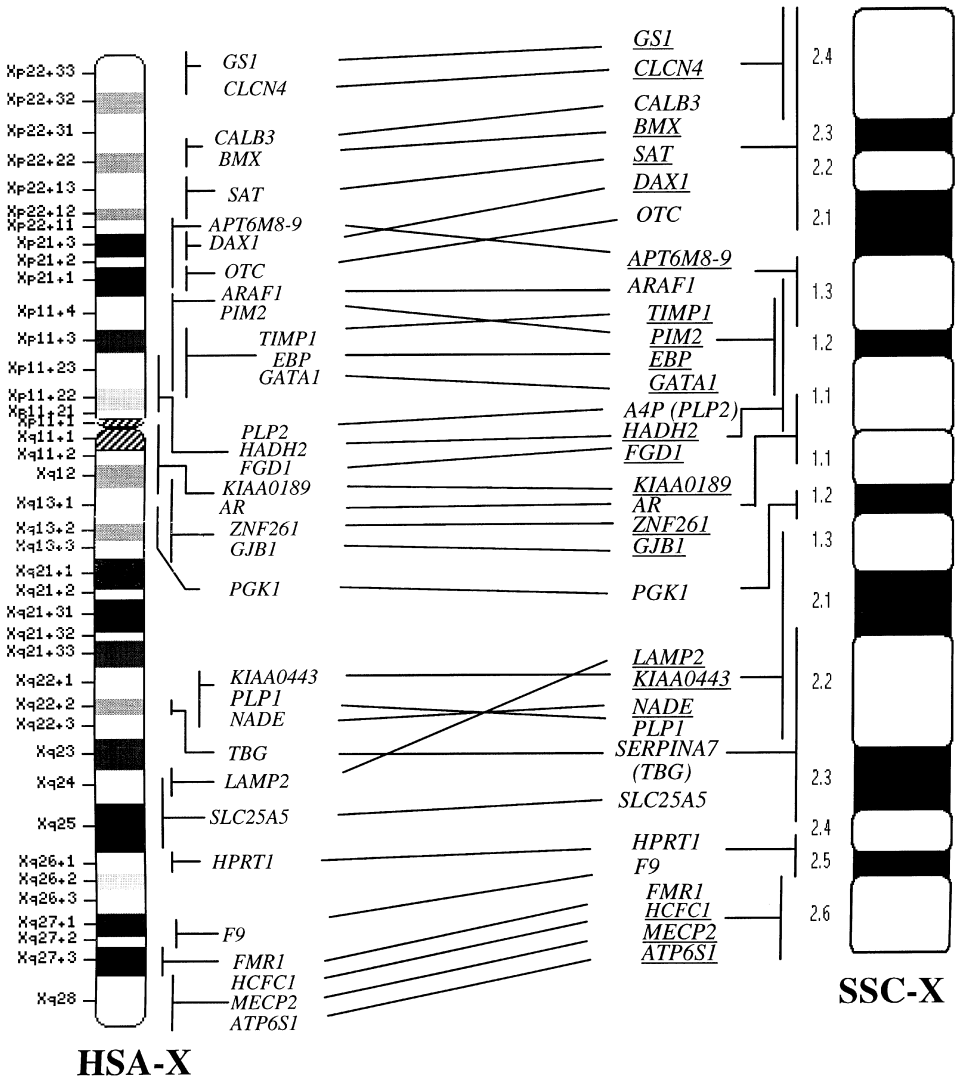

Figure 2 An integrated map of genetic markers assigned to porcine chromosome X (SSC-X) and compared with the order of these on human chromosome X (HSA-X). Newly assigned markers are underlined.

availability of thyroid hormones to target tissues, and in rodents, thyroid hormones directly influence testicular development (Cooke *et al.* 1993; De Franca *et al.* 1995). Additionally, GATA1 is expressed in mouse Sertoli cells in an age-specific manner (Ito *et al.* 1993; Yomogida *et al.* 1994) and can regulate testicular gene expression (Feng *et al.* 2000). Gap junction protein β1 (GJβ1 or connexin 32), a component of gap junctions, potentially affects Sertoli-germ cell communications within the testis (Risley 2000). In addition, there are genes still to be identified and characterized on chromosome X that affect testicular size (Wang *et al.* 2001).

