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Rapid Communication: A HincII Polymorphism in the Porcine Calpain, Large Polypeptide L3 (CAPN3) Gene

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Abstract

Source and Description of Primers. Primers were designed from a published, partial porcine cDNA sequence (Genbank accession no. U05678) in positions corresponding to exons 11 and 13 of the human CAPN3 gene (Genbank accession no. X85030). Sequences were obtained from the ends of the PCR fragment and compared with the porcine cDNA sequence showing 98.1% identity in a 108-bp overlap at the exon 11 end and 99.2% identity in a 124-bp overlap at the exon 13 end. Sequences produced in this study have been submitted to Genbank (accession no. AF025660-AF025661).

Keywords

Swine, PCR-RFLP, Calpain, CAPN3

Rapid Communication: A *Hinc*II Polymorphism in the Porcine *Calpain, Large Polypeptide L3* (*CAPN3*) Gene[1]

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Primer Sequences. The forward primer was 5′-AGGATGATGACCCTGACGA-3′, and the reverse primer was 5′-GGTGGAGGGCACAATGAC-3′.

Method of Detection. A 1.6-kb fragment was PCR-amplified using 12.5 ng of porcine genomic DNA, 1× PCR buffer, 1.5 m*M* $MgCl_2$, .125 m*M* dNTP, .3 μM of each primer, and .35 U *Taq* DNA polymerase (Promega, Madison, WI) in a 10-μL reaction volume. The PCR profile included 2 min at 94°C; 35 cycles of 30 s at 94°C, 1 min 30 s at 62°C, 2 min at 72°C; and 5 min at 72°C in a Robocycler (Stratagene, La Jolla, CA). The 1.6-kb PCR product was digested with *Hinc*II, and fragments were separated by electrophoresis on 1% agarose gels.

Description of Polymorphism. The *Hinc*II digestion of the PCR product produced allelic fragments of 1.25 kb vs 1.0 kb + 260 bp, and a constant fragment of 345 bp (Figure 1). Polymorphisms producing a more complicated fragment pattern were observed with the enzymes *Aci*I and *Msp*A1I. These polymorphisms seemed in complete linkage disequilibrium with the *Hinc*II polymorphism and were not pursued further.

Inheritance Pattern. Autosomal segregation in accordance with Mendelian expectations was observed in four three-generation European PiGMaP families (Archibald et al., 1995).

Frequency. Allele frequencies were determined in grandparental animals of the European PiGMaP families and in unrelated animals from Iowa State University. Allele 1, comprising the 1.25-kb and the 345-bp fragments, was observed with a frequency of .39 in Meishan (n = 9) and .5 in Fengjing (n = 4). Allele 1 was not observed in Large White (n = 11), wild boar (n = 2), Yorkshire (n = 7), Hampshire (n = 7), Chester White (n = 5), Landrace (n = 8), or Minzu (n = 3).

Chromosomal Location. Two-point linkage analysis was performed on genotypes in the PiGMaP families (Archibald et al., 1995) using the CRI-MAP program (Green et al., 1990). This demonstrated that the *CAPN3* gene is closely linked to several markers previously located on porcine chromosome (SSC) 1. The most closely linked marker at 8 cM (Kosambi) with a supporting LOD score of 8.64 is *S0313* (Anderson-Dear and Miller, 1994). The *CAPN3* gene has previously been mapped to SSC1q15-17 by *in situ* hybridization (Briley et al., 1996).

Comments. The *CAPN3* gene shows muscle-specific expression. The gene product is a non-lysosomal

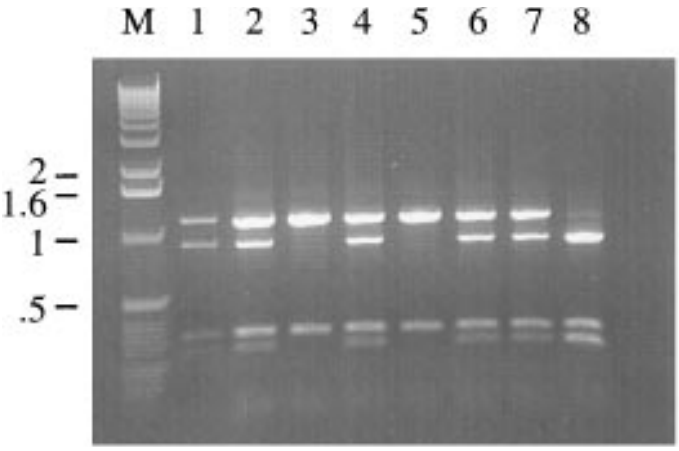

Figure 1. The *CAPN3-Hinc*II digests of two parental animals (lanes 1 and 2) and six offspring (lanes 3–8). Genotypes are 1/1 (lanes 3 and 5), 1/2 (lanes 1, 2, 4, 6, and 7), and 2/2 (lane 8). Low levels of incomplete digestion are seen in several lanes, most obviously in lane 8. The **M** is the "1 kb ladder" molecular standard (Gibco-BRL, Gaithersburg, MD), and the approximate size of four of the fragments are given to the left.

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intracellular cysteine protease, the concentration of which is tightly regulated in the cell. It is thought to be involved in the control of gene expression by degrading transcription factors, etc. Inherited mutations in the human *CAPN3* gene are a cause of limb-girdle muscular dystrophy type 2A (LGMD2A) (Richard et al., 1995).

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