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Rapid communication: Porcine vitamin D-25-hydroxylase maps to chromosome 5[1]

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Locus Name. Vitamin D-25-hydroxylase.
Species. Sus scrofa.
Locus Symbol. CYP25.
Source of Primers. PCR primers were designed from the published sequence for porcine vitamin D-25-hydroxylase (CYP25) cDNA (GenBank accession no. Y16417). The position of the forward and reverse primers corresponds to 506 to 530 bp and 830 to 854 bp, respectively, of the cDNA. The primer pair amplified a 348-bp fragment of the cDNA and an 862-bp fragment from the pig genomic DNA. The 862-bp fragment (GenBank accession no. AF284378) was subcloned into a pNoTA vector and identity was confirmed by sequencing. Genomic sequences from 1 to 74, 169 to 329, and 749 to 862 showed 100% homology to the cDNA.
Primer Sequences. Primers derived from the porcine sequences were as follows: forward, 5′-GGG CAA GAA GGG GTC GTT GGA GGA GTC-3′, and reverse, 5′-ACT CGA CTA GTG TCT CGT GTT CTA C-3′.
Method of Detection. PCR was performed in 50-μL reactions that included 1 μL *AdvanTaq* (solution diluted 1:50) DNA polymerase (Clontech, Palo Alto, CA), 16 m*M* $(NH)_4SO_4$, 67.5 m*M* Tris buffer pH 8.8, 100 μ*M* dNTPs, 1.5 m*M* $MgCl_2$, 0.01% Tween-20, and 50 ng of genomic DNA. PCR was carried out in a Perkin Elmer GeneAmp 9600 PCR System (Norwalk, CT). The cycling conditions included initial denaturation at 95°C for 3 min followed by 30 cycles at 95°C for 45 s, 60°C for 1 min, 72°C for 2 min, and final extension at 72°C for 10 min. From the cDNA information it can be deduced that the 862-bp fragment of the porcine *CYP25* gene produced by PCR contained two introns and portions of two exons and a full exon.
Chromosome Location. The 862-bp fragment of the porcine *CYP25* gene was used for physical mapping by using the INRA-University of Minnesota porcine Radiation Hybrid panel (Hawken et al., 1999). LOD values of 2.5 or greater obtained by the two-point analysis procedure are presented in Table 1; values of 4.8 were considered significant evidence for linkage. The results showed that the *CYP25* gene locus is located on the end of the short arm of pig chromosome 5 near the aconitase gene locus (*ACO2*). The retention fraction was 34%. Based on previous physical mapping results for *ACO2*, the position of the pig *CYP25* gene is likely on the chromosome 5p14–15 region.
Comments. A porcine vitamin D-25-hydroxylase previously was cloned and characterized (Postlind et al., 1997). The gene codes for a cytochrome P450 microsomal enzyme that is expressed in liver and kidney. Porcine CYP25 cDNA consists of 1,652 bp, and the purified protein consists of 500 amino acids and catalyzes equally the conversion of vitamin D_2 and vitamin D_3 to 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3, respectively (Axen et al., 1994; Postlind et al., 1997). Porcine CYP25 therefore was proposed to be the true vitamin D-25-hydroxylase. Porcine CYP25 is 83% homologous with human CYP2D6, an enzyme involved in the metabolism of debrisoquine and other drugs (Postlind et al., 1997). No CYP2D6 activity was detected in pig liver (Skaanild and Friis, 1999). As indicated in the present report, the porcine *CYP25* gene locus maps to pig chromosome 5p14–15, close to the *ACO2* gene locus (Yasue et al., 1999). This region is syntenic to human chromosome 22q12–13 containing the loci for the human *CYP2D6* and *ACO2* genes (Gonzalez et al., 1988; Gough et al., 1993). The location of the *CYP25* gene in the RH map is in a telomeric region with few markers mapped, and this makes it difficult to ascertain the exact position of the gene. The biological relationship, if any, between porcine CYP25 and human CYP2D6 remains to be determined.

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Table 1. Mapping the *CYP25* gene with the INRA-University of Minnesota porcine Radiation Hybrid panel: two-point analysis[a]

Order	Chromosome	Marker	– –	– +	+ –	+ +	Distance (ray)	LOD[b]
1	5	*ACO2*	72	4	19	21	0.64	6.17
2	5	*SW1482*	67	9	22	18	1.00	2.93
3	5	*DK*	52	25	13	28	1.03	2.77

[a]+ +, Hybrids in which both markers were detected; – –, Hybrids in which both markers were not detected; – + and + –, Hybrids in which one of the markers was detected and one not.
[b]LOD values of 4.8 or greater are considered significant.

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