In summary, this study highlights the value of an integrated approach to comparative mapping of chromosome X for ordering of genes along a chromosome and for identification of candidate genes for QTL. This approach enabled the construction of a high-resolution comparative map of SSC-X and HSA-X. Alignment of chromosome X maps of these two species predicts striking homology between genomes, and thus, facilitates employment of a positional candidate gene cloning approach to identify genes related to traits of interest for which QTL have been identified.

Acknowledgements

The authors acknowledge the skilful technical assistance of Jeni Kuzak, Tracy Happold, Linda Flathman, Bree Quigley

and Kris Simmerman. Part of this work was supported by a USDA grant USDA-NRI no. #99-35205-8619 (LJA).

References

Bhasin S., Mallidis C. & Ma K. (2000) The genetic basis of infertility in men. *Baillière's Best Practice and Research in Clinical Endocrinology and Metabolism* **14**, 363–88.

Cepica S., Rohrer G.A., Knoll A., Masopust M. & Malek O. (2001) Linkage mapping of four genes (OTC, SERPINA7, SLC25A5 and FMR1) on porcine chromosome X. *Animal Genetics* **32**, 106–9.

Cooke P.S., Kirby J.D. & Porcelli J. (1993) Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment: optimization of the propylthiouracil dose and effects of methimazole. *Journal of Reproduction and Fertility* **97**, 493–9.

De Franca L.R., Hess R.A., Cooke P.S. & Russell L.D. (1995) Neonatal hypothyroidism causes delayed Sertoli cell maturation in rats treated with propylthiouracil: evidence that the Sertoli cell controls testis growth. *Analytical Record* **242**, 57–69.

Ewing B. & Green P. (1998) Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Research* **8**, 186–94.

Ewing B., Hillier L., Wendl M.C. & Green P. (1998) Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Research* **8**, 175–85.

Fahrenkrug S.C., Smith T.P., Freking B.A. *et al.* (2002) Porcine gene discovery by normalized cDNA-library sequencing and EST cluster assembly. *Mammalian Genome* (in press).

Feng Z.M., Wu A.Z., Zhang Z. & Chen C.L. (2000) GATA-1 and GATA-4 transactivate inhibin/activin beta-B-subunit gene transcription in testicular cells. *Molecular Endocrinology* **14**, 1820–35.

Ford J.J., Wise T.H., Lunstra D.D. & Rohrer G.A. (2001) Interrelationships of porcine X and Y chromosomes with pituitary gonadotropins and testicular size. *Biology of Reproduction* **65**, 906–12.

Gordon D., Abajian C. & Green P. (1998) Consed: a graphical tool for sequence finishing. *Genome Research* **8**, 195–202.

Green P., Falls K. & Crooks S. (1990) *Documentation for CRI-MAP*. University School of Medicine, St Louis, WA.

Hawken R.J., Murtaugh J., Flickinger G.H., Yerle M., Robic A., Milan D., Gellin J., Beattie C.W., Schook L.B. & Alexander L.J. (1999) A first-generation porcine whole-genome radiation hybrid map. *Mammalian Genome* **10**, 824–30.

Heaton M.P., Grosse W.M., Kappes S.M., Keele J.W., Chitko-McKown C.G., Cundiff L.V., Braun A., Little D.P. & Laegreid W.W. (2001) Estimation of DNA sequence diversity in bovine cytokine genes. *Mammalian Genome* **12**, 32–7.

