2000

Rapid communication: Linkage and physical mapping of the porcine melanocortin-4 receptor (MC4R) gene

K. S. Kim
Iowa State University

N. K. Larsen
Iowa State University

Max F. Rothschild
Iowa State University, mfrothsc@iastate.edu

Rapid communication: Linkage and physical mapping of the porcine melanocortin-4 receptor (MC4R) gene

Abstract

Genus and Species. Sus scrofa. Locus. Pig Melanocortin-4 Receptor (MC4R) gene. Source and Description of Primers. Primers were designed from well-conserved sequence regions between the human and rat MC4R genes (Genbank accession no. S77415 and U67863, respectively). The primers were used to amplify approximately 760 bp of the porcine MC4R gene from genomic DNA. The sequence of the PCR product showed 92.2 and 97.6% identities at the nucleotide and amino acid levels, respectively, to the corresponding human sequence. The porcine MC4R sequence has been submitted to GenBank (accession no. AF087937). Using this sequence, pig-specific primers were designed.

Keywords

Melanocyte Stimulating Hormone Receptor, Pigs, Gene Mapping

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics

Comments

This is an article from *Journal of Animal Science* 78 (2000): 791, doi:/2000.783791x. Posted with permission.

Rapid communication: Linkage and physical mapping of the porcine *melanocortin-4 receptor* (*MC4R*) gene[1]

K. S. Kim, N. J. Larsen[2], and M. F. Rothschild[3]

Department of Animal Science, Iowa State University, Ames 50011

Genus and Species. Sus scrofa.

Locus. Pig *Melanocortin-4 Receptor (MC4R)* gene.

Source and Description of Primers. Primers were designed from well-conserved sequence regions between the human and rat *MC4R* genes (Genbank accession no. S77415 and U67863, respectively). The primers were used to amplify approximately 760 bp of the porcine *MC4R* gene from genomic DNA. The sequence of the PCR product showed 92.2 and 97.6% identities at the nucleotide and amino acid levels, respectively, to the corresponding human sequence. The porcine *MC4R* sequence has been submitted to GenBank (accession no. AF087937). Using this sequence, pig-specific primers were designed.

Primer Sequences. Primers derived from human and rat sequences: Forward primer: 5′-TGG CAA TAG CCA AGA ACA AG-3′ and reverse primer: 5′-CAG GGG ATA GCA ACA GAT GA-3′. Pig-specific primers: Forward primer: 5′-TTA AGT GGA GGA AGA AGG-3′ and reverse primer: 5′-CAT TAT GAC AGT TAA GCG G-3′.

Method of Detection. The PCR reaction was performed using 12.5 ng of porcine genomic DNA, 1× PCR buffer, 1.5 m*M* $MgCl_2$, .125 m*M* dNTP, .3 μ*M* of each primer, and .35 U *Taq* DNA polymerase (Promega, Madison, WI) in a 10-μL final volume. The PCR profile included 2 min at 94°C; 35 cycles of 30 s at 94°C, 1 min at 56°C, 1 min 30 s at 72°C; and a final 15-min extension at 72°C in a Robocycler (Stratagene, La Jolla, CA). The PCR product was digested with *Taq*I and incubated overnight at 65°C. The digested fragments were separated by electrophoresis in a 3% agarose gel.

Description of Polymorphism. The *Taq*I digestion of the PCR product produced fragments of 466, 225, and 76 bp in allele 1 vs 542 and 225 bp in allele 2. The heterozygous genotype has fragments of both allele 1 and allele 2 (Figure 1).

Pattern of Inheritance. Autosomal segregation of Mendelian inheritance was observed in three three-generation European PiGMaP families (Archibald et al., 1995).

Allele Frequencies. Allele frequencies were determined by genotyping of 6 grandparental animals of the European PiGMaP families and 49 unrelated animals from several breeds in the ISU herd. Allele 1 was observed with a frequency of 1.0 in the Meishan pigs (n = 8), .15 in Yorkshire (n = 10), .2 in Duroc (n = 9), .56 in both Landrace (n = 8) and Chester White pigs (n = 8), and was not observed in Hampshire pigs (n = 12).

Chromosomal Location. The *MC4R* gene was physically mapped to porcine chromosome 1 (SSC1) q22–q27 by observing amplification product in clones 7, 8, 16, 18, and 19 of the pig/rodent somatic cell hybrid panel (Yerle et al., 1996). Two-point and multi-point linkage analyses were performed on the genotypes of PiGMaP families using the CRI-MAP program (Green et al., 1990). The *MC4R* gene was significantly linked to several markers on porcine chromosome 1 (**SSC1**). The two most closely linked markers (recombination fraction and LOD score) determined by two-point linkage analysis were SO313 (0, 17.76) and S0082 (.05, 14.74). A multi-point linkage analysis produced the best map order of the *MC4R* gene between linked markers (with distance in Kosambi cM): *FGF*7-5.6-*MEF2A*-5.8-*MC4R*-5.9-*SO313*-0-*S0082*.

