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Abstract

Source and Description of Primers. The conserved sequences of human and rabbit cholesteryl ester transfer protein gene (CETP) (GenBank accession numbers NM000078 and M27486, respectively) were used to design primers to PCR amplify porcine CETP. The primers amplified a 970-bp fragment of porcine CETP gene spanning an intron between exon 1 and exon 2. The porcine CETP sequence (GenBank accession number AF333037) determined from this fragment showed 80.9%(34/42) and 78.6% (33/42) nucleotide identity to the corresponding human and rabbit CETP exon 2 sequence, respectively. To physically map porcine CETP by PCR testing pig/ rodent somatic cell hybrid panel (SCHP) and to develop PCR-RFLP marker for linkage mapping in PigMaP reference families, a pig-specific reverse primer was designed based on the new generated porcine intron 1 sequence.

Keywords

Pigs, Cholesteryl Ester Transfer Protein, Polymorphism, Mapping

Comments

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Rapid communication: Genetic linkage and physical mapping of the porcine *cholesteryl ester transfer protein* (*CETP*) gene[1]

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Source and Description of Primers. The conserved sequences of human and rabbit *cholesteryl ester transfer protein* gene (***CETP***) (GenBank accession numbers NM000078 and M27486, respectively) were used to design primers to PCR amplify porcine *CETP*. The primers amplified a 970-bp fragment of porcine *CETP* gene spanning an intron between exon 1 and exon 2. The porcine *CETP* sequence (GenBank accession number AF333037) determined from this fragment showed 80.9% (34/42) and 78.6% (33/42) nucleotide identity to the corresponding human and rabbit *CETP* exon 2 sequence, respectively. To physically map porcine *CETP* by PCR testing pig/rodent somatic cell hybrid panel (**SCHP**) and to develop PCR-RFLP marker for linkage mapping in PigMaP reference families, a pig-specific reverse primer was designed based on the new generated porcine intron 1 sequence.

Primer Sequences. Primers designed from human and rabbit *CETP* sequences are 5′-TCGTGTGCCGCATCACCAAG-3′ (**PF1**, forward) and 5′-CCGTGATATCTGGGTAGC TG-3′ (**PR1**, reverse). Pig-specific reverse primer is 5′-TGTGCTCAGGCTAACCCAAC-3′ (**PR2**).

Method of Detection. An approximately 970-bp fragment was amplified by using primer pair PF1 and PR1. The amplification was performed in a 50-μL volume of reaction containing 1× PCR magnesium-free buffer, 1.5 m*M* $MgCl_2$, 0.2 m*M* of each dNTP, 1.0 unit of *Taq* polymerase (Promega, Madison, WI), 50 ng of porcine genomic DNA, and 0.15 μ*M* of each primers. The PCR profile included 3 min pre-denature at 94°C, followed by 40 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1.5 min, and ended with final extension at 72°C for 8 min. The PCR products were isolated from a 1.2% agarose gel and purified with QIAEX II gel extraction and purification kit (Qiagen, Valencia, CA). The purified PCR product was sequenced using both primers at the Iowa State University DNA sequencing and synthesis facility (Ames, IA). The amplification with primer pair PF1 and PR2 produced a 436-bp band. In this amplification, 10 μL of reaction volume containing 25 ng of porcine genomic DNA and 0.5 unit of *Taq* polymerase were used. The PCR condition was 94°C for 3 min, then 35 cycles of 94°C for 45 s, 62°C for 45 s and 72°C for 1 min, finished by 72°C for 5 min. Both PCR amplifications were performed in an MJ Research model PTC-200 (Watertown, MA). The 436-bp PCR product produced with the primer pair PF1 and PR2 was digested with restriction enzyme *Ava*II according to instructions of the manufacturer (New England Biolab, Beverly, MA). The digested fragments were separated with electrophoresis on 2.5% agarose gels with ethidium bromide staining.

Description of Polymorphism. Sequence analysis of PCR products generated with primer pair PF1 and PR2 from pigs of the Yorkshire and Meishan breeds revealed nine single nucleotide polymorphisms (**SNP**) within intron 1 (T19C, G69A, C132T, G175T, C203G, C224G, G234C, G853T, and T858C based on GenBank accession number AF333037). The polymorphism at position 224 was situated within an *Ava*II recognition site and was selected as a PCR-RFLP marker for further use in linkage analysis and population variation investigations. The *Ava*II PCR-RFLP exhibited three polymorphic fragments with sizes of 436 bp (allele 1) and 241 bp plus 195 bp (allele 2) (Figure 1).

Pattern of Inheritance. Autosomal Mendelian segregation of porcine *CETP* gene revealed by *Ava*II PCR-RFLP was observed in five three-generation European PiGMaP families (Archibald et al., 1995).

