Rapid Communication: The Porcine Inter-α Trypsin Inhibitor-Heavy Chain 4 (ITIH4) Gene Maps to Chromosome 13

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Polymorphism. A restriction fragment length polymorphism was detected within PCR amplification products of the porcine Inter-α-trypsin inhibitor-heavy chain 4 (ITIH4) gene using the restriction endonuclease AciI.

Primer Sequences. Forward primer: 5′-TGCCACTGCCTGGGCAGAGT-3′; reverse primer: 5′-TGAAAGGTCACTTCGATCCAT-3′.

Method of Detection. The PCR (15 μL final volume) was performed using 50 ng of porcine genomic DNA, 100 μM of each dNTP, 300 nM each primer, .5 units of Taq polymerase, 1.5 mM MgCl2, and PCR buffer (Tris-HCl, 10 mM; KCl, 50 mM; pH 8.3). Thermal cycling began with an initial denaturation at 95°C (2 min), followed by 35 cycles of 94°C (30 s), 58°C (45 s), and 72°C (1 min), and concluded with a final extension at 72°C (10 min).

Amplification of porcine genomic DNA resulted in a single 1,200-bp product. This product represented 296 bp of coding region corresponding to bases 2,180-2,476 of the human sequence for PK-120, a plasma kalikrein-sensitive glycoprotein (Nishimura et al., 1995; GenBank accession no. D38535) and ∼900 bp of intron sequence. Terminal end sequencing of the PCR product showed 100% homology with previously published porcine coding region sequence for a porcine homologue of human HRP, the Inter-α-trypsin inhibitor family heavy chain-related protein (Hashimoto et al., 1996; GenBank accession no. S82800). Digestion of the PCR product with AciI revealed a polymorphism with two alleles (Figure 1) characterized by bands of 600, 345, 180, and 75 bp (A allele) and 600, 420, and 180 bp (B allele).

Inheritance Pattern. Mendelian inheritance of ITIH4 was confirmed in seven full-sib families (71 offspring).

Chromosome Location. Individuals from the three-generation PiGMaP reference families of European Wild Boar × Large White and Meishan × Large White (Archibald et al., 1995) were genotyped for ITIH4 to determine linkage relationships between the ITIH4 gene and previously mapped loci. Significant linkage to several microsatellite markers on porcine Chromosome 13 (SSC 13) was found (Table 1). In addition, ITIH4 was linked to several other genes, including the transferrin (TF2) and cholecystokinin (CCK) loci with recombination frequencies (sex averaged) of .06 and .04, and LOD scores of 3.7 and 7.0, respectively. Interestingly, ITIH4 had no recombination (LOD = 4.5) with ITIH, a previously mapped Inter-α-trypsin inhibitor locus (Archibald et al., 1995; Couperwhite et al., 1995).

Figure 1. AciI restriction fragment length polymorphism in ITIH4 PCR products. A DNA molecular weight marker is represented in the left lane (pGEM; Promega, Madison, WI). The remaining lanes represent the distribution of the three genotypes in F1 parents of the PiGMaP reference families. The AA genotype (600, 345, 180, and 75 bp) is observed in lanes 8 and 11 to the right of the molecular weight marker lane, and the BB genotype (600, 420, and 180) is seen in lane 13. Heterozygotes are in lanes 2, 3, 4, 5, 6, 7, 9, 10, 12, and 14 to the right of the molecular weight marker lane, and the genotype in lane 1 cannot be determined from this reaction.
Frequency. Frequency of the A allele is .57 in the 14 F1 parents of the PiGMaP families.

Comments. Coding sequence of the present marker shows complete homology with a previously characterized porcine locus (Hashimoto et al., 1996). IHRP and PK-120 (Nishimura et al., 1995) are old terms for the ITIH4 gene and its glycoprotein product, respectively. We have shown that our marker is within the ITIH4 locus through extensive protein purification, NH2-terminal amino acid sequencing, and cDNA sequencing of products generated through RT-PCR (R. D. Geisert and D. Pomp, unpublished data).

The previously mapped porcine ITIH marker (Archibald et al., 1995; Couperwhite et al., 1995) was an RFLP developed from a pig liver cDNA clone that exhibited 63 and 58.9% homology with human ITIH3 and ITIH1, respectively (Couperwhite et al., 1995). Because ITIH1, ITIH3, and ITIH4 are colocalized to a tightly linked region in humans and mice (Jean et al., 1997), it is possible that the prior ITIH marker could represent any of these three loci in pigs. However, a BLAST sequence similarity search of the sequence from the cDNA clone (016n-4D/lambda2; GenBank accession no. M29507) used to develop the prior ITIH marker revealed 97 and 84% homology to recently mapped (Jean et al., 1997) in humans (ITIH4 D38595) and in mice (ITIH4 Nishimura et al., 1995; GenBank accession no. S82800) and human (Nishimura et al., 1995; GenBank accession no. D38595) ITIH4, respectively. Therefore, it is likely that the ITIH and ITIH4 RFLP markers are at the ITIH4 locus. Even though the ITIH1 and ITIH3 loci have yet to be mapped in pigs, it is likely that they are tightly linked to ITIH4 on SSC 13.

Inter-α-trypsin inhibitor, heavy chain 4 was recently mapped (Jean et al., 1997) in humans (ITIH4) to HSA 3 (3p21.2-p14.1) and in mice (Itih4) to MMU 14 (12 cM). Localization of ITIH4 to SSC 13 is consistent with previous information indicating significant synteny between this chromosome and HSA 3. Many other genes, including CCK, PIT1, TF2, and CP map to SSC 13 and HSA 3, although gene order is not conserved in all cases. In humans and pigs, the CCK and ITIH4 loci are closely linked, but these two loci are on different mouse chromosomes (MMU 9 and 12, respectively). In fact, regions of synteny between SSC 13 and HSA 3 are represented by at least seven murine chromosomes.

The ITIH4 gene product is different from the other three Inter-α-trypsin inhibitor heavy chains in that it lacks a binding site for bikunin, which contains the serine protease inhibitory activity, and is sensitive to deavag by kallikrein (Hashimoto et al., 1996). All ITIH heavy chains possess a von Willebrand type-A domain that serves as adhesion molecules for integrins, collagen, proteoglycans, and heparin. We found that uterine gene expression of ITIH4 is enhanced on d 10 through 15 of the estrous cycle and d 10 through 18 of pregnancy in pigs (R. D. Geisert and D. Pomp, unpublished data). Endometrial expression of ITIH4 may therefore provide a supportive role for early conceptus attachment to the uterine epithelium in pigs.

Key Words: Pigs, Inhibitor Genes, Gene Mapping

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Table 1. Linkage between the Inter-α-trypsin inhibitor-heavy chain 4 (ITIH4) locus and microsatellite markers on Porcine Chromosome 13.