Brief Gene Mapping Report

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Assignment[1] of the phosphoinositide-3-kinase, class 3 (PIK3C3) gene to porcine chromosome 6q22→q23 by somatic cell and radiation hybrid panel mapping

J.H. Kim,[a*] Y.S. Lee,[b*] E.W. Park,[c] B.Y. Seo,[a] I.C. Cho,[e] J.G. Lee,[a] S.J. Oh,[c] J.H. Lee[d] and J.T. Jeon[a]

[a] Division of Applied Life Science, Gyeongsang National University, Jinju; [b] Korea Research Institute of Bioscience and Biotechnology, Daejeon; [c] National Livestock Research Institute, Rural Development Administration, Suwon; [d] Division of Animal Science and Resources, Chungnam National University, Daejeon; [e] National Institute of Subtropical Agriculture, Rural Development Administration, Cheju (Korea)

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[1] To our knowledge this is the first time this gene has been mapped in pig.

Rationale and significance

PI3-kinases (phosphatidylinositide 3-kinases) constitute a lipid kinase family characterized by their ability to phosphorylate the inositol ring 3′-OH group in inositol phospholipids to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3). PI3-kinases are one of the most important regulatory proteins that are considered as main intracellular factors responsible for the transmission of anti-apoptotic signals that control the growth of cells (Krasilnikov et al., 2000; Cantley, 2002). Also, these kinases participate in mitogenesis, glucose transport, regulation of hepatic glucose output, glycogen synthesis, and antilipolysis in typical insulin target cells such as liver, muscle, and fat (Czech and Corvera, 1999; Rajala et al., 2004). There are multiple isoforms of PI3Ks in mammalian cells, and these are divided into three classes based on their structure and substrate specificity (Domin and Waterfield, 1997). Among them, PIK3C3 (class 3 PI3-kinase) is typified by the yeast protein encoded by the VPS34 gene and phosphorylates only PtdIns to produce PtdIns(3)P; they are thought to regulate vesicular trafficking, osmoregulation and endocytosis (Volinia et al., 1995).

Materials and methods

In order to determine the chromosomal position of PIK3C3, we performed PCR analysis of a porcine × rodent somatic cell hybrid panel (Yerle et al., 1996) as well as a porcine radiation hybrid (RH) panel (Yerle et al., 1998). An alignment result between a human PIK3C3 cDNA sequence (GenBank accession no. NM_002647) and a porcine EST sequence (GenBank accession no. CD572011) was used to design a primer set for SCH and RH mapping. The following primers were used in the mapping of PIK3C3: 5′-CAA CTT CAG AAG CTG TGT GG-3′ and 5′-CAT GGT TCT GGA ACA AGC TTG-3′. Each PCR reaction was carried out 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 $MgCl_2$, 0.2 µM of each primer, 100 µM of each dNTP and one unit of *Taq* polymerase (Promega). A 5-min denaturation at 94°C was followed by 35 cycles (30 s at 94°C, 45 s at 57°C and 45 s at 72°C) and a final extension of 10 min at 72°C. PCR reactions were carried out in a PTC-100 programmable Thermal Controller (MJ Research). A 223-bp product was obtained from the PCR. The PCR product was sequenced on an ABI 3700 sequencer (Applied Biosystems). With the primer set, the chromosomal localization of the porcine PIK3C3 was detected by PCR analysis using a porcine × rodent somatic cell hybrid panel and a porcine whole genome radiation hybrid panel. 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).

* These authors contributed equally to the work.

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Corresponding author: Dr. Jin-Tae Jeon
Division of Animal Science and Technology
College of Agriculture and Life Science
Gyeongsang National University, Jinju 660-701 (Korea)
telephone: +82-55-751-5516, fax: +82-55-756-7171
e-mail: jtjeon@nongae.gsnu.ac.kr

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Results

Regional mapping results

Analysis of the 27 hybrid clones from the somatic cell hybrid panel produced the following vector: 00000 00100 00000 00000 00001 00 that assigned the PIK3K3 gene to SSC6 with the highest probability and correlation values (74 and 100%) for the region q22→q23. The radiation hybrid panel showed the following distribution of positive and negative amplifications within the 118 clones: 10000 10100 01001 00110 01000 00000 01001 00111 10000 00101 01000 10111 01000 10100 00011 00100 10010 00111 01010 00000 01110 01100 00010 000, and the retention frequencies for PIK3C3 were 33%. Two-point analysis revealed close linkage of PIK3C3 to S0228 (LOD scores 14.51, 26 cRs away) on chromosome 6 (Hawken et al., 1999). The localization of porcine PIK3C3 is consistent with the conserved segment in the human chromosome 18q12.3. De Koning et al. (1999) and Ovilo et al. (2002) detected a QTL associated with the intramuscular fat content (IMF) on SSC6q. In addition, Grindflek et al. (2001) reported that the highest probability of the QTL position affecting IMF was found between markers SW1823 and S0003. Linkage of PIK3C3 to SW0003 was estimated as 103 cR and the location of this gene was inferred to be between the two markers in this study. Finally, PIK3C3 would be a possible positional candidate related to IMF in domestic pigs.

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