Allele Frequencies. Allele frequencies were determined in 15 Large White pigs, 12 Meishan pigs, eight Yorkshire

Table 1. Recombination frequencies and LOD scores of *CETP* with loci on porcine chromosome 6

Locus	Recombination frequencies	LOD scores
S0016	0.20	5.38
S0087	0.17	4.50
S0325	0.12	3.03
SW1057	0.07	10.79
SW1353	0.18	3.35
SW1841	0.15	3.49
SW2406	0.17	7.31

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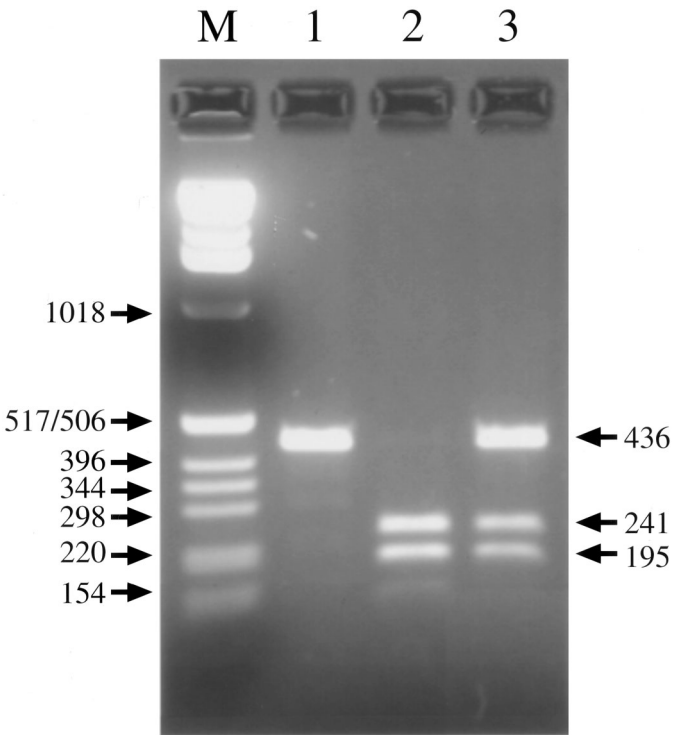

Figure 1. Demonstration of the *CETP* polymorphism by using *Ava*II digestion of PCR fragments. Line 1 is 1 kb ladder, line 2 is the homozygous genotype 1 1, line 3 is the homozygous genotype 2 2, and line 4 is the heterozygous genotype 1 2.

pigs, two Hampshire pigs, and two European wild boars. Allele 1 was observed with a frequency of 0.74 in Large White, 0.17 in Meishan, 0.89 in Yorkshire, 1.00 in Hampshire pigs, and 0.25 in wild boars.

Chromosomal Location. The porcine *CETP* locus was physically assigned to distal chromosome 6p (SSC6p) by analysis of a pig/rodent SCHP comprised of 27 clones (Yerle et al., 1996). A product of expected size amplified with primer pair PF1 and PR2 was observed in clones 12, 18, 19, and 26. The data analysis showed that the *CETP* locus was mapped to porcine chromosome 6 (SSC6) with 100% probability and in region (1/2 p14)–p15 with 87.48% concordance, with less than 0.5% error (http://www.toulouse.inra.fr/lgc/pig/pcr/pcr.htm).

Linkage analysis was performed by genotyping five three-generation PiGMaP reference families (Archibald et al., 1995) by using *Ava*II PCR-RFLP marker and the CRI-MAP program (Green et al., 1990). Two-point linkage results indicated that the porcine *CETP* was closely linked to genes/markers previously mapped to SSC6, consistent with the result of physical mapping. The recombination frequencies and LOD scores with seven microsatellites on SSC6 are presented in Table 1. The best map order produced by multipoint linkage analysis with other linked markers is as follows (with distance in Kosami centimorgans): S0016-43.5-S0087-6.5-S0325-8.2-S0333-23.6-SW1057-2.0-*CETP*-5.3-SW1841-10.9-SW1353-3.7-SW2406.

Comments. Cholesteryl ester transfer protein (*CETP*) is a plasma glycoprotein that mediates the transfer of cholesteryl ester from high-density lipoproteins (**HDL**) to triglyceride-rich lipoproteins in exchange for triglycerides. In humans, the structure of *CETP* has been analyzed in detail (Agellon et al., 1990), and the *CETP* gene had been assigned to chromosome 16q (HSA6q) (Lusis et al., 1987). Kuivenhoven et al. (1997) studied the human *CETP* gene and found a restriction polymorphism *Taq*IB in intron 1 of the *CETP* gene. The *Taq*IB polymorphism had been shown to be associated with an effect on lipid-transfer activity and on HDL cholesterol concentrations. In addition, *CETP* belongs to the lipid transfer/lipopolysaccharide-binding protein family that includes the phospholipid transfer protein, the bactericidal permeability increasing protein, and the lipopolysaccharide-binding protein (Lagrost et al., 1998). Structure similarities suggest that *CETP* might be involved both in lipoprotein metabolism and in antimicrobial defense. In the present study, porcine *CETP* was mapped to SSC6, consistent with the bidirectional painting between human HSA16q and pigs SSC6p (Goureau et al., 1996). Restriction polymorphism *Ava*II in intron 1 of porcine *CETP* will facilitate further investigation of the association between *CETP* variants and lipoprotein metabolism and(or) host defense in pigs.

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