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Characterization and linkage mapping of 15 porcine STS markers to fine-map chromosomal regions associated with hernia inguinalis/scrotalis

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Source/description: Recently, a genome scan has revealed chromosomal regions associated with porcine hernia inguinalis/scrotalis on SSC3, 6, 7, 12 and 15.¹ In humans, it has been proposed that the smooth muscles around the processus vaginalis must undergo apoptosis to prevent congenital herniation.² In subsequent studies^{3,4} we successfully assigned genes involved in apoptosis to the regions. Here we report the identification and linkage mapping of region-specific STS markers.

Isolation of PAC clones and STS-marker characterization: The porcine P1 derived artificial chromosome library (PAC) TAI-GP714⁵ was screened by PCR with primers listed in Table S1. STS markers were isolated from the 13 clones by TOMMI⁶ (targeted oligonucleotide-mediated microsatellite identification). The evaluation of microsatellites and the size determination of alleles were performed on an ABI PRISM[®] 3100 DNA analyzer with ABI software GENESCAN (3.7) and GENOTYPER (3.6) using GeneScan[™] 500 ROX[™] as an internal size standard. Microsatellites were further evaluated by genotyping a DNA repository of 123 pigs of different breeds (Table S2).

Linkage mapping on the USDA-MARC reference panel: Genomic DNA (12.5 ng) from 95 animals of the USDA-MARC Swine Reference Population⁷ was amplified. Briefly, radiolabelled products were size-fractionated on 6% polyacrylamide denaturing gels and product sizes were determined using an M13 sequencing reaction. Polymorphism of the microsatellites was confirmed in nine parents of the MARC Swine Reference Population. Each marker was initially assigned to a chromosome

based on TWOPOINT results of CRIMAP,⁸ and then multipoint linkage analyses determined the final location of each marker. Genotypic data were evaluated with CROMPIC and corrections were made if necessary. The position reported (Table 1) is based on the current USDA-MARC swine linkage map.

Comments: Linkage-mapping results of the STS markers are presented in Table 1. Four markers (*S0894*, *S0898*, *S0899* and *S0903*) were either uninformative or could not be reliably genotyped on the MARC panel under standard conditions. RH mapping of the adjunct genes⁴ used for PAC library screening (Table 1) assigned *S0894*, *S0898* (W. Laenoi, K. Gatphayak, B. Brenig & C. Knorr, unpublished data) and *S0903* to SSC6 and *S0899* to SSC7. Excluding *S0897* and *S0900*, all other microsatellites were mapped to genomic regions associated with hernia inguinalis/scrotalis in pigs¹ and will be useful to fine-map these regions.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 PAC library screen information, including gene/clone ID, primer sequences, annealing temperatures and fragment sizes.

Table S2 Summary of breed sample sizes in DNA repository.

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Table 1 Characterization of porcine STS markers S0891–S0905.

Locus ID	Gene/clone (library TAIGP714)	Primer sequence (5'–3')	T _A (°C)	Allele sizes (bp)	Alleles ¹	Unique alleles (breed/bp size)	PIC	H _T	Linkage position ² (SSC: cM)	No. meioses ²	Closest marker ²	Accession no.
S0891 ³	SOX9/ M19053Q	AAACTCCCTGGCTGGTC TCAGAAATTCGGTACGCTCC	55	145–170	10/4	CT/195	0.80	0.82	12: 26.94	38	S0229/ SW957	AM950278
S0892 ³	SOX9/ M19053Q	GAGTTTGCAGATGATG AAGAGAAATGCAGGGGAA	63	160–192	13/3	RC/160 GL/192	0.78	0.80	12: 26.94	37	S0229/ SW957	AM950280
S0893	BCL2L1/ D06043Q	CTATCACAGCCAGCAACAGGA GCCATTCTGGCTCTTGACTG	53	161–163	2/2	N/A	0.22	0.31	3: 65.97	70	S0229/ SW957	AM981392
S0894	CARD8/ M19035Q	CACTGAGAGCCACAGACAAA CATACTCCTGCCCTCCAAAG	55	201–219	2/1	N/A	0.25	0.30	N/A	N/A	N/A	AM981394
S0895 ⁴	RPSAP4/ O12058Q	CAGGACAGGGTCTATTGTGA TAGAAACCTGCCCATTTGAGG	54	303–346	6/6	N/A	0.34	0.41	6: 106.47	156	SW353/ SW1055	AM981395
S0896 ⁴	RPSAP4/ O12058Q	GTCACCTGGTACCACGGTTA TTGGGGTATGGATGTTTGT	54	303–346	6/2	N/A	0.34	0.41	6: 106.47	131	SW353/ SW1055	AM981395
S0897	SW1409/ C20108Q	TTCTACAAATATTAGCATTCAGCA CAGGGAAAAGTGAAATGTGA	52	140–166	5/6	N/A	0.47	0.51	13: 68.58	78	SWR1008/ SWR2054	AM981397
S0898	BAX1/ C15114Q	CATGGTAAACCGCAGGATCT CCCTTGGAAAATCCCTCAA	53	215–217	2/1	RC/217	0.49	0.55	N/A	N/A	N/A	AM981399
S0899	ITPR3/ M06089Q	CCTCTGGTCCCTTGATAA GGCTGGGAACCTCCATA	56	172–178	4/–	CM/198 CT/202	0.29	0.32	N/A	N/A	N/A	AM981398
S0900	TAC1/ P15081Q	TGTTTGGAAAACATAAAGCCTCA TTTAGTTCAAATGTCAAGATTGATGATCA	52	100–126	6/6	N/A	0.23	0.42	9: 87.16	98	SW01/ SWR915	AM981396
S0901	CASP9/ L15049Q	CTTTGCACGCTGGTAAAGTT TGCACTCACAGGTTCAATTC	53	192–228	6/9	N/A	0.48	0.52	6: 87.39	168	SWR987	AM981391
S0902	COL1A1/ D12150Q	GCAGTTGCTTCAACTTCTG TCCATATGCTGGGAGTGTAGC	53	118–148	5/2	CT/118 CM/132	0.32	0.35	12: 65.36	83	SW168	AM981400
S0903	PDCD5/ D17091Q	CCCTAGAACCACCTCCACA GTGGTGTAGGTCGAAGACG	53	220–222	2/1	N/A	0.21	0.31	N/A	N/A	N/A	AM981393
S0904	SEPT4/ L10088Q	GTTGCACACTCCAACCTCCA CATATGCACTGCAGGTACCG	63	124–144	7/7	CM/124 CT/134 SHS/144	0.51	0.55	12: 69.2	155	SWR1802/ S0090	AM930852
S0905	ERN1/ A21137Q	TTTGTTTTGAAGTTGCATTGA TTGGGAAACTCTGATACCC	63	183–187	3/3	N/A	0.35	0.41	12: 35.27	102	SW957/ SW1307	AM930853

GL, German Landrace; SHS, Swabian Haellian Swine; CT, Chinese Tibetan; RC, Rongchang; CM, Chinese Minipig; PIC, polymorphism information content; H_T, heterozygosity.

¹Number of alleles in DNA repository/number of alleles in MARC Swine Reference Population.

²Results on the current MARC-USDA linkage map.

³Distance between S0891 and S0892: 5148 bp.

⁴Original sequence was split into two loci for the MARC-USDA linkage map. One locus was amplified for the DNA repository (5'-TATCTTCTGGTCCGCCACT-3' and 5'-TTGGGGGTATGGATGTTTTGT-3'). Distance between S0895 and S0896: 3 bp.