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Rapid communication: Mapping of the beta Tropomyosin (TPM2) gene to pig chromosome 1

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Locus. Pig Tropomyosin beta (TPM2) gene.

Genus and Species. Sus Scrofa.

Source and Description of Primers. Primers were derived by using well-conserved regions of published human and mouse sequence (GenBank accession nos. AF209746 and NM_009416). The primers were used to amplify a 1.4-kp fragment of the porcine TPM2 gene covering exons 3 through 8. The porcine cDNA sequence (GenBank accession no. AF420022) showed 95% exonic identity (325 bp) at the nucleotide level to the human skeletal muscle beta tropomyosin (GenBank accession no. X06825). Additionally, pig specific primers were designed to amplify a 246-bp piece that spans exons 3 to 4 (GenBank accession # AF420023) to be used for linkage and physical mapping.

Primer Sequences. Primers derived from human and mouse were as follows: forward, 5′-CTGAACCGCCG-CATTCAGC-3′ and reverse, 5′-TTTCTCAAACCTTGTGC-CACAGA-3′. Pig-specific primers: forward 5′-AGCTGATGAGAGTGAAAGGTGG-3′ and reverse 5′-GGCTGTCACCTCACCTCC-3′.

Method of Detection. The PCR amplification was performed using 1× PCR Buffer, 1.5 mM MgCl2, 0.125 mM dNTP, 2.5 pmol of each pig-specific primer, 0.2 U Taq Polymerase (Promega, Madison, WI), and 12.5 ng of porcine genomic DNA in a 10-μL reaction volume. Thermocycling was carried out in a MJ Research, PTC-100 instrument (Watertown, MA). The cycling conditions included an initial denaturation of 3 min at 94°C followed by 40 cycles of 93°C for 1 min, 58°C for 1 min, and 72°C for 2 min, and a final extension for 12 min at 72°C. The PCR product was digested using the AvaII enzyme, and the fragments were separated using electrophoresis on 3% agarose gels.

Description of Polymorphism. Digestion of the resulting porcine PCR product (246 bp) with AvaII confirmed a polymorphism with two alleles. Allele 1 remained uncut after the digestion, producing a single 246-bp piece, and allele 2 produced two fragments that were 167 bp and 79 bp. Heterozygous individuals contained fragments from both allele 1 and allele 2 (Figure 1).

Pattern of Inheritance. Autosomal segregation of Mendelian inheritance was confirmed in three three-generation European PiGMaP families (Archibald et al., 1995), including both of the Edinburgh families and the Swedish family.

Allele Frequencies. Allele frequencies were determined from 73 animals without common grandparents from Iowa State University representing five different breeds. Allele 2 was fixed in four out of the five breeds, including Hampshire (n = 20), Duroc (n = 20), Yorkshire (n = 16), and Landrace (n = 10). Allele 1 was fixed in Meishan (n = 7).

Chromosomal Location. Positive results were obtained for hybrids 7, 8, 16, 18, and 19 in the pig/rodent somatic cell hybrid panel (Yerle et al., 1996). The PCR results were submitted and analyzed as is described on the Web site located at http://www.toulouse.inra.fr/lgc/pig/hybrid.htm. These results revealed that the TPM2 gene was physically mapped to the porcine chromosome 1 (SSC1) region q23–27 (P = 0.86). Marker genotypes from

Figure 1. Agarose gel image showing the different PCR-RFLP genotypes of TPM2 using AvaII. Lane 1 includes the 1-kb ladder with predicted sizes indicated on the left; lane 2, the heterozygote; lane 3, allele 2 homozygote; lane 4, allele 1 homozygote. The arrows indicate each allele.
the three-generation PiGMaP families (Archibald, et al., 1995) were used in two-point and multi-point analyses (CRI-MAP; Green et al., 1990) to map TPM2. Based on the linkage results, porcine TPM2 was shown to have significant linkage with several previously mapped markers on porcine chromosome 1. Most significant linkages between TPM2 and PiGMaP gene markers were obtained from microsatellite S0155 (recombination fraction = 0.02 and LOD = 24.48) and S0311 (recombination fraction = 0.04 and LOD = 22.58). These results confirmed the position obtained by the physical map.

Comments. Tropomyosin is an alpha helical coiled coil protein (Smillie, 1999). It binds end-to-end to form a continuous fiber that follows the helical symmetry of the actin filament. Tropomyosin plays an important regulatory role in muscle contraction by blocking the myosin-binding site on actin and either preventing or allowing muscle contraction depending on the intracellular ion concentration. In the absence of calcium, tropomyosin inhibits the binding of myosin heads to actin filaments. Tropomyosin’s interaction with other muscle proteins and its role in muscle contraction make it a very important protein for muscle contraction. Previous work revealed that TPM2 was mapped to human chromosome 9p13.2–p13.1 (Tiso et al., 1997). The position of TPM2 on SSC1q23–27 corresponds to this region in humans based on comparative mapping studies. The agreement between the human map location and the localization of TPM2 to SSC 1 can improve comparative mapping between humans and pigs.

Literature Cited


Green, P., K. Falls, and S. Crooks. 1990. Documentation for CRI-MAP, version 2.4. Washington Univ. School of Medicine, St. Louis, MO.


Key Words: Polymorphism, Gene Mapping, Pigs, Tropomyosins