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References

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Linkage mapping of a SNP in the porcine MADH1 gene to a region of chromosome 8 that contains QTL for uterine capacity
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Source/description of primers: Homo sapiens mothers against decapentaplegic homolog 1 (Drosophila) (MADH1), also reported as Smad1, belongs to a family of proteins that mediate signal transduction from TGF-β family ligands, including TGF-β and bone morphogenetic proteins. Comparison of porcine and human genetic maps suggest that MADH1 is located near the uterine capacity quantitative trait locus (QTL) on chromosome 8. In addition, MADH1 mRNA is expressed in the vascular endothelial cells of mouse decidua and MADH1-mutant mice die due to the defects in allantois formation. A cDNA clone of MADH1 (2077 bp) containing the full coding region of the porcine MADH1 was isolated (GenBank accession no. AY245888) from the ‘Meat Animal Research Center (MARC) 2PIG’ expressed sequence tag (EST) primary library by iterative screening and sequenced. The forward (MADH1 f1) and reverse (MADH1 r1) primers that were used for PCR amplification to screen the MARC 2PIG EST library were derived from ovine MADH1 sequence (GenBank accession no. AY035385) and corresponded to bases 560–578 (with one mismatch) and 932–950 (with two mismatches) of the porcine cDNA sequence (GenBank accession no. AY245888), respectively. For single nucleotide polymorphism (SNP) detection, forward (MADH1 f2) and reverse (MADH1 r2) primers [bases 1628–1647 and 2046–2026 of the porcine MADH1 cDNA (GenBank accession no. AY245888), respectively] were designed to amplify a 419-bp product in the 3′ untranslated region of the cDNA. This region was evaluated for SNP in the eight parents (seven F1 sows and one white composite boar) of the MARC Swine Reference Population by sequencing PCR products amplified from genomic DNA.

PCR primer sequences and flanking sequence for a single nucleotide polymorphism: MADH1 f1: CATTCTCTGCTCCCTGGAC
MADH1 r1: AGGAGATTTGGAATACGGTG
MADH1 f2: AGAATACCACCGCCAGGATG
MADH1 r2: AGATGATTGCCTCCCTGGTG.

Sequence flanking polymorphism: CATCTGAAC(T/C)ACAAA GGAGGC
MADH1 probe primer: TCAGACCATCTGAACT.

PCR conditions: Polymerase chain reaction reactions were performed in a 25-μl volume containing 100 ng genomic DNA, 1.5 mM MgCl2, 20 pmol of each primer, 100 μM dNTP and 0.35 U Hotstar™ Taq polymerase (Qiagen Inc., Valencia, CA, USA). Amplification was performed under the following PCR conditions: 15 min at 94 °C; 45 cycles of 30 s at 94 °C, annealing for 45 s at 61 °C, 1.5 min at 72 °C and a final extension of 5 min at 72 °C. Both strands of the amplified genomic DNA of parents from the MARC Swine Reference Population were sequenced and evaluated for polymorphisms.

Polyorphism and chromosomal location: A C/G single nucleotide polymorphism was detected at position 1842 of the porcine MADH1 cDNA (GenBank accession no. AY245888). This polymorphism was heterozygous in three of the seven F1 sows. An assay was designed to genotype this polymorphism using primers MADH1 f2 and r2, primer extension with the MADH1 probe primer and analyte detection on a MALDI-TOF mass spectrometer (Sequenom Inc., San Diego, CA, USA) with 50 μl of PCR product. Polymerase chain reaction conditions were same as above. This marker generated 38 informative meioses in the MARC Swine Reference Population. The MADH1 gene was mapped to chromosome 8 at position 78 cM, which is similar to the marker KS139 on the current MARC swine chromosome 8 linkage map (http://www.marc.usda.gov/) using CRI-MAP. The most significant two-point linkage detected was with KS139 (LOD = 10.24) at 0 recombinant. MADH1 maps are within the uterine capacity QTL on chromosome 8. The human MADH1 gene is located on chromosome 4q28, which shares homology with swine chromosome 8.

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References
1 Huang S. et al. (2000) Gene 258, 43–53.
Linkage mapping of the bovine bone morphogenetic protein receptor-1B (BMPR1B) to chromosome 6


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Source/description of primers: A mutation in the ovine bone-morphogenetic protein receptor-1B (BMPR1B) gene is associated with an increased ovulation rate phenotype in Booroola Merino sheep1–3. Therefore, BMPR1B is considered as a candidate gene for ovulation rate in cattle. A cDNA was obtained for porcine BMPR1B and the gene was mapped in the porcine genome4. Comparison of the ovine and porcine BMPR1B cDNA sequences indicated that they are highly conserved. Therefore, a pair of primers derived from the porcine BMPR1B cDNA, which amplified across intron 8 in the pig, were used to amplify the bovine genomic DNA. The forward (NZ-F1)1 and reverse (exon9-R1) primers correspond to bases 1041–1064 and 1289–1265 of the porcine BMPR1B cDNA (GenBank accession no. AF432128), respectively. Agarose gel electrophoresis and sequencing of the polymerase chain reaction (PCR) amplicons of bovine genomic DNA indicated that the size of this product was 1253 bp (GenBank accession no. AY242067).

PCR primer sequences and a flanking sequence for a single nucleotide polymorphism: BMPR1B NZ-F1: GTGCATATGGGAAGTTTGG ATG BMPR1B exon9-R1: GGTGCTGACCTCAGGTAATCATAG Sequence flanking polymorphism: GTGGTAAAGA(A/G)TCTA CATTAG CTTG

PCR conditions: Polymerase chain reactions were carried out in a 25-μl volume containing 100 ng genomic DNA, 1.5 mM MgCl2, 20 pmol of each primer, 100 μM dNTP and 0.35 U Taq polymerase. Amplification was performed under the following PCR conditions: 2 min at 94 °C; 45 cycles of 30 s at 94 °C, annealing for 1 min at 61 °C, 1 min at 72 °C and a final extension of 5 min at 72 °C. Both strands of the amplified genomic DNAs were sequenced and evaluated for polymorphisms5 in four bulls from the Meat Animal Research Center (MARC) Bovine Reference Population6.

Polymorphism and chromosomal location: An A/G single nucleotide polymorphism was detected in intron 8 (GenBank accession no. AY242067), position 658 from the exon/intron boundary. This polymorphism was heterozygous in two of the four bulls from the MARC Bovine Reference Population. An assay was designed to genotype this polymorphism using primer extension with analyze detection on a MALDI-TOF mass spectrometer (Sequenom Inc., San Diego, CA, USA). This marker generated 50 informative meioses (40 phases known) in the MARC Bovine Reference Population. The BMPR1B gene was mapped to chromosome 6 position 42.4 cM on the current MARC bovine chromosome 6 linkage map (http://www.marc.usda.gov/) using CRI-MAP near the quantitative trait locus for milk production7. The most significant two-point linkage detected was with BMS2508 (LOD = 11.40) at 0.04 recombination. The BMPR1B gene in human is located on chromosome 4q22.3, which shares homology with bovine chromosome 6.

References

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Mutations in the limbin gene previously associated with dwarfism in Japanese brown cattle are not responsible for dwarfism in American Angus breed

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Source/description: Bovine chondrodysplastic dwarfism in Japanese brown cattle has been mapped to the distal end of bovine chromosome 6 by linkage analysis. Disease–specific mutations in limbin were identified in affected dwarf calves1. Disproportionate dwarfism has been reported in many cattle breeds including Dexter, Holstein, Aberdeen Angus, Hereford and Shorthorn breeds2,3. Dwarfism in American Angus has not been reported since the 1970’s until recently when