Comments. The melanocortin-4 receptor is a G protein-coupled, seven-transmembrane receptor expressed in the brain. Huszar et al. (1997) found that inactivation of *MC4R* gene resulted in a maturity onset obesity syndrome in mice and demonstrated a major role of MC4R protein in regulation of energy balance. The *MC4R* gene has been mapped to human chromosome 18q21.3 (Gantz

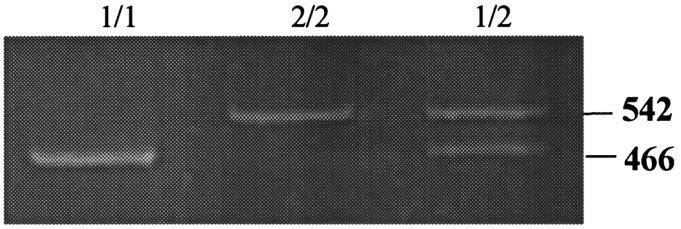

Figure 1. *Taq*I digestion of *MC4R* fragment, analyzed by agarose-gel electrophoresis. Allele 1 produced a 466-bp fragment and allele 2 produced a 542-bp fragment as the PCR-RFLP. Both alleles produce a 225-bp fragment, and allele 1 has an additional 75-bp fragment (not shown).

[1]The authors thank John Newton for collection of samples and Jeannine Helm, Zhiliang Hu, and Stefan Marklund for technical support. This work is supported in part by PIC USA and PIC Group and the Iowa Agric. and Home Econ. Exp. Sta., Ames, paper no. J-18325, project no. 3148, as well as by Hatch Act and State of Iowa funds.

[2]Present address: The Panum Institute, Copenhagen Univ., Blegdamsvej 3b, 2200 Copenhagen N, Denmark.

[3]To whom correspondence should be addressed.

Received April 5, 1999.

Accepted September 22, 1999.

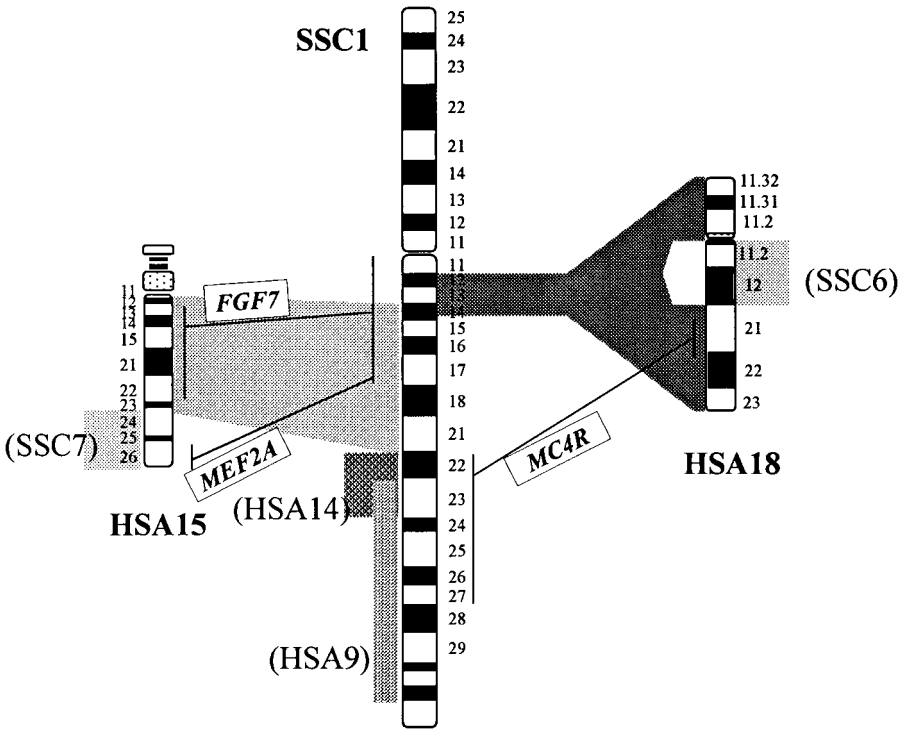

Figure 2. Comparