Assignment¹ of phosphatidylinositol glycan, class K (PIGK) gene to porcine chromosome 6q32 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 in pigs.

Rationale and significance

GPI (glycosylphosphatidylinositol)-anchored proteins are cell surface antigens, ectoenzymes, receptors, or cell-adhesion molecules expressed in eukaryotes from yeast to human (Englund, 1993; McConville and Ferguson, 1993). The generation of inositol phosphoglycan, called insulin mediator, from membrane GPI has been shown to be stimulated by insulin (Romeo and Larner, 1993). In addition, the structure and turnover of GPI are critically involved in some of the cellular actions of insulin (Lazar et al., 1994). GPI anchors and proteins are synthesized separately in the endoplasmic reticulum (ER) (Kinoshita et al., 1995). The final step of this synthesis process consists of a transamidation reaction. PIGK (phosphatidylinositol glycan, class K) is a subunit of GPI transamidase called GPI8 in yeast that cleaves the signal and replaces it with GPI (Yu et al., 1997; Nagamune et al., 2003). Therefore, PIGK would be essential for mammalian development and many specific cellular functions as well as for metabolic activity of insulin associated with GPI.

Materials and methods

In order to screen the KNP BAC library (Jeon et al., 2003), a primer set was designed from an alignment between PIGK cDNA sequences of human (GeneBank accession no. NM-005482) and mouse (GeneBank accession no. NM_178016). The BAC 1441E3 containing porcine PIGK was selected and sequenced. The primer sequences namely PIGK: 5'-AGC ACA ACC CGT GAA TTT CC-3' and 5'-GCT CTT GTG TGA CTT TGT GA-3' were designed from flanking intron sequences of exon 8 on the basis of the BAC sequence. The PCR analysis of a porcine × rodent somatic cell hybrid (SCH) panel (Yerle et al., 1996) and a radiation hybrid (RH) panel of the porcine genome (Yerle et al., 1998) were performed to determine the chromosomal localization of porcine PIGK. A PCR was performed in duplicate of a total 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). The mixture was incubated at 94 °C for 5 min, and then subjected to 35 cycles with each cycle containing 30 s of denaturation at 94 °C, 30 s of annealing at 58 °C, and 30 s of extension at 72 °C. An extra 5-min incubation at 72 °C was done at the end of the reaction. 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: 00000 00011 00001 00100 00000 10 that assigned the PIGK gene to SSC6 with the highest probability and correlation values (95 and 100%) for the region 6q32. The radiation hybrid panel showed the following distribution of positive and negative amplifications within the 118 clones: 00100 00101 11000 00111 11000 10011 00000 01101 10000 00000 00000 00010 01000 11000 00010 00010 00000 11011 01000 00000 01111 01100 00011 100, and the retention frequencies for PIGK were 31 %. Two-

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point analysis revealed close linkage of PIGK to SW1647 (LOD scores 19.37, 15 cRs away) on chromosome 6 (Hawken et al., 1999). The localization of porcine PIGK is consistent with the conserved segment in the human chromosome 1p31.1. De Koning et al. (2000) and Grindflek et al. (2001) detected a QTL associated with the intramuscular fat content (IMF) on SSC6q. In addition, Ovilo et al. (2002) reported that maximum values of F obtained for IMF were located between the markers S0228 and SW1881, with confidence intervals 96–108 cM. PIGK located between these two markers. In conclusion, PIGK would be a possible positional and functional candidate gene related to IMF in domestic pigs.

References

- de Koning DJ, Rattink AP, Harlizius B, Van Arendonk JAM, Brascamp EW, Groenen MAM: Genome-wide scan for body composition in pigs reveals important role of imprinting. *Proc Natl Acad Sci USA* 97:7947–7950 (2000).
- Englund PT: The structure and biosynthesis of glycosyl phosphatidylinositol protein anchors. *Annu Rev Biochem* 62:121–138 (1993).
- Grindflek E, Szyda J, Liu Z, Lien S: Detection of quantitative trait loci for meat quality in a commercial slaughter pig cross. *Mamm Genome* 12:299–304 (2001).
- Hawken RJ, Murtaugh J, Flickinger GH, Yerle M, Robic A, Milan D, Gellin J, Beattie CW, Schook LB, Alexander LJ: A first generation porcine whole genome radiation hybrid map. *Mamm Genome* 10:824–830 (1999).
- Jeon JT, Park EW, Jeon HJ, Lim TH, Lee KT, Cheong IC: A large-insert porcine library with sevenfold genome coverage: a tool for the positional cloning of candidate genes for major quantitative traits. *Mol Cells* 16:113–116 (2003).
- Kinoshita T, Inoue N, Takeda J: Defective glycosyl phosphatidylinositol anchor synthesis and paroxysmal nocturnal hemoglobinuria. *Adv Immunol* 60:57–103 (1995).
- Lazar DF, Knez JJ, Medof ME, Cuatrecasas P, Saltiel AR: Stimulation of glycogen synthesis by insulin in human erythroleukemia cells requires the synthesis of glycosyl-phosphatidylinositol. *Proc Natl Acad Sci USA* 91:9665–9669 (1994).
- McConville MJ, Ferguson MAJ: The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. *Biochem J* 294:305–324 (1993).
- Nagamune K, Ohishi K, Ashida H, Hong Y, Hino J, Kangawa K, Inoue N, Maeda Y, Kinoshita T: GPI transamidase of *Trypanosoma brucei* has two previously uncharacterized (trypanosomatid transamidase 1 and 2) and three common subunits. *Proc Natl Acad Sci USA* 100:10682–10687 (2003).
- Ovilo C, Oliver A, Noguera JL, Clop A, Barragan C, Varona L, Rodriguez C, Toro M, Sanchez A, Perez-Enciso M, Silio L: Test positional candidate genes for body composition on pig chromosome 6. *Genet Sel Evol* 34:465–479 (2002).
- Romero G, Lerner J: Insulin mediators and the mechanism of insulin action. *Adv Pharmacol* 24:21–50 (1993).
- Yerle M, Echard G, Robic A, Mairal A, Dubut-Fontana C, Riquet J, Pinton P, Milan D, Lahbib-Mansais Y, Gellin J: A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. *Cytogenet Cell Genet* 73:194–202 (1996).
- Yerle M, Pinton P, Robic A, Alfonso A, Palvadeau Y, Delcros C, Hawken R, Alexander L, Beattie C, Schook L, Milan D, Gellin J: Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenet Cell Genet* 82:182–188 (1998).
- Yu J, Nagarajan S, Knez JJ, Udenfriend S, Chen R, Medof ME: The affected gene underlying the class K glycosylphosphatidylinositol (GPI) surface protein defect codes for the GPI transamidase. *Proc Natl Acad Sci USA* 94:12580–12585 (1997).