Rapid communication: Physical and linkage mapping of the porcine calcitonin (*CALC*) gene[1]

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Genus and Species. Sus scrofa.

Locus. Porcine calcitonin (***CALC***) **gene.**

Source and Description of Primers. Primers, CCA1F and CCA1R, were designed from canine calcitonin sequence (GenBank Accession no. AJ271090) to amplify genomic porcine DNA. Using sequence obtained from the amplified PCR product, additional pig-specific primers (CIPBF, CIPBR, CIPCF, CIPCR) were designed.

Primer Sequences. CCA1F: 5′-CAC TTT GGA TTG GCC GCG C-3′; CCA1R: 5′-ACC AGG GCA GCC AGC AGG A-3′; CIPBF: 5′-AAC TTC CCA CTC TGC ACA CT-3′; CIPBR: 5′-AGA CCA AAC TTC AGC AGG AT-3′; CIPCF: 5′-TTC TCC TTC CTC TGC TTC TG-3′; CIPCR: 5′-GCA AAC CCA ATA CAG GCT CT-3′.

Method of Detection. A PCR was performed using the CCA1F and CCA1R primers in 10-μL reactions of the following: 1× PCR buffer, 1.5 m*M* MgCl, 200 μ*M* of each dNTP, 2.5 pmol of each PCR primer, 0.35 units *Taq* DNA polymerase (Promega, Madison, WI), and 12.5 ng of genomic DNA. Genomic DNA from four individuals for two swine breeds (Hampshire and Yorkshire) was used. The PCR was performed in a Robocycler (Stratagene, La Jolla, CA) under the following thermocycling conditions: initial denaturation at 94°C for 4 min, 40 cycles of 94°C for 45 s, 64°C for 1 min, 72°C for 2:10 min, and a final extension time of 12 min at 72°C. For each breed, the PCR products from the four individuals were pooled. These pools were then directly sequenced using dye terminators and an ABI 377 sequencer (Perkin-Elmer, Foster City, CA) at the Iowa State University DNA Sequencing and Synthesis Facility. The CCA1F and CCA1R primers produced a 1,600-bp fragment covering exons 1, 2, and 3. This fragment's sequence was used to design the pig specific primers: CIPBF, CIPBR, CIPCF, and CIPCR. The CIPBF and CIPBR primers produced a 340-bp product used for physical mapping. The CIPCF and CIPCR primers produced a 220-bp fragment used for linkage mapping. All mapping was completed using AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA) and the following thermocycling conditions: initial denaturation at 94°C for 10 min, 36 cycles of 94°C for 45 s, 56°C for 45 s, 72°C for 45 s, and a final extension time of 5 min at 72°C. An intronic single nucleotide polymorphism (**SNP**) was found and subsequently confirmed using the *Hae*III restriction enzyme, and this marker was used for linkage mapping. For the PCR-RFLP assays, 5 μL of PCR products were digested with 3 units *Hae*III in 1× digestion buffer with 1× BSA added in a total volume of 10 μL. Following digestion for 4 h at 37°C, digested products were loaded onto a 4% NuSieve (BMA, Rockland, ME) agarose gel containing ethidium bromide, electrophoresed, and photographed.

Sequencing and Polymorphisms. Sequence analysis confirmed the identity of the porcine *CALC* by comparison to human and canine calcitonin. Exons 1 and 2 within the 1,600-bp fragment amplified using primer set CCA1F and CCA1R showed an 85% sequence homology to the human *CALC* mRNA sequence (GenBank Accession no. XM_006209). Comparison of the 220-bp fragment amplified using primer set CIPCF and CIPCR and DNA from five commercial swine lines revealed one intronic SNP. A single G to C base pair substitution at position 98 from primer CIPCF resulted in the formation of a *Hae*III restriction enzyme site. A *Hae*III PCR-RFLP test was designed using this restriction enzyme site and the CIPCF and CIPCR primers. Resulting allelic fragment sizes were 220 bp (allele 1) and 98 bp and 122 bp (allele 2) (Figure 1).

Inheritance Pattern. In the five PiGMaP families (Archibald et al., 1995) the *Hae*III PCR-RFLP segregated in accordance with autosomal Mendelian inheritance.

Allele Frequencies. In 35 unrelated animals the intronic SNP was found to be informative in only two breeds, Yorkshire and Berkshire. Allele frequencies were 0.37 and 0.15 for allele 1 in Yorkshire and Berkshire pigs, respectively. Among the remaining 24 Duroc, Hampshire, and Landrace animals all were monomorphic for allele 2.

Chromosomal Location. Using the INRA pig/rodent somatic cell hybrid panel (Yerle et al., 1996) and the

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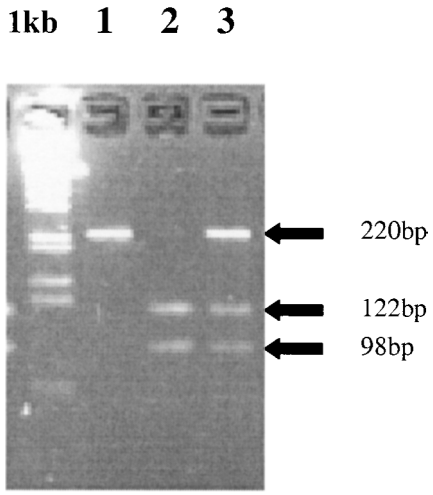

Figure 1. *Hae*III PCR-RFLP genotypes of the *CALC* gene are illustrated. Lane 1 contains uncut PCR product, 11 genotype, following the *Hae*III digestion. Lanes 2 and 3 indicate the 22 and 12 genotypes, respectively. The fragment sizes of alleles are listed.

340-bp PCR fragment, *CALC* was physically mapped to SSC2 p14–17 with a probability of 0.79. Linkage mapping was performed using the CRIMAP (Green et al., 1990) analysis and the genotypes for the PiGMaP families. Using two-point linkage analysis, seven markers were found to be significantly linked to *CALC*. These markers were (LOD score and recombination frequencies in parenthesis): *SW240* (4.24, 0.23), *CAST* (4.40, 0.22), *S0226* (3.78, 0.21), *FSHB* (8.31, 0.09), *SW776* (10.14, 0.09), *SW395* (7.86, 0.07), and *S0091* (10.79, 0.04). Using CRIMAP, a multipoint map of SSC2 including *CALC* was constructed and the following gene order was found (with distance in Kosambi centimorgans): *SW240*-0.0-*FSHB*-19.3-*CALC*-9.9-*S0091*-3-*SW395*-0.9-*SW776*-0.0-*S0226*-15.6-*CAST*-0.0. These results confirm the position obtained from physical mapping.

Comments. Calcitonin is a mulifaceted gene that produces three separate peptides within the human body. These peptides, calcitonin mRNA, alpha-calcitonin gene-related peptide, and beta-calcitonin gene-related peptide, are expressed throughout the body, especially in thyroid and neural tissues. Calcitonin has been identified as an agent in the treatment of diseases such as Paget's disease, osteoporosis, and inhibition of pain perception (MacIntyre, 1992).

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