Assignment¹ of the β -glucuronidase (GUSB) gene to porcine chromosome SSC3p16→p14 by FISH and confirmation by hybrid panel analyses

J. Beck,^a C. Knorr,^a F. Habermann,^b R. Fries^b and B. Brenig^a

^aInstitute of Veterinary Medicine, Georg-August-University of Göttingen, Göttingen;

^bInstitute of Animal Breeding and Molecular Genetics, Technical University of Munich, Munich (Germany)

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

Rationale and significance

The acid hydrolase GUSB (β -D-glucuronoside glucuronosyl hydrolase, E.C. 3.2.1.31) plays an essential role in catabolism of glucuronic acid containing glycosaminoglycans (GAGs). It cleaves β -glucuronosyl residues at the non-reducing end of oligosaccharides from GAGs. GUSB degrades sulphated glycosaminoglycans such as heparan, dermatan and chondroitin and is involved in the catabolic pathway of hyaluronic acid. GUSB deficiency causes the lysosomal storage disorder Mucopolysaccharidosis type VII. Clinical and biochemical findings of MPSVII in human were first described by Sly et al. (1973). Fyfe et al. (1999) reported a G→A transition at position 1074 of an MPSVII affected cat GUSB cDNA. GUSB is already assigned to human chromosome 7q21.11. Here we report the localization of the porcine GUSB gene to chromosome SSC3p16→p14 by FISH and confirm the position by screening of hybrid panels.

Materials and methods

Isolation of the porcine GUSB gene from a PAC-library

PCR amplification to generate a probe for screening of a porcine PAC-library (Al Bayati et al., 1999) was done with primers adopted from Fyfe et al. (GenBank Accession No. AF012424). The primers 1 (5'-GTC TGT GTC

TGA CTT CTA CAC TCT C-3') and 2 (5'-GCG GTC ACA GGC TGC ATC ACC T-3') derived from exon 6 and 7, spanning intron 6. PCR conditions were 30 cycles of 95 °C for 50 s, 62 °C for 60 s and 72 °C for 30 s with an initial denaturing of the DNA for 5 min at 95 °C and a final extension step at 72 °C for 5 min. 50 ng of porcine DNA in a total volume of 25 μ l were used for PCR amplification. The resulting fragment of 504 bp was blunt-end cloned in a pGEM-T Vector System (Promega, Madison) and bidirectionally sequenced. A similarity of 93% between the exon sequences of the probe and the feline GUSB mRNA verified sequence identity. A PAC clone of approximately 120 kb was isolated from the PAC-library by PCR screening with the primers mentioned above. Various subclones of the PAC were generated to obtain the complete genomic sequence of the porcine GUSB gene.

Fluorescence in situ hybridization

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 GUSB by PCR. Primers 3 (5'-TGA GTT GCT CCC CGC CCC CGT-3') and 4 (5'-TCG GAT CTA GGG GAC AGC AGA ACA G-3') were designed from intron 6 included in the pGEM-T cloned 504-bp fragment as described above. PCR amplifications with these primers led to a 245-bp fragment and were performed in a total volume of 25 μ l using 25 ng of panel DNA as template. PCR conditions were 95 °C for 50 s, 62 °C for 60 s and 72 °C for 30 s repeated in 30 cycles with an initial denaturing of DNA for 5 min at 95 °C and a final extension step at 72 °C for 5 min. PCR results were evaluated using the interpreting webpages at INRA: <http://imprh.toulouse.inra.fr> for radiation hybrid panel and <http://www.toulouse.inra.fr/lgc/pig/hybrid.htm> for somatic cell hybrid panel.

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Corresponding author: Dr. Bertram Brenig, Institute of Veterinary Medicine
Groner Landstrasse 2, D-37073 Göttingen (Germany)
telephone: +49-551-393383; fax: +49-551-393392; e-mail: bbrenig@gwdg.de

Fig. 1. Chromosomal assignment of GUSB by FISH. Detection of signals on porcine chromosome SSC3.

Results

Fluorescence in situ hybridization

Most precise location: SSC3p15→p14

Number of chromosomes measured: 30

Flqter: 0.89 ± 0.04 (Fig. 1)

Somatic cell hybrid panel

Somatic hybrid panel analysis gave the following vector: 11000 11111 11000 11010 00000 00. Statistical evaluations revealed a significant correlation of 1.0 between the GUSB gene and SSC3 (error risk lower than 0.1 % and maximum correlation of 1.0). Within SSC3, chromosomal region p17→p16 indicated the highest probability of 0.87 with correlation of 1.0.

Radiation hybrid panel

Radiation hybrid panel analysis resulted in the following vector: 00001 10000 11100 01110 00000 00000 00100 00010 00100 10110 01000 10101 10010 00001 00010 10000 00001 11000 01000 10001 11101 10000 11010 100. The most significantly linked marker (two-point-analysis) is SW251 on SSC3 (55 cR and LOD Score of 7.79). Multipoint-analysis led to linkage group SW2429–SW833–SW72–GUSB–SW251–SW1443–SW0206. Whereas SW2429 maps to SSC3p15 and SW0206 maps to SSC3p14, this verifies the localization of porcine GUSB to SSC3p16→p14.

Comments

Further chromosome paintings between human and pig suggest that porcine chromosome SSC3pter→p15 is homologous to human 7q21→qter. Therefore our results in mapping of GUSB reported here concur with that evidence.

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