Assignment¹ of the β -N-acetylhexosaminidase gene (HEXB) to porcine chromosome SSC2q21 $\rightarrow$ q22 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 pig.

Rationale and significance

The enzyme β -N-acetylhexosaminidase B (EC: 3.2.1.52) is a tetramer of two β -a and two β -b chains which are derived from the cleavage of a precursor chain, which is a product of the β -N-acetylhexosaminidase B gene (HEXB) (Mahuran, 1999). It catalyses hydrolysis of terminal non-reducing N-acetyl-D-hexosamine residues. Mutations in HEXB gene cause Sandhoff disease in humans (Srivastava and Beuter, 1973). The human HEXB gene stretches over 37 kb and consists of 14 exons (Proia, 1988). Human HEXB has been assigned to chromosome 5q13 (Boedecker et al., 1975). Here we report the localization of the porcine HEXB gene to chromosome 2 confirming homology between the q arms of chromosomes HSA5 and SSC2.

Materials and methods

Isolation of the porcine HEXB gene from a PAC library

PCR amplification to generate a probe for screening a porcine genomic PAC library (Al-Bayati et al., 1999) was done with primers A (forward: 5'-GGA AGC TAT TCT TTG TCT CAT GT-3') and B (reverse: 5'-CTT TTC

CCC AAG ACC GTG AAT-3') designed from the porcine HEXB mRNA (EMBL accession no: X92379). PCR amplifications were performed using 50 ng of porcine DNA in a total volume of 25 μ l. DNA was preheated at 95 °C for 5 min. The following PCR profile was used: 35 cycles of 94 °C for 60 s, 63 °C for 60 s and 72 °C for 60 s. The final cycle had an extension time of 10 min. Sequencing of the 132-bp PCR product confirmed sequence identity with the porcine mRNA. A PAC library screen with the above mentioned primers identified a clone of approximately 170 kb harboring exons 1–13 of porcine HEXB.

Fluorescence in situ hybridization (FISH)

FISH was performed 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 HEXB by PCR. Primers (forward: 5'-GGA AGC TAT TCT TTG TCT CAT GT-3' and reverse: 5'-CTT TTC CCC AAG ACC GTG AAT-3') originated from exon 8 of porcine HEXB. PCR amplifications of a 129-bp fragment were performed in a total volume of 25 μ l with 20 ng of panel DNA as template. Cycling conditions were 95 °C for 60 s, 63 °C for 60 s and 72 °C for 60 s for 35 cycles. PCR involved a preheating step at 95 °C for 5 min plus a final extension at 72 °C for 10 min. 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 IRNA.

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Fig. 1. Chromosomal assignment of HEXB by FISH. Signals were detected on porcine chromosome SSC2q21 → q22.

Results

FISH

Most precise location: SSC2q21 → (distal) q22

Flqter: 0.40 ± 0.04 (Fig. 1)

Chromosomes measured: 30

Mean chromosome length: $7.1 \mu\text{m}$

Range: $5.6\text{--}9.6 \mu\text{m}$

Standard deviation: $1.1 \mu\text{m}$

Somatic hybrid cell panel

Somatic cell hybrid panel analysis of the 27 pig × rodent hybrid cell clones gave the following vector: 00000 10000 00010 00000 10001 00. Statistical evaluations revealed a significant correlation of 1.00 between HEXB and SSC2 (error risk lower than 0.5% and maximum correlation of 1.00). Within SSC2, chromosome region q11 → (1/2 q21) indicated the highest probability of 0.79 with a correlation of 1.00.

Radiation hybrid panel

Radiation hybrid panel analysis resulted in the following vector: 00000 00000 01000 00000 00000 01100 00000 00100 00000 00000 01001 00010 00000 00000 00000 00000 10111 00000 01010 10010 10000 00000 00010 010. The most significantly linked marker (two-point-analysis) is SW766 on SSC2 (108 cR and LOD Score of 2.26), which is in agreement with the known homology between the q arms of chromosome HSA5 and SSC2. Multi-point-analysis leads to linkage group SW2442–ADM–SW1466–SSC2F05–SWR1342–SW776–SW395–SW1655–HEXB–SW834–S0091–SW14. The cytogenetic localization of SW2442 is SSC2q21 (Alexander et al., 1996), confirming the localization of HEXB to SSC2q21 → q22.

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