Assignment¹ of two isoforms of the AMP-activated protein kinase γ subunits, PRKAG1 and PRKAG2 to porcine chromosomes 5 and 18, respectively by radiation hybrid panel mapping

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¹ To our knowledge this is the first time that these genes have been mapped in the pig.

Rationale and significance

The AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme complex comprising catalytic α -subunits and regulatory β - and γ -subunits (Hardie et al., 2003). Each subunit exists as different isoforms encoded by two or three genes ($\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$, $\gamma 3$). AMPK has a key role in the regulation of energy metabolism in eukaryotic cells. AMPK is regulated by phosphorylation on the α -subunit and by AMP allosteric control thought to be mediated by both α - and γ -subunits (Adams et al., 2004).

A $\gamma 1$ mutation, R70Q, causes a loss of AMP dependence (Hamilton et al., 2001). Mutations in human $\gamma 2$ and pig $\gamma 3$ genes were previously identified to cause an unusual cardiac phenotype and a glycogen storage disease, respectively (Gollob et al., 2002; Milan et al., 2000a). The mapping of the porcine PRKAG1 and PRKAG2 genes is the first step for further investigations helping to understand the regulation of the AMP-activated protein kinase family, an enzyme family with relevance to type 2 diabetes and the metabolic syndrome.

Materials and methods

To map the porcine PRKAG1 and PRKAG2 genes the sequences from humans (GenBank accession no. NM_002733, NM_016203) were used for identifying porcine ESTs (GenBank accession no. BI401973, BI326904) in order to design primers. The primers were developed out of conserved regions between humans and mice

(PRKAG1, F 5'-AGACGTTTGTGTGCCTGTG-3', R 5'-ATCCTCG-CACCTTGAGGAAAA-3'; PRKAG2, F 5'-CTTGCTGCGAGAAAACATAC-3', R 5'-ATCTCCAGCTTACTGCACTT-3'). The product sizes for PRKAG1 and PRKAG2 were 201 bp and 146 bp, respectively.

PCR for ImpRH mapping was performed in a total volume of 15 μ l containing 10 ng of template DNA, 1.5 μ l of Invitrogen Standard buffer, 1.5 mM MgCl₂, 0.2 μ M of each primer, 200 μ M of dNTPs, and 0.3 units of Taq polymerase (Invitrogen). PCR were conducted on a MJ Research PTC 200 thermocycler started with 3 min of denaturation at 94 °C followed by 40 cycles of amplification. Each cycle consists of 30 s denaturation at 94 °C, 1.30 min annealing at 62 °C for PRKAG1 and 58 °C for PRKAG2 and a 2 min extension at 72 °C. Further, a final extension step for 10 min at 72 °C was performed.

The 118ImpRH (Yerle et al., 1998) served for high resolution gene mapping along with porcine and hamster control DNAs. These results were analysed using the ImpRH mapping tool (<http://imprh.toulouse.inra.fr>) developed by Milan et al. (2000b), relative to markers previously mapped on the ImpRH panel.

The results were verified by generating PCR products with the primers described above and subsequent sequencing of these products with an ABI 377 DNA sequencer (Perkin Elmer Cetus). Analysis was performed with the Sequence Analysing Software ABI Prism Version 3.2 (Perkin Elmer).

Results

The 118 clones of the ImpRH panel showed for PRKAG1 (BI401973) the following vector: 00000 00000 01010 00000 00001 0?000 10?10 00100 10000 01000 10000 00001 00000 01000 00000 00010 00101 11001 11001 01111 00100 10110

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10011 101. For PRKAG2 (BI326904) the distribution of positive and negative amplifications was as follows: 00000 0?000 01000 10000 11010 01010 00000 01011 10010 00110 00000 00010 00000 11000 00010 01000 01101 ?1100 ?001? 11111 1000? 110?? 1??11 100.

PRKAG1 was mapped on porcine chromosome 5 with the most significantly linked marker SW439 (LOD score 4.08). The second gene PRKAG2 could be mapped on chromosome 18 close to the marker TCRB (LOD score 6.53).

A BLAST analysis of the sequences of PRKAG1 and PRKAG2 (product sizes: PRKAG1 180 bp, PRKAG2 87 bp) resulted in a high agreement with the originally ESTs.

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