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Abstract

Source and Description of Primers. Primers for the Cocaine- and Amphetamine-Regulated Transcript (CART) gene were designed from homologous regions of published human and rat CART sequences (GenBank accession nos. U20325 and U10071, respectively). The primers were used to amplify a 468-bp fragment of the porcine CART gene spanning exons 1 and 2. Porcine CART sequences were sequenced from animals of the Meishan and Hampshire breeds (GenBank accession nos. AFO81918 and AFO81919, respectively) and showed 96% exonic nucleotide identity to the corresponding human CART sequence. Additionally, pig-specific primers were designed for use of the pig/rodent somatic cell hybrids to physically map the CART gene.

Keywords

Pigs, Gene Mapping, Chromosomes

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics

Comments

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Rapid Communication: Genetic Linkage Mapping of the Porcine *Cocaine- and Amphetamine-Regulated Transcript* (*CART*) Gene[1]

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Primer Sequences. Primers derived from human and rat sequences were as follows: forward, 5′-ACA TCT ACT CTG CCG TGG A-3′ and reverse, 5′-GTT TAC TCT TGA GCT TCT TCA-3′. Pig-specific forward primers were 5′-GCT GTC ACC TGA AGA ATG TC-3′, and reverse primers were 5′-TGA GCT TCT TCA GGA CTT CC-3′.

Method of Detection. The PCR amplification was performed using 12.5 ng of porcine genomic DNA, 1× PCR buffer, 1.5 m*M* $MgCl_2$, .125 m*M* dNTPs, .6 μM of each primer, and .7 U *Taq* polymerase (Promega, Madison, WI) in a 20-μL reaction volume. The PCR profile included 2 min at 94°C, 40 cycles of 45 s at 94°C, 1 min at 52°C and 90 s at 72°C, and a final 5-min extension at 72°C in a Robocycler (Stratagene, La Jolla, CA). The PCR product was digested with *Hae*III, and fragments were separated with electrophoresis on 3% agarose gels.

Description of Polymorphism. Sequence analysis of the PCR products from animals of the Meishan and Hampshire breeds revealed six nucleotide substitutions within intron 1 and one in exon 2. One polymorphism was situated within a *Hae*III recognition site and was selected for further use in linkage analysis as a PCR-RFLP. The *Hae*III digestion of the PCR product produced fragments of 298 and 170 bp in allele 2, but allele 1 did not contain a *Hae*III site (Figure 1).

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

Allele Frequencies. Allele frequencies were determined in eight grandparental animals from the European PiGMaP families and in 57 unrelated animals from the ISU reference families. Allele 2 was observed with a frequency of .11 in Large White (n = 9), .23 in Meishan (n = 11), .72 in Duroc (n = 16), .39 in Landrace (n = 9), .17 in Yorkshire (n = 9), and .41 in Hampshire (n = 11).

Chromosomal Location. Two-point linkage analysis of the *CART* polymorphism was performed on the genotypes of three informative European PiGMaP families using the CRI-MAP program (Green et al., 1990). The *CART* gene was most closely linked to *S0077* on SSC16 with a recombination frequency of 0 and a LOD score of 3.91. The best map order relative to the *CART* gene was produced by multipoint linkage analysis with other linked markers and is as follows (with distance in Kosambi centimorgans): *S0077*-5.5-*GHR*-.4- *CART*-2.2-*C9*-4.2-*S0298* (Figure 2). The

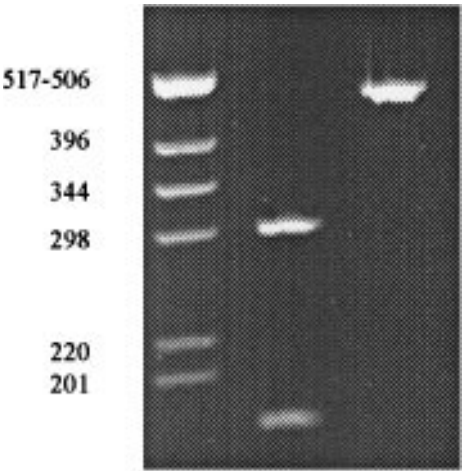

Figure 1. The *CART* polymorphism with *Hae*III digestion. Lane 1 is the molecular marker, lane 2 is the homozygous allele 2, and lane 3 is the homozygous allele 1.

