Assignment of cellular retinoic acid-binding protein 1 (*CRABP1*) and 2 (*CRABP2*) to porcine chromosome 7q12→q23 and 4q21→q23 by somatic cell and radiation hybrid panel mapping

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Rationale and significance

CRABPs (cellular retinoic acid binding proteins) belong to members of a superfamily of lipid-binding proteins (Mansfield et al., 1998) that are thought to act by maintaining tolerable concentrations of intracellular RA (retinoic acid), as modulators of RA catabolism (Gorry et al., 1994) and as intracellular transporters for RA from the cytoplasm to the nuclear receptor (Flagiello et al., 1997). Based on their significance roles in binding lipids, they are thought to be candidate genes for meat quality related traits in domestic animals. The mapping of the porcine *CRABP1* and *CRABP2* genes are one step towards further investigation on their possible roles in meat quality traits in pig.

Materials and methods

In order to design primer sets for mapping the porcine *CRABP1* and *CRABP2*, mRNA sequences from human (GenBank accession no. NM_004378), mouse (GenBank accession no. NM_013496), rat (GenBank accession no. XM_236253), cattle (GenBank accession no. NM_181028)

were aligned for *CRABP1* and sequences from human (GenBank accession no. NM_001878), mouse (GenBank accession no. NM_007759), rat (GenBank accession no. NM_017244) were aligned for *CRABP2* using ClustalW (<http://www.ebi.ac.uk/clustalw>) program. Two primer sets, one for *CRABP1* and the other for *CRABP2*, were selected from the consensus sequences (*CRABP1*-F; 5'-TTCGCAGGCACCTGGAAGAT-3', *CRABP1*-R; 5'-GTCCACTGTCTCCTCTTCAA-3' and *CRABP2*-F; 5'-GCTGAGGAAGAT-TGCTGTGG-3', *CRABP2*-R; 5'-AATTCTCTGGTCCAGGAGGT-3'). PCR was performed in a total volume of 25 µl containing 25 ng of template DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM MgCl₂, 0.2 µM of each primer, 100 µM of each dNTP and one unit of *Taq* polymerase (Applied Biosystems, CA, USA). Reaction profiles included a 10-min denaturation step at 94 °C followed by 38 cycles, each consisting of 30 s denaturation at 94 °C, 30 s annealing at 68/69 °C, 40 s of extension at 72 °C, and then a final 10-min extension step at 72 °C using a PTC-100 Programmable Thermal Controller (MJ Research, Inc., USA). To confirm further the PCR products, DNA sequencing was carried out on an Applied Biosystems 377 DNA sequencer and analyzed with SedEd software (Applied Biosystems, CA, USA). Chromosomal localizations of *CRABP1* and *CRABP2* were detected by PCR analysis of a porcine × rodent somatic cell hybrid panel (Yerle et al., 1996) as well as a porcine whole genome radiation hybrid panel (Yerle et al., 1998). PCR results were analyzed using the interpreting web pages at INRA (<http://www.toulouse.inra.fr/lgc/pig/per/pcr.htm>, and <http://imprh.toulouse.inra.fr>).

Results

Regional mapping results

Analysis of the 27 hybrid clones from the somatic cell hybrid panel produced the following vector: 00000 00001 11000 10000 10101 01, which assigned the *CRABP1* gene to SSC7 with the highest probability and correlation values (100%) for the region 7q12→q23. *CRABP2* gave the following

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vector: 10000 10010 01001 00111 00001 01, which mapped to SSC4 with the highest probability and correlation values (92 %) for the region 4q21 → q23. The radiation hybrid panel showed the following distributions of positive and negative amplifications within the 118 clones: 00101 00000 10000 00000 01000 01000 00000 00000 00100 00100 00000 00000 00000 00000 01100 01000 00000 00000 00001 00001 00001 00001 100 for *CRABP1*, with the most significantly linked marker (LOD score 3.99) being SW175 (80 cRs away) on SSC7 and 00001 10010 01000 11111 01000 10001 10000 00000 00001 01010 00010 00000 00010 01000 01001 00000 00100 01001 11000 00000 01101 00100 10011 110 for *CRABP2*, with the most significantly linked marker (LOD score 14.50) being SW286 (26 cRs away) on SSC4 (Hawken et al., 1999). The assignment of porcine *CRABP1* and *CRABP2* to 7q12 → q23 and 4q21 → q23 is consistent with comparative human locations at HSA15q24 and 1q21.3, respectively (Fröncke et al., 1996). Previous results indicated that SSC7q and SSC4q contained fat and glucose related quantitative traits loci (Marklund et al., 1999; Pérez-Enciso et al., 2000; De Koning et al., 2001); *CRABP1* and *CRABP2* are possible positional candidate genes.

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