Hisamatsu N., Sawazaki T., Itoh T., Mikawa S., Kobayashi E., Wada Y. & Yasue H. (1997) Assignment of two microsatellites and extension of current linkage map on porcine chromosome X. *Proceedings of International Conference on Animal Biotechnology*, 36–41.

Hu Z., Rohrer G.A., Murtaugh M.P., Stone R.T. & Beattie C.W. (1997) Mapping genes to swine X chromosome provides reference loci for comparative mapping. *Mammalian Genome* **8**, 608–10.

Ito E., Toki T., Ishihara H., Ohtani H., Gu L., Yokoyama M., Engel J.D. & Yamamoto M. (1993) Erythroid transcription factor GATA-1 is abundantly transcribed in mouse testis. *Nature* **362**, 466–8.

Keele J.W., Wray J.E., Behrens D.W., Rohrer G.A., Sunden S.L., Kappes S.M., Bishop M.D., Stone R.T., Alexander L.J. & Beattie C.W. (1994) A conceptual database model for genomic research. *Journal of Computer Biology* **1**, 65–76.

Lange K., Boehnke M., Cox D.R. & Lunetta K.L. (1995) Statistical methods for polyploid radiation hybrid mapping. *Genome Research* **5**, 136–50.

Levine E., Cupp A.S. & Skinner M.K. (2000) Role of neurotropins in rat embryonic testis morphogenesis (Cord Formation). *Biology of Reproduction* **62**, 132–42.

Little D.P., Cornish T.J., O'Donnell M.J., Braun A., Cotter R.J. & Koster H. (1997) MALDI on a chip: analysis of arrays of low-femtomole to subfemtomole quantities of synthetic oligonucleotides and DNA diagnostic products dispensed by a piezoelectric pipette. *Analytical Chemistry* **69**, 4540–6.

Nickerson D.A., Tobe V.O. & Taylor S.L. (1997) PolyPhred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. *Nucleic Acids Research* **25**, 2745–51.

Parma P., Pailhoux E. & Cotinot C. (1998) Detection and characterization of a mutation in the porcine DAX1 gene. *Animal Genetics* **29**, 401–2.

Refetoff S., Robin N.I. & Fang V.S. (1970) Parameters of thyroid function in serum of 16 selected vertebrate species: a study of PBI, serum T4, free T4 and the pattern of T4 and T3 binding to serum proteins. *Endocrinology* **86**, 793–805.

Risley M.S. (2000) Connexin gene expression in seminiferous tubules of the Sprague-Dawley Rat. *Biology of Reproduction* **62**, 748–54.

Rohrer G.A., Alexander L.J., Hu Z., Smith T.P., Keele J.W. & Beattie C.W. (1996) A comprehensive map of the porcine genome. *Genome Research* **6**, 371–91.

Rohrer G.A., Alexander L.J., Keele J.W., Smith T.P. & Beattie C.W. (1994) A microsatellite linkage map of the porcine genome. *Genetics* **136**, 231–45.

Rohrer G.A., Wise T.H., Lunstra D.D. & Ford J.J. (2001) Identification of genomic regions controlling plasma FSH concentrations in Meishan-White composite boars. *Physiological Genomics* **6**, 145–51.

Wang P.J., McCarrey J.R., Yang F. & Page D.C. (2001) An abundance of X-linked genes expressed in spermatogonia. *Nature Genetics* **27**, 422–6.

Yerle M., Pinton P., Robic A. *et al.* (1998) Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenetics and Cell Genetics* **82**, 182–8.

Yomogida K., Ohtani H., Harigae H., Ito E., Nishimune Y., Engel J.D. & Yamamoto M. (1994) Developmental stage- and spermatogenic cycle-specific expression of transcription factor GATA-1 in mouse Sertoli cells. *Development* **120**, 1759–66.

Yong E.L., Lim J., Qi W., Ong V. & Mifsud A. (2000) Molecular basis of androgen receptor diseases. *Annals of Medicine* **32**, 15–22.