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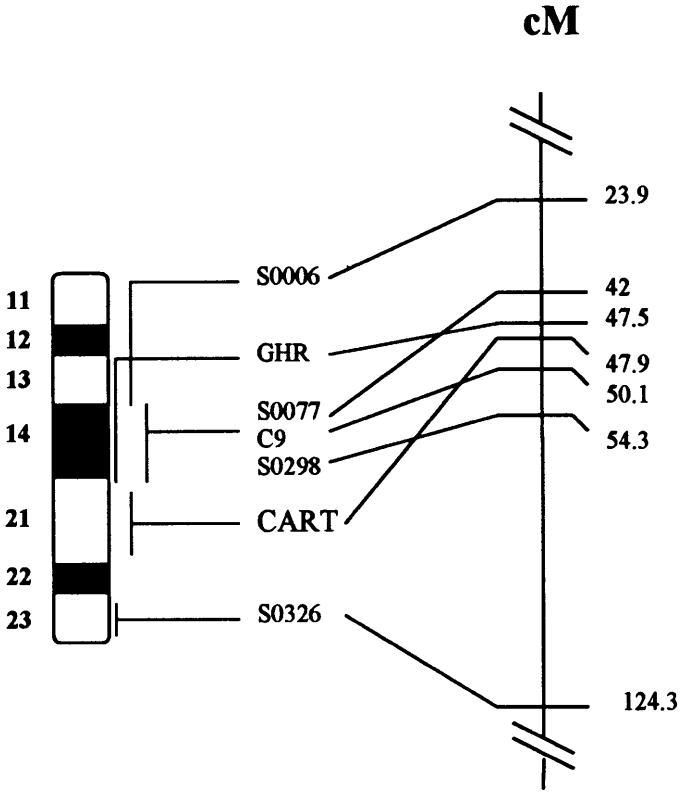

Figure 2. Summary of the physical and linkage maps of *CART* and other genetic markers on SSC 16.

CART gene was also localized to porcine chromosome 16 (SSC16) q21 by the analysis of 12 pig-specific amplifications obtained from 27 pig/rodent somatic cell hybrids with a probability of .818 and a correlation coefficient of 1.0 (Yerle et al., 1996). The disagreement in the gene order between the linkage and the physical data shown in Figure 2 may be due to limited linkage data or to the fact that only one hybrid represents SSC 16q21. Therefore, the regional localization of the *CART* gene with the panel might not be precise enough to provide gene order with certainty. With regard to other markers mapped to SSC16q14-21, there were at least two markers (SW419 and SW81) assigned to the region with some discordance (Robic et al., 1996, 1997).

Comments. Cocaine- and amphetamine-regulated transcript (CART) is a peptide in the brain that acts as a satiety factor associated with food intake regulators, leptin and neuropeptide Y (Kristensen et al., 1998). Feeding inhibition was the first trait observed with putative behavior effects of CART peptides that may be associated with molecular and cellular events caused by administrating the psychomotor stimulants cocaine and amphetamine. Characterization of the *CART* sequence shows a high degree of similarity at both the nucleotide and amino acid levels among mammalian species, implying a conserved function as a neuroendocrine signaling molecule (Douglass and Daoud, 1996). Furthermore, the observation of seven nucleotide substitutions in the 468-bp pig sequence obtained from this study may be of particular interest because an alternative splicing event generated in the rat *CART* gene had been reported (Douglass and Daoud, 1996). All of the seven nucleotide substitutions are pyrimidine (Cytosine or Thymine) base differences, and three of them are close to the intron-exon boundaries. Further studies are needed to determine the importance of these mutations and alternative splicing because experiments have demonstrated the important effects of pyrimidine loss and single base-pair substitutions in mRNA splice junctions (Krawczak et al., 1992).

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