Assignment¹ of protein kinase, AMP-activated, beta 2 non-catalytic subunit (PRKAB2) gene to porcine chromosome 4q21→23 by somatic cell and radiation hybrid panel mapping

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

Rationale and significance

PRKAB2 (protein kinase, AMP-activated, beta 2 non-catalytic subunit) belongs to a protein kinase family that has a key role in regulation of energy metabolism in eukaryotic cells. PRKAB2 is activated by an increase in the ratio of adenosine monophosphate to adenosine triphosphate. Activated PRKAB2 turns on ATP producing pathways and inhibits ATP-consuming pathways (Thornton et al., 1998). PRKAB2 can inactivate by phosphorylation glycogen synthase, the key regulatory enzyme of glycogen synthesis (Hardie et al., 1998), and stimulate fatty acid oxidation (Yamauchi et al., 2003). Therefore, it plays significant roles in fat and glucose metabolism, which are the major determinants for meat quality in domestic animals. The mapping of the porcine PRKAB2 gene is one step towards further investigation on its possible role in meat quality traits in pigs.

Materials and methods

In order to design a primer set for mapping the porcine PRKAB2, two PRKAB2 cDNA sequences from rat (Genbank accession no. AF182717) and human (Genbank accession no. NM_005399) were aligned using Blast2 Sequences (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>).

Information for the exon-intron organization of PRKAB2 was collected from a *Homo sapiens* chromosome 1 working draft sequence segment (Genbank accession no. NT_004434). Two primer sequences conserved in rat and human were selected from each exon 3 and 4 (namely PRKAB2-F with the sequence 5'-TGT CCA TCC ACA AAG AAC TTG-3' and PRKAB2-R with 5'-ATT GGT CTT TCA ACA ATT GGA-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), 0.5 mM MgCl₂, 0.2 µM of each primer, 100 µM of each dNTP and one unit of Taq polymerase (Promega, USA). Reaction profile included a 5-min of denaturation step at 94 °C followed by 35 cycles, each consisting of 30 s denaturation at 94 °C, 30 s annealing at 60 °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).

A 940-bp product containing a part of exon 3, whole intron 3 and a part of exon 4 of the porcine PRKAB2 was yielded from the PCR. To confirm further the PCR product, DNA sequencing was carried out on an Applied Biosystems 377 DNA sequencer and analyzed with SeqEd software (Applied Biosystems, CA, USA). With the primer set, chromosomal localization of the PRKAB2 gene was 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/pcr/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: 10000 10010 01000 00111 00001 01, which assigned the PRKAB2 gene to

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SSC4 with the highest probability and correlation values (89 and 100%) for the region q21 → 23. The radiation hybrid panel showed the following distribution of positive and negative amplifications within the 118 clones: 00000 10010 01000 01111 00000 00001 00101 01000 10011 00011 11101 11000 01000 01000 01010 11000 00101 00000 00010 00000 10101 00100 10001 110, with the most significantly linked marker (LOD score 9.13) being SW512 (47 cRs away) on SSC4 (Hawken et al., 1999). The assignment of porcine PRKAB2 to 4q21 → q23 is consistent with the Zoo-FISH determined homology between HSA1 and SSC4 (Fronicke et al., 1996). Since SSC4q contained fat and glucose related quantitative traits loci (Marklund et al., 1999; Pérez-Enciso et al., 2000), the PRKAB2 is a possible positional candidate gene.

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