Assignment¹ of lymphocyte antigen 64 homolog, radioprotective 105 kDa (LY64) gene to porcine chromosome band 16q14 with somatic cell hybrids and linkage mapping

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

Rationale and significance

Lymphocyte antigen 64 homolog, radioprotective 105-kDa gene (LY64) encodes LY64, a leucine-rich repeat molecule expressed on B-cell surfaces. This gene has a striking similarity to Toll-like receptor 4 (TLR4). An extracellular molecule MD-1 is required for LY64 to signal LPS presence, as MD-2 is required for TLR4. B-cells lacking LY64 are severely impaired in LPS-induced proliferation and antibody production (Miyake et al., 2000; Nagai et al., 2002). The mouse and human LY64 was mapped to chromosome 13D1 and 5q12 respectively (Miyake et al., 1996). The mapping of porcine LY64 will help in investigation of its role in disease resistance of the innate immune system.

Materials and methods

Full cDNA sequence of the porcine RP105 gene (GenBank accession number AY323207) was obtained by designing primers from conserved regions between human and mouse cDNA and by sequencing the PCR products from porcine genomic DNA or spleen cDNA. The RACE technique was used to obtain the 5' and 3' untranslated region (UTR) sequences. Primers used for physical mapping and linkage mapping were from the 3' UTR. PCR was performed using primers RP1053UF1 (TCCCGGGAAGGTAAACAGA) and RP1053UR1 (CCAACCTATTTTCCAAATGGCT) to amplify a 319-bp product from the porcine somatic cell hybrid panel (Yerle et al., 1996). LY64 was localized by using the INRA-Toulouse interactive server (<http://w3.toulouse.inra.fr/lgc/pig/pcr/pcr.htm>). For linkage mapping, RP1053UF2 (GCTGTGGGCATTTTCTTCT) and RP1053UR1 were used to amplify a 578-bp product. The *DdeI* digestion of the 578-bp PCR product produced allelic fragments of 182, 161, 76, 65, 62, and 32-bp (allele 1), or 146, 161, 76, 65, 36, 62 and 32-bp (allele 2). This PCR restriction fragment length polymorphism (RFLP) was used to genotype animals from the PiGMAP reference families (Archibald et al., 1995). Mendelian segregation of the *DdeI* PCR-RFLP was observed in six three-generation PiGMAP families. Two-point and multipoint linkage analyses were performed using the genotypes of the PiGMAP families and the CRIMAP program (Green et al., 1990). The PCR profile for physical mapping included 3 min at 94°C; 35 cycles of 45 s at 94°C, 45 s at 58°C, 45 s at 72°C, and a final 5 min extension at 72°C; PCR profile for linkage mapping included 3 min at 94°C; 3 cycles of 45 s at 94°C, 45 s at 60°C, 45 s at 72°C; 3 cycles of 45 s at 94°C, 45 s at 55°C, 45 s at 72°C; 30 cycles of 45 s at 94°C, 45 s at 53°C, 45 s at 72°C; and a final 5-min extension at 72°C. Reaction volume was 10 µl for all PCR with 10 ng template DNA for the somatic hybrid panel and 25 ng for linkage mapping.

Results

Regional mapping results

The following result was obtained from the somatic porcine × rodent cell hybrid panel: 01010 11011 11000 10010 00000 00. Statistical evaluation showed significant correlation be-

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tween the LY64 PCR product and chromosome 16 (SSC16). The regions with the highest probability were q14 and q22 → q23 with identical probability (0.473) and correlation values (0.925) with an error risk less than 0.1%. The distribution of fragments of porcine chromosome 16 does not enable the distinction between these two regions. To precisely map LY64, we identified an SNP within the 3' UTR and performed segregation analysis with PiGMap families. Linkage analysis showed that the LY64 gene was significantly linked to several genes and markers mapping to chromosome 16q14 (S0077, CART, C9, S0298). The maximum LOD score is 5.51 to the Cocaine- and Amphetamine-regulated Transcript (CART) gene with a recombination of 0.06. While CART has been physically mapped to q21, the linkage mapping showed this gene is closely linked with markers on q14 (Kenealy et al., 1999). The gene order relative to the LY64 gene produced by multipoint linkage analysis is as follows (with distance in Kosambi centimorgans): S0077–19.0–LY64–6.0–CART–18.1–C9–0.0–S0298. S0298 and C9 have been physically mapped to 16q14 (Wintero et al., 1998; Hoyheim et al., 1995). Thus because none of the loci linked to LY64 have been mapped to 16q22 → q23, and the linkage mapping data maps LY64 closely to q14 loci, this combined data excludes 16q22 → q23 for the location of LY64.

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