Assignment¹ of the sperm protein zona receptor tyrosine kinase gene (SPRMTK) to porcine chromosome SSC3q11→q12 by fluorescence in situ hybridization and by analysis of somatic cell and radiation hybrid panels

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¹ To our knowledge, this is the first time this gene has been mapped in swine.

Rationale and significance

The recognition of carbohydrate sequences by complementary receptors has been shown to be a critical factor when the plasma membrane of the sperm head binds to the zona pellucida (ZP), an extracellular coat surrounding the oocyte. ZP3, one of the three glycoproteins in the ZP, is the egg protein recognized by sperm. A human sperm surface protein, HUMSPRMTK (Mr = 95,000) homolog has been identified on the basis of its specific affinity for ZP3 (Burks et al., 1995). It is expressed only in testicular germ cells, which has been assigned to chromosome HSA2q. Detailed mapping and the availability of the primary sequence would further our understanding of the molecular basis of infertility and for development of a sperm-targeted contraceptive. The latter based on the fact that synthetic blocking of the intracellular domain inhibits phosphotyrosine activity (Leyton and Saling, 1989), thus preventing fertilization. Here we report the localization of the porcine sperm protein zona receptor tyrosine kinase gene to chromosome 3q11→q12.

Materials and methods

Isolation of the porcine SPRMTK gene from a PAC library

A porcine genomic PAC library (IVM PAC 714, Al-Bayati et al., 1999) was screened by PCR using primers A (forward: 5'-GCG AGA TGA CAT GAC TGT CT-3') and B (reverse: 5'-CTG TAC CCA TGG CAC GCA CA-3') designed from the *Homo sapiens* HUMSPRMTK-mRNA (EMBL accession no: L08961). Briefly, PCR amplifications were performed using 25 ng of porcine DNA in a total volume of 25 µl. The PCR program used was as follows: an initial denaturation step of 5 min at 95°C followed by 35 core cycles of 30 s at 95°C, 30 s at 57°C and 30 s at 72°C. Final step was an incubation of 10 min at 72°C.

Amplified products were separated on 1.5% agarose gels by electrophoresis for 1 h at 120 V in 0.5 BE buffer. Sequencing of the 254-bp PCR product confirmed sequence identity with *Homo sapiens* mRNA. The PAC library screened with the aforementioned primers identified a clone of approximately 150 kb harboring exons 1–7 of the porcine SPRMTK gene.

Fluorescence in situ hybridization (FISH)

FISH analysis was applied as described previously by Toldo et al. (1993) and Solinas-Toldo et al. (1995) using swine metaphase spreads (prepared from peripheral lymphocytes) obtained from a normal, healthy boar. Prior to FISH, the QFQ-banded spreads were photographed using a cooled CCD camera. Hybridization signals were detected and amplified by incubation with streptavidin-Cy3 (Rockland, Gilbertsville). The chromosomes were then DAPI counterstained (Sigma, Deisenhofen). The positions of the signals on chromosomes were measured considering relative fractional length from the short arm telomere to the hybridization signal enabling the calculation of the fractional length (Flqter).

Hybrid panel analyses

A porcine rodent somatic cell hybrid panel (Yerle et al., 1996) and a porcine whole genome radiation hybrid panel (Yerle et al., 1998) were screened for porcine SPRMTK by PCR. Primers (forward: 5'-GCG AGA TGA CAT GAC TGT CT-3') and (reverse: 5'-CTG TAC CCA TGG CAC GCA CA-3') were designed from exon 6 of *Homo sapiens* SPRMTK. PCR amplifications of a 254-bp fragment were performed in a total volume of 25 µl with 25 ng of panel DNA as template. Cycling conditions same as above. PCR results were evaluated using the interpreting web-pages <http://imprh.toulouse.inra.fr> (radiation hybrid panel) and <http://toulouse.inra.fr/lgc/pig/hybrid.htm> (somatic cell hybrid) at INRA.

