2002

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Abstract

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.

Keywords

Polymorphism, Gene Mapping, Pigs, Tropomyosins

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics

Comments

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Rapid communication: Mapping of the *beta Tropomyosin* (*TPM2*) gene to pig chromosome 1[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′-CTGAACCGCCGCATTCAGC-3′ and reverse, 5′-TTTCTCCAACTTTGCCACAGA-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 m*M* $MgCl_2$, 0.125 m*M* 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 *Ava*II 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 *Ava*II 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).

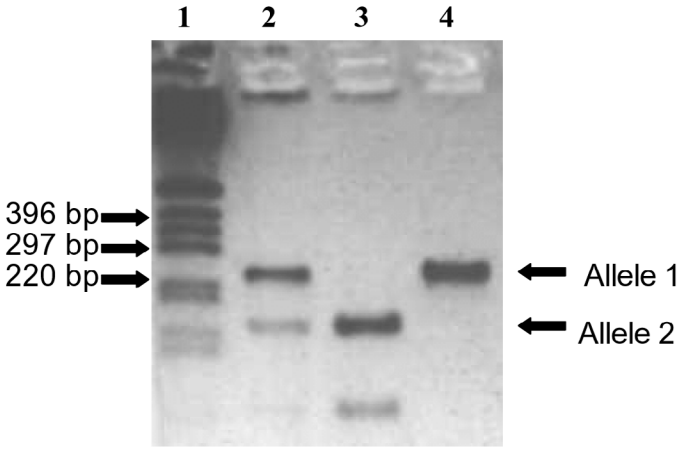

Figure 1. Agarose gel image showing the different PCR-RFLP genotypes of *TPM2* using *Ava*II. Lane 1 includes the 1-kb ladder with predicted sizes indicated on the left; lane 2, the heterozygote; lane 3, allele 2 homozygote; lane 4, allele 1 homozygote. The arrows indicate each allele.

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

[1]We thank Jeannine Helm and Kwan-Suk Kim for their advice and suggestions, Alan Archibald for use of the PiGMaP DNA and the ResPig database, and Martine Yerle for use of the French SCH panel. Support was provided by an Iowa State Univ. Assistantship Program, PIC International Group and the Iowa Agric. and Home Econ. Exp. Stn., Ames, paper no. J-19323, project no. 3600, as well as by Hatch Act and State of Iowa funds.

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Received April 25, 2001.

Accepted January 16, 2002.

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.

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