Rapid communication: Nucleotide sequence and physical mapping of the porcine cyclin-dependent kinase inhibitor 3 (CDKN3) gene

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Name of the Sequence. Cyclin-dependent kinase inhibitor 3 (CDKN3; CDK2-associated dual specificity phosphatase).

Genus and Species. Sus scrofa.

Origin of Clone. We used the differential display/reverse transcriptase PCR (DD/RT) approach to isolate potential candidate genes for congenital splay leg in piglets. A total of 16 cDNA fragments with apparent differential expression in skeletal muscle (biceps femoris) between each two healthy and affected piglets were detected in this investigation (Maak et al., 2001a,b). Among them, a 301-bp cDNA fragment from a male splay-leg piglet (German Landrace) was isolated by reverse transcription of total RNA with primer D8 and subsequent amplification with primers D8 and U17 (GeneExScreen kit, Biometra, Goettingen, Germany). We observed a more intense band in preparations from both the splay-leg piglets compared to those from healthy control animals. Homology search revealed significant similarity of the porcine cDNA to the 3'-UTR of human protein phosphatase (KAP1) gene (GenBank accession no. L27711). This gene was designated CDKN3 by the HUGO gene nomenclature committee. A primer was designed on the basis of the human sequence (forward: 5'-ATA GAC AGC CTG CGA GAC C-3') and used with a porcine primer (reverse 5'-TTG CCA ATA AAA GCT TTA GGA A-3') for amplification of 3'-UTR and partial codons. With a second primer set (human-derived forward: 5'-GTG AGA CTG CCA GCC ATG AAG-3'; reverse: 5'-TTG ATG ATA GTG CAG CTA ATT T-3') the complete coding region of the porcine gene was amplified. The PCR were performed with 100 ng porcine cDNA, 50 pmol of each primer, and a PCR bead (RTG PCR Beads, AmershamPharmacia Biotech, Freiburg, Germany) in a total volume of 25 µL. Thermocycling was carried out in a TC1 machine (Perkin Elmer, Ueberlingen, Germany). Initial denaturation (94°C for 5 min) was followed by 35 cycles consisting of denaturation (94°C for 1 min), annealing (60°C for 1 min), and extension (70°C for 2 min). The PCR products were cloned into pGEM-T vector (Promega, Mannheim, Germany) and both strands were sequenced on an automated sequencer (ALFexpress, AmershamPharmacia Biotech, Freiburg, Germany).

Comparison with Related Sequences. The porcine CDKN3 cDNA contains a 636-bp open reading frame and has 92% identity with the human sequence (GenBank accession no. NM_005192). The conceptual translation predicts a protein of 212 amino acids. The identity between the human and porcine amino acid sequences is 92%. Despite two nucleotide differences in the stretch coding for the catalytic core the respective amino acid sequences are identical.

Sequence Data. The putative genomic organization of the gene was derived from a human chromosome 14 contig (GenBank accession no. AL049778) containing the complete CDKN3 cDNA. On this basis, primers sets were designed that flanked each one of the putative introns (data not shown). PCR amplification with porcine genomic DNA, cloning, and sequencing was done essentially as described above. After confirmation of the exon/intron boundaries in our genomic fragments sequencing of the remaining intronic sequence was performed by a commercial sequencing facility (SeqLab, Goettingen, Germany). All introns except intron 6 were completely sequenced. The remaining gap is estimated to be less than 500 bp. All splice acceptor/donor sites conform to the GT-AG rule. Our data demonstrate an identical genomic organization of the porcine and human CDKN3 genes (8 exons). Due to length differences, especially in introns 3 and 5, the human gene covers approximately 23 kb genomic sequence compared to 13 kb of the porcine gene. Although sequence and length of the porcine and human introns differ considerably, conserved stretches of 20 to 170 bp (each with homology of 81 to 100%) were observed in all introns. This sequence conservation suggests a functional meaning of these motifs. A porcine short interspersed element (SINE) PRE-1 (Singer et al., 1987) is present in introns 2, 4, 5, and 6 in different orientations. We could demonstrate expression of the gene in skeletal muscle, liver, kidney, brain, and small intestine of newborn piglets by RT-PCR (data not shown). Comparative sequencing of the cDNA of the four investigated piglets revealed one limited polymorphism (nt 653–670: A18 vs A17). All information on genomic organization, protein
sequence, and localization of the catalytic motif is included in the EMBL/GenBank entries listed below.

