Assignment¹ of the porcine hyaluronidase-3 (HYAL3) gene to SSC13q21 by FISH and confirmation by hybrid panel analyses

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

Rationale and significance

Hyaluronidases are enzymes responsible for the breakdown of hyaluronan (HA). The human genome contains six hyaluronidase-like genes sharing about 40% amino acid identity with one another. The genes are organized into two groups: HYAL1, HYAL2, and HYAL3 are clustered on HSA3p21.3 and are equivalent to the genes for tumor suppressor for small cell lung carcinoma LUCA1, LUCA2, and LUCA3, respectively (Wei et al., 1996). This cluster has been reported to be part of the Tumor Suppressor Genes clone (TSGs) spanning ~ 370 kb containing the gene encoding putative tumor suppressor protein FUS2 (Lerman and Minna, 2000). The other group containing HYAL4, SPAM1/PH-20 and the expressed pseudogene HYALP1 was assigned to HSA7q31.3. Knowledge about the genes' functions resulted from the identification of mutations in the HYAL1 gene causing mucopolysaccharidosis type IX in man. In this report we document the mapping of the porcine HYAL3 gene by FISH and hybrid panel analyses and reveal the location on SSC13q21.

Materials and methods

Isolation of a PAC clone harboring the porcine HYAL3 gene

The porcine PI-derived artificial chromosome library (IVM PAC 714) constructed in pCYPAC2 was screened by PCR (Al-Bayati et al., 1999). The PCR for isolation of a single clone harboring HYAL3 was performed with HL3a primers (forward: 5' GAT TGG GAG GAG TGG TGT C 3' and reverse: 5' GAG GTA GAT GCT GGG GAA G 3') generating a 363-bp fragment. The primers were designed based on the published human LUCA14 DNA clone of 37 kb length (Wei et al., 1996; accession no. U73167). PCR conditions included an initial 3 min denaturation at 92 °C followed by 34 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. A positive signal was selected and the 316-bp fragment was sequenced showing 87% homology to the human HYAL3 gene, accession no. NM 003549). The isolated DNA was linearized with *SacI*, *BamHI*, *XbaI*, *PstI*, and *HindIII* (New England Biolabs, Schwalbach). Fragments containing the HYAL3 gene were confirmed by Southern Blot hybridization using the PCR fragment as a probe and cloned into the multiple cloning site of the pGEM4Z vector (Stratagene, Amsterdam). Various subclones of the PAC were generated by further linearization with restriction enzymes and sequenced to obtain the complete genomic sequence of the porcine HYAL3 gene. The full size of the original PAC-clone (0056/O11) was determined by cleavage with *NotI* and the resulting fragments were separated by pulsed field gel electrophoresis (CHEF-DR II; BIO-RAD, Munich) on a 1.0% agarose gel.

Hybrid panel analyses

The radiation hybrid panel (ImpRH panel) and a somatic cell hybrid panel were used for physical mapping of the HYAL3 gene (Yerle et al., 1996, 1998). HL3b primers (forward: 5' GAC AGT AGT AGC CTA AGC CCA 3' and reverse: 5' GTC TGT CTA GTT CAC TAC TGC 3') from intron 1 of the porcine HYAL3 sequence were used to amplify a 425-bp PCR fragment on 118 clones of the ImpRH panel and on 27 clones of the somatic cell hybrid panel including hamster, mouse and pig genomic DNA as control (the PCR conditions were as above). Obtained PCR results were evaluated with the INRA Toulouse web site, available at <http://imprh.toulouse.inra.fr> for RH panel and with <http://www.toulouse.inra.fr/lgc/pig/hybrid.htm> (somatic cell hybrid panel).

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Fig. 1. Chromosomal assignment of porcine HYAL3 by FISH.

Fluorescence in situ hybridization (FISH)

FISH analysis was performed as described 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 relative positions of the signals on the chromosomes were measured considering the distance to the telomere and the length of the entire chromosome enabling the calculation of the fractional length (Flqter).

Results and discussion

Fluorescence in situ hybridization

Precise localization: 13q21 (Fig. 1)

Number of chromosomes measured: 27

Flqter: 0.78 ± 0.03

Mean chromosome length: $6.22 \mu\text{m}$

Range: $4.26\text{--}8.73 \mu\text{m}$

Standard deviation: $0.99 \mu\text{m}$

Somatic cell hybrid panel

The physical mapping of HYAL3 with the somatic cell hybrid panel analysis gave the following vector as result: 01100 11010 01010 10000 01000 00. Statistical analysis showed a significant correlation between HYAL3 and SSC13 of 0.99 (error risk about 0.5%, maximal correlation 0.67). The most probable region within chromosome 13 is $q42 \rightarrow (1/2) q46$.

Radiation hybrid panel

The RH panel analysis resulted in the vector 11011 00001 01000 11001 11000 01011 01000 00000 00010 00000 10000 10010 00010 00000 10001 01000 00000 00001 11000 01011 10100 00001 10010 100. The evaluation data (two point analysis) showed that the most significant linked markers are SSC24F05 and SW864 on SSC13 (17 cR and 33 cR distance, LOD-scores were 16.23 and 12.46, respectively). Multipoint analysis led to linkage group SW1400–SW864–SSC24F05–HYAL3–SW1495–SW882–SW992–SW2459. STS-marker SW1400 (Alexander et al., 1996) and EST SSC24F05 in the DAG1-gene (Jorgensen et al., 1997; Thomsen et al., 1998) were assigned to SSC13q21 $\rightarrow$ q22 and SW2459 to SSC13q31 (Alexander et al., 1996), verifying the localization of the HYAL3 gene to SSC13q21 $\rightarrow$ q22.

Pulsed-field gel analysis showed that PAC clone 0056/O11 contains a single *NotI* restriction site and therefore most likely harbors further genes or even the complete gene cluster consisting of HYAL1, 2, and 3, according to the previously established human HYAL gene family which clusters on HSA3p21.3 (Kreil, 1995; Csoka et al., 2001). According to the ~ 370 kb TSGs deletion region studied by Lerman and Minna (2000) the cluster contains a set of 19 genes, CACNA2D2–PL6–101F6–NPRL2–ZYMND10–RASSF1–FUS1–HYAL2–HYALI–FUS2–HYAL3–IFRD2–SEMA3B–GNI2–SLC38A3–GNAT1–SEMA3F–RBM5–RBM6. The sequence characterization of PAC-clone 0056/O11 revealed the identical gene order of this cluster in pigs and humans with respect to FUS1–HYAL2–HYALI–FUS2–HYAL3. The porcine LUCA1 gene was recently mapped to SSC13q23 $\rightarrow$ (1/2)q41 by Lahbib-Mansais et al. (1999) using the somatic cell hybrid panel. The porcine FUS1 gene has been mapped by somatic cell hybrid panel analysis to SSC13q23 $\rightarrow$ (1/2)q41 (Gatphayak et al., 2003). Considering the gene order in the cluster, the somatic cell hybrid panel results for LUCA1, FUS1, and HYAL3 seem to be in concordance, however, RH-mapping assigns the cluster more proximal on the chromosome and the FISH image proves the exact localization of HYAL3 on SSC13q21. We presume, the differences in localization are due to the limited resolution power of the somatic cell hybrid panel. Other than this, the results are in total agreement with the available comparative mapping data between HSA3 and SSC13 (<http://www.toulouse.inra.fr/lgc/pig/compare/SSCHTML/SSC13B.HTM>).

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