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Results

Fluorescence in situ hybridization

Most precise location: SSC3q11 → (distal) q12

Flqter: 0.56 ± 0.03 (Fig. 1)

Chromosomes measured: 21

Mean chromosome length (μm): 4.78

Range: 3.55–7.64 μm

Standard deviation: 1.02 μm

Somatic cell hybrid panel

Somatic cell hybrid panel analysis of the 27 pig × rodent hybrid cell clones gave the following vector: 10100 11111 10000 11100 00100 00. Statistical evaluations revealed a significant correlation of 1.00 between SPRMTK and SSC3 (error risk lower than 0.5 % and maximum correlation of 0.78). Within SSC3, chromosome region q11 indicated the highest probability of 0.84 with a correlation of 0.77.

Radiation hybrid panel

Radiation hybrid panel analysis resulted in the following vector: 00000 10001 00100 00110 00101 10000 00101 10100 01000 00001 01010 00001 01000 00001 00010 00000 00001 00001 01110 01100 01101 10110 01011 010. The most significantly linked marker (two-point-analysis) is SWR978 (Rohrer et al., 1994) on SSC3 (39 cR; LOD Score of 11.08). Multi-point-analysis leads to linkage group SWR2069–SW2597–SWR978–SPRMTK–IL1B–SW1045–SW1436.

The cytogenetic localization of SW2597 (Alexander et al., 1996) and IL1B (Mellink et al., 1994) are 3q11 → q12 and 3q11 → q14 respectively, thus confirming the localization of SPRMTK to 3q11 → q12.

References

- Alexander LJ, Troyer DL, Rohrer GA, Smith TP, Schook LB, Beattie CW: Physical assignments of 68 porcine cosmid and lambda clones containing polymorphic microsatellites. *Mammal Genome* 7:368–372 (1996).
- Al Bayati HK, Duscher S, Kollers S, Rettenberger G, Fries R, Brenig B: Construction and characterization of a porcine P1-derived artificial chromosome (PAC) library covering 3.2 genome equivalents and cytogenetical assignment of six type I and type II loci. *Mammal Genome* 10:569–572 (1999).
- Burks DJ, Carballada R, Moore HD, Saling PM: Interaction of a tyrosine kinase from human sperm with the zona pellucida at fertilization. *Science* 269:83–86 (1995).
- Leyton L, Saling P: 95 kDa sperm proteins bind ZP3 and serve as tyrosine kinase substrates in response to zona binding. *Cell* 57:1123–30 (1989).
- Mellink C, Lahbib-Mansais Y, Yerle M, Gellin J: Mapping of the regulatory type I alpha and catalytic beta subunits of cAMP-dependent protein kinase and interleukin 1 alpha and 1 beta in the pig. *Mammal Genome* 5:298–302 (1994).
- Rohrer GA, Alexander LJ, Keele JW, Smith TP, Beattie CW: A microsatellite linkage map of the porcine genome. *Genetics* 136:231–245 (1994).
- Solinas-Toldo S, Mezzelani A, Hawkins GA, Bishop MD, Olsaker I, Mackinlay A, Ferretti L, Fries R: Combined Q-banding and fluorescence in situ hybridization for the identification of bovine chromosomes 1 to 7. *Cytogenet Cell Genet* 69:1–6 (1995).
- Toldo SS, Fries R, Steffen P, Neiberghs HL, Barendse W, Womack JF, Hetzel DJ, Stranzinger G: Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. *Mammal Genome* 4:720–727 (1993).
- Yerle M, Echard G, Robic A, Mairal A, Dubut-Fontana C, Riquet J, Pinton P, Milan D, Lahbib-Mansais Y, Gellin J: A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. *Cytogenet Cell Genet* 73:194–202 (1996).
- Yerle M, Pinton P, Robic A, Alfonso A, Palvadeau Y, Delcros C, Hawken R, Alexander L, Beattie C, Schook L, Milan D, Gellin J: Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenet Cell Genet* 82:182–188 (1998).

Fig. 1. Chromosomal assignment of SPRMTK by FISH. Signals were detected on porcine chromosome SSC3q11 → q12.