**Chromosomal Location.** For physical mapping of CDKN3 a pig/rodent somatic cell hybrid panel comprising 27 cell lines was used (Yerle et al., 1996). In two independent experiments a fragment containing the complete intron 3 (575 bp, forward: 5'-ATT CTC AGT TTC TTG GTT TA-3', reverse: 5'-TGG TAT ATT TCT TCT AAC ATC-3') and a fragment of the 3'-UTR (174 bp, forward: 5'-TGC ACA TCT ATC ATC AAG-3'; reverse: 5'-TGC ACA TAT ACA TTT ACA TTT-3') were mapped, respectively. The reactions contained 20 ng genomic DNA, 2 μM of each primer, and a ReadyToGo-PCR bead (Amersham, Freiburg, Germany) in a total volume of 25 μL. Cycling conditions were as follows: initial denaturation at 94°C (4 min) followed by 35 cycles with denaturation at 94°C (1 min), annealing at 64°C/55°C (1 min), and extension at 70°C (2 min). Analysis of 27 porcine-rodent somatic cell hybrids for both fragments revealed no products of identical size with pure hamster and murine DNA and positive results for clones 7, 8, 16, 18, and 19. This amplification pattern allowed assignment of CDKN3 to porcine chromosome 1 (probability 1.00) and to region q23–q27 with 100% concordance (Chevalet et al., 1997).

**EMBL/GenBank Accession Numbers.** AJ404882 (cDNA), AJ404883, and AJ404884 (genomic DNA).

**Comments.** The CDKN3 gene encodes a protein (E.C. 3.1.3.48/3.1.2.16) that belongs to a family of dual-specificity protein phosphatases interacting with cyclin-dependent kinases (Gyuris et al., 1993). Other cyclin-dependent kinase inhibitors are the proteins p21, p27, p57 (CDKN1A, B, C) and p16, p18, and p19 (CDKN2A, B, C, D). Cyclin-dependent kinases (CDK) are key regulators of cell cycle progression. Their co-ordinated inactivation is a prerequisite for withdrawal of several types of cells from the cell cycle. The ability of CDKN3 to interact with cyclin-dependent kinases suggests a potential role in the regulation of the cell cycle (Hannon et al., 1994).

CDKN3 was shown to facilitate the Thr^{160} dephosphorylation of CDK2 when the associated cyclin subunit is degraded or dissociates (Poon and Hunter, 1995). Expression of CDKN3 was observed at the G1 to S transition and overexpression of wild type CDKN3 delays progression through the cell cycle in yeast and HeLa cells (Gyuris et al., 1993). The human CDKN3 gene was mapped to Hsap 1q23–q27 by FISH (Demetrick et al., 1995). According to Goureau et al. (1996), this region corresponds to Sscr 1q23–q27. This is in contrast to our mapping result. However, Goureau et al. (2000) found a correspondence between Sscr 1q23 and Hsap 1q23–q27 is MGAT2 (Leeb et al., 1997). MGAT2 and CDKN3 were assigned to different bands on Hsap 14 but are located within an interval of 1.37 to 3.80 Mbp on Hsap 14 (Unified Database for Human Genome Mapping; URL: http://bioinfo.weiz-mann.ac.il/udb). Additionally, Lahbib-Mansais et al. (1999) assigned a human EST (GenBank accession no. H74180), mapping to the same interval like CDKN3 on human GB4 map, to Sscr 1q23–q27. Our mapping result provides further evidence for the synteny between segments of Hsap 14 and Sscr 1q23–q27. According to the results of Goureau et al. (2000) we assign porcine CDKN3 to Sscr 1q23 rather than to Sscr 1q24–q27 by deduction. The EST isolated in our experiment are the first collection of potential candidate genes for congenital splay leg. With the structural characterization of the porcine CDKN3 gene described in this paper we provide a basis for further analysis of the gene as a potential candidate for the disease.

**Literature Cited**


**Key Words:** Gene Mapping, Cell Cycle, Splayleg, Pigs