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## References

- 1 Buitkamp J. *et al.* (2000) *Anim Genet* **31**, 347–51.
- 2 Solinas-Toldo S. *et al.* (1993) *Mamm Genome* **4**, 720–7.
- 3 Womack J. E. *et al.* (1997) *Mamm Genome* **8**, 854–6.
- 4 Hofmann F. *et al.* (1999) *Rev Physiol Biochem Pharmacol* **139**, 33–87.
- 5 Doyle J. L. *et al.* (1998) *Trends Genet* **14**, 92–8.
- 6 Powers P. A. *et al.* (1994) *Genomics* **19**, 192–3.
- 7 Georges M. *et al.* (1993) *Proc Natl Acad Sci USA* **90**, 1058–62.

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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- $\beta$  family ligands, including TGF- $\beta$  and bone morphogenetic proteins<sup>1</sup>. Comparison of porcine and human genetic maps suggest that *MADH1* is located near the uterine capacity quantitative trait locus (QTL) on chromosome 8<sup>2</sup>. In addition, *MADH1* mRNA is expressed in the vascular endothelial cells of mouse decidua<sup>3</sup> and *MADH1*-mutant mice die due to the defects in allantois formation<sup>4</sup>. A cDNA clone (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<sup>5</sup> 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<sup>6</sup> by sequencing PCR products amplified from genomic DNA.

*PCR primer sequences and flanking sequence for a single nucleotide polymorphism:* *MADH1* f1: CATTCTCGCTCCCTGGAC  
*MADH1* r1: AGGAGAGTTGGGGTAACTGCTG  
*MADH1* f2: AGAATACCACCGCCAGGATG  
*MADH1* r2: AGATGATTTGTCCCTGGCTTG.

Sequence flanking polymorphism: CATCTGAACT(C/G)ACAAA GGAGC

*MADH1* probe primer: TCAGACCATCTGAACT.

*PCR conditions:* Polymerase chain reaction reactions were performed in a 25- $\mu$ l volume containing 100 ng genomic DNA, 1.5 mM MgCl<sub>2</sub>, 20 pmol of each primer, 100  $\mu$ M dNTP and 0.35 U Hotstar<sup>TM</sup> *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<sup>6</sup> were sequenced and evaluated for polymorphisms<sup>7</sup>.

*Polymorphism 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<sup>8</sup> (Sequenom Inc., San Diego, CA, USA) with 50  $\mu$ l 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 recombination. *MADH1* maps are within the uterine capacity QTL on chromosome 8<sup>2</sup>. 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.
- 2 Rohrer G.A. *et al.* (1999) *J Anim Sci* **77**, 1385–91.
- 3 Ying Y. *et al.* (2000) *Biol Reprod* **63**, 1781–6.

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- 4 Lechleider L.J. *et al.* (2001) *Dev Biol* **240**, 157–67.  
 5 Fahrenkrug S.C. *et al.* (2002) *Mamm Genome* **13**, 475–8.  
 6 Rohrer G.A. *et al.* (1994) *Genetics* **136**, 231–45.  
 7 Fahrenkrug S.C. *et al.* (2002) *Anim Genet* **33**, 186–95.  
 8 Heaton M.P. *et al.* (2002) *Mamm Genome* **13**, 272–81.

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## 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 sheep<sup>1–3</sup>. 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 genome<sup>4</sup>. 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)<sup>3</sup> 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: GTCGCTATGGGGAAGTTTGG ATG

*BMPR1B* exon9-R1: GGTGGTGGACTTCAGTAATCATAG  
 Sequence flanking polymorphism: GTGGTAAAGA(A/G)TCTA CCTGCC

*PCR conditions:* Polymerase chain reactions were carried out in a 25- $\mu$ l volume containing 100 ng genomic DNA, 1.5 mM MgCl<sub>2</sub>, 20 pmol of each primer, 100  $\mu$ M 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 polymorphisms<sup>5</sup> in four bulls from the Meat Animal Research Center (MARC) Bovine Reference Population<sup>6</sup>.

*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<sup>6</sup>. An assay was designed to genotype this polymorphism using primer extension with analyte 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 production<sup>7</sup>. 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

- Mulsant P. *et al.* (2001) *Proc Natl Acad Sci USA* **98**, 5104–9.
- Souza C.H.I. *et al.* (2001) *J Endocrinol* **169**, R1–R6.
- Wilson T. *et al.* (2001) *Biol Reprod* **64**, 1225–35.
- Kim J.G. *et al.* (2003) *Biol Reprod* **68**, 735–43.
- Fahrenkrug S.C. *et al.* (2002) *Anim Genet* **33**, 186–95.
- Bishop M.D. *et al.* (1994) *Genetics* **136**, 619–39.
- Olsen H.G. *et al.* (2002) *J Dairy Sci* **85**, 3124–30.

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## Mutations in the *limbin* gene previously associated with dwarfism in Japanese brown cattle are not responsible for dwarfism in the 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 calves<sup>1</sup>. Disproportionate dwarfism has been reported in many cattle breeds including Dexter, Holstein, Aberdeen Angus, Hereford and Shorthorn breeds<sup>2,3</sup>. Dwarfism in American Angus has not been reported since the 1970's until recently when

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