Assignment¹ of the CALC-A/ α -CGRP gene (CALCA) to porcine chromosome SSC2p13→p11 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

Calcitonin and α -CGRP are products of the alternatively spliced gene CALCA. Calcitonin (CALC) mRNA is predominantly found in thyroïdal C cells and mRNA encoding the neuropeptide calcitonin gene-related peptide (CGRP) in neural tissues (Amara et al., 1982, Rosenfeld et al., 1983). In vivo, calcitonin reduces serum calcium and acts as an antagonist to the parathyroid hormone. The human CALC-A/ α -CGRP gene stretches over 7.5 kb and consists of six exons (Steenbergh et al., 1986, Broad et al., 1989). CALCA and CALCB, the pseudogene CALCP, and the gene encoding the islet amyloid polypeptide IAPP are members of the calcitonin/CGRP family. Human CALCA, CALCB, and CALCP have been assigned to chromosome HSA11p15.2→p15.1 (Hoovers et al., 1993). Here we report the localization of the porcine CALC-A/ α -CGRP gene to chromosome 2 confirming homology between the p arms of chromosomes HSA11 and SSC2.

Materials and methods

Isolation of the porcine CALC-A/ α -CGRP gene CALCA

PCR amplification to generate a probe for screening of a porcine genomic phage library (Stratagene) was done with primers A (forward: 5'-CCC TCA CTC TTA CCT CTA ACC-3') and B (reverse: 5'-AGC TAA GCG GTG CAG TAA TC-3') designed from the canine CALCA mRNA (EMBL accession no: X56994). PCR amplification was performed using 50 ng of porcine DNA in a total volume of 25 μ l. DNA was preheated at 95 °C for 2 min. The following PCR profile used was: 30 cycles of 95 °C for 60 s, 55 °C for 60 s, and 72 °C for 60 s. The final cycle had an extension time of 10 min. The resulting fragment of 398 bp was bidirectionally sequenced (with tagged primers) and a similarity of 86 % between the probe and the canine mRNA confirmed the sequence identity. A GenBank screen with the above mentioned primers identified a clone of approximately 12 kb harboring exons 1 to 5 of porcine CALCA.

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 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).

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 CALCA by PCR. Primers (forward: 5'-CTG AAG CCA TGA GAG CTT TC-3'; reverse: 5'-CAA TAC AGG CTC TAA GCC AC-3') originated from intron 2 of porcine CALCA. PCR amplifications of a 212-bp fragment were performed in a total volume of 25 μ l with 25 ng of panel DNA as template. Cycling conditions were 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 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://www.toulouse.inra.fr/lgc/pig/hybrid.htm> (somatic cell hybrid) at INRA.

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Fig. 1. Chromosome assignment of CALCA by FISH. (A) Q-banding of the metaphase spread. (B) Detection signals on porcine chromosome SSC2.

Results

Fluorescence in situ hybridization

Most precise location: SSC2p13→p11

Flqter: 0.70 ± 0.057 (Fig. 1)

Chromosomes measured: 19

Somatic cell hybrid panel

Somatic hybrid panel analysis of the 27 pig × rodent hybrid cell clones gave the following vector: 00000 10000 00000 00000 11101 00. Statistical evaluations revealed a significant correlation of 0.99 between CALCA and SSC2 (error risk lower than 0.5% and maximum correlation of 0.87). Within SSC2, chromosome region p17→p14 indicated the highest probability of 0.78 with a correlation of 0.8748.

Radiation hybrid panel

Radiation hybrid panel analysis resulted in the following vector: 00000 10000 01000 01000 00000 01101 00000 00000 10010 00000 10110 00101 11000 00100 00100 10111 11010 01000 11000 10101 11111 01100 11011 110. The most significantly linked marker (two-point-analysis) is PTH3 on SSC2 (25 cR and LOD Score of 15.60), which is in agreement with the tight linkage between these genes observed in humans (Holm et al., 1985). Multi-point-analysis leads to linkage group SW2167–S0170–SW1026–SW747–CALCA–SW1857–PTH3–SWR1338–SW2442–ADM. The cytogenetic localization of S0170 is SSC2p11 (Ellegren et al., 1994) confirming the localization of CALCA to SSC2p11→p13.

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