Assignment¹ of the patatin-like phospholipase domain containing 2 gene (*PNPLA2*) to porcine chromosome 2p17 with radiation hybrids

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Received 31 August 2005; manuscript accepted by A. Kallioniemi, 6 September 2005.

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

Rationale and significance

Recently, a novel triacylglycerol lipase, patatin-like phospholipase domain-containing 2 (*PNPLA2*) also called adipose triglyceride lipase (ATGL) (Zimmermann et al., 2004), the ζ -isoform of a calcium-independent phospholipase A2 (*iPLA2*zeta) (Jenkins et al., 2004) and desnutrin (Villena et al., 2004) was identified and characterized simultaneously by several laboratories. The concept of hormone-sensitive lipase (HSL) being the sole rate-determining lipolytic enzyme in adipocytes has been challenged. As we all know, HSL is highly expressed in adipose tissue and catalyzes the breakdown of triglycerides (TAG) and diacylglycerides (DAG) into glycerol and free fatty acids (FFA) (Kraemer and Shen, 2002), however, there is another lipase called *PNPLA2*. It represents a novel type of 'patatin domain-containing' triacylglycerol hydrolase that is more closely related to plant lipases than to other known mammalian metabolic triacylglycerol hydrolases (Zimmermann et al., 2004). *PNPLA2* predominantly hydrolyses the first ester bond of triglycerides, which results in

the conversion of TAG to DAG, and then HSL breaks down DAG into FFA. Thus, fat mobilization has been further modified to be a two-step affair, and it is believed that *PNPLA2* and HSL coordinately catabolize stored triglycerides in adipose tissue of mammals (Zimmermann et al., 2004). The *PNPLA2* gene has been mapped on human chromosome 11p15.5, on mouse chromosome 7F5 and on rat chromosome 1q41 (http://www.informatics.jax.org/searches/homology_report.cgi?Marker_key=50833), but has not previously been mapped in the pig. The mapping of the porcine gene will enable further investigations on its possible role in porcine fat deposition.

Materials and methods

Isolation and sequencing of the three porcine gene fragments

The cDNA sequences of human and mouse *PNPLA2* genes were obtained from NCBI (GenBank accession numbers are NM_020376 and NM_025802, respectively) and were compared with all sequences in the EST-other databases by using the BLAST (<http://www.ncbi.nlm.nih.gov/blast/>) algorithm. Pig ESTs with at least more than 80% similarity with the corresponding human mRNA were chosen and aligned together using SeqMan. The primers (Forward, 5'-CCTGCCTCTCTACGAAC-CA-3'; and reverse, 5'-CCCTGTAACCCTGCTTGC-3') were designed based on the pig EST (BX922511). PCR was conducted in a 25- μ l reaction volume consisting of 50 ng of porcine genomic DNA, 1 $\times$ PCR buffer, 0.3 μ M of each primer, 0.1 mM of dNTPs, 1.5 mM MgCl₂ and 1 U Taq DNA polymerase (Sangon, Shanghai, China). Samples were denatured at 94°C for 4 min followed by 35 cycles under the following conditions: denaturation for 40 s at 94°C, annealing for 40 s at 60°C and extension for 40 s at 72°C. The PCR was completed with a final cooling at 4°C for 10 min. The PCR products were separated on a 1.5% agarose gel and stained with 0.5 μ g/ml ethidium bromide. The PCR products were purified, cloned and sequenced. Comparisons of pig exon sequences of the gene's PCR products with the corresponding human mRNA sequences in GenBank were done using the BLAST2 software provided by the

This work was supported by grants from National Natural Science Foundation of China (30371028), the National 863 and 973 Project (2004AA213110, TG2000016105) and Natural Science Foundation of Hubei Province (2005ABA135).

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NCBI server (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>) to confirm that the expected pig sequences were isolated. The porcine PCR product including an intron of 191 bp was 406 bp, and showed high identity of 92% between human and porcine exon sequence (GenBank accession No. DQ157429).

Radiation hybrid (RH) mapping

The 118IMpRH panel (Yerle et al., 1998; Hawken et al., 1999) served for high resolution gene mapping along with porcine and hamster control DNAs. Same primers were used in mapping. DNA from each hybrid was amplified in 15- μ l reactions containing 10 ng of template DNA, 1 $\times$ PCR buffer, 1.5 mM MgCl₂, 0.2 μ M of each primer, 200 μ M of dNTPs, 0.5 U Taq DNA polymerase. The controls consisted of parental DNA: porcine and hamster genomic DNA for IMpRH. A negative control (no DNA) was also added in all cases. The PCR conditions were the same as described above. Each clone in the RH panel was amplified twice in order to get reliable results.

Results and discussion

The presence and absence of PCR product was scored and the results were analyzed by the tools provided at <http://imprh.toulouse.inra.fr/>. The survey of the whole genome radiation hybrid panel showed the following distribution within the 118 lines: 00000 00001 01000 00000 00000 00111 00000 00011 00100 00000 00000 10101 00000 00001 00000 10000 01001 00000 00110 00100 10001 01000 00000 010. The retention frequency of the sequence tagged site (STS) marker was 18%. Two-point analysis showed the most significant linked marker was *SW2443* on chromosome 2 at a distance of 36cR (LOD = 10.26). Other significantly linked microsatellite markers were *SWC9* (LOD = 7.83, 49cR), *SWR2516* (LOD = 7.72, 51cR) and *SW2623* (LOD = 5.01, 72cR) on the current RH map. *SW2443*, *SWR2516*, *SWC9* and *SW2623* all have been assigned to SSC2p17 (Alexander et al., 1996). Thus, we can localize porcine *PNPLA2* to SSC2p17, the distal tip of the p-arm of SSC2.

Our mapping of porcine *PNPLA2* on SSC2p17 agrees with comparative mapping data. The mapping of porcine *PNPLA2* to the distal tip of the p-arm of SSC2 is similar to that of human *PNPLA2* on 11p15.5. Interestingly, a quantitative trait locus (QTL) for backfat thickness is reported to be on the p-arm of SSC2 where *IGF2* is located (Rattink et al., 2001). Mapping of *PNPLA2* to this region strongly suggests it might be a candidate for the QTL. In addition, considering its important role in fat mobilization, we suggest that *PNPLA2* could be an important candidate gene for fat deposition traits.

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