Assignment¹ of the gene (MBD3) encoding methyl-CpG binding domain protein 3 to porcine chromosome 2q21 → q22 by somatic cell and radiation hybrid panel mapping

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¹ To our knowledge this is the first time this gene has been mapped in swine.

Rationale and significance

DNA methylation is the major modification of eukaryotic genomes and plays an essential role in mammalian development. Human proteins MeCP2, MBD1, MBD2, MBD3, and MBD4 comprise a family of nuclear proteins related by the presence of a methyl-CpG binding domain (MBD). Each of these proteins, with the exception of MBD3, is capable of binding specifically to methylated DNA. MBD3 is a subunit of the NuRD, a multisubunit complex containing nucleosome remodeling and histone deacetylase activities. MBD3 mediates the association of metastasis-associated protein 2 (MTA2) with the core histone deacetylase complex. The human MBD3 gene was localized on 19p13 (Hendrich et al., 1999). In this study, using somatic cell hybrid cells and a radiation hybrid panel we have mapped gene MBD3 to porcine chromosome 2, thus confirming the expected localization of the gene in the swine genome.

Materials and methods

Primer design

From the website (<http://genopole.toulouse.inra.fr/lccare/>), the porcine sequence orthologous to human gene MBD3 was selected to design the primer pair inside the putative exon, which was deduced from the exon/intron

structure of the corresponding human gene. Primers were designed using Primer 3 software (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>) (Rozen and Skaletsky, 2000).

PCR conditions/sequencing

The PCR conditions were optimized on 25 ng and 35 ng genomic DNAs from pig and Chinese hamster, respectively, in 15 µl of 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl₂, 50 mM KCl, 200 µM of each dNTP, 1.0 µM of primer pair, and 0.375 U AmpliTaq Gold (BE, Applied Biosystems, USA). The primers used for PCR analysis were 5'-GCAAGCCTGACTTGAACACA-3' and 5'-TTCTGGGGGTCCTCTTAC-3'. The mixture was treated at 95 °C for 9 min, then with 35 cycles of denaturation at 95 °C for 30 s, 65 °C for 30 s, and extension at 72 °C for 30 s, followed by a final elongation step at 72 °C for 5 min. All PCRs were carried out using the "GeneAmpPCR System 9700" (Perkin-Elmer, CT, USA). The 115-bp porcine-specific PCR product was detected by agarose gel electrophoresis. The resultant PCR product was sequenced using ABI PRISM Big Dye Terminator Sequencing Ready Reaction Kit and a 3700 DNA Analyzer (Applied Biosystems, CA, USA) to confirm that the sequence was identical to the sequence used for primers design.

RH panel mapping

Using the primer pair thus prepared as well as the 90 selected IMPRH 7000-rad hybrid cell panel DNAs, which were provided by INRA (France) and the University of Minnesota (USA) (Yerle et al., 1998), PCR was performed under the conditions described above. PCR products were separated in a 2.0% agarose gel, and scored for mapping as described previously (Kiuchi et al., 2002). Assignment of the gene to the IMPRH map was performed with the IMPRH mapping tool (Milan et al., 2000), which is accessible on the INRA web server (<http://imprh.toulouse.inra.fr>).

SCH mapping

The somatic cell hybrid-panel DNAs (Yerle et al., 1996) provided by INRA (France) were used for mapping the MBD3 gene. The PCR conditions for SCH mapping were the same as for RH mapping except that 20 ng of panel DNA was used for each PCR. The assignment was performed with software that calculates the likelihood of the localization of a marker in 115 individual regions of the porcine genome on the INRA web server (<http://www.toulouse.inra.fr/lgc/pig/pcr/pcr.htm>).

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Results

Regional mapping results

Somatic porcine × rodent cell hybrid panel analysis of 27 hybrids resulted in the following vector: 01000 10000 00000 00000 10001 00. Statistical evaluation showed a correlation between the MBD3 gene and chromosome 2 (1/2 q21)→(1/2 q22) with the highest probability and correlation values (69 and 85%, respectively; error risk <1%). The IMpRH panel was employed to determine the precise location of the MBD3. The survey of the RH panel showed the following distribution within the 90 lines: 00000 00010 00001 01100 00011 00000 00001 00000 00010 00000 00100 01001 00110 00000 10110 01001 00010 01011, with the most significantly linked markers (LOD score 5.88 and 5.49) SW776 and SW395 (Hawken et al., 1999). Localization of MBD3 locus to SSC2q21→q22 using both SCHP and IMpRH, in the same region, confirmed the conservation of synteny between SSC2 and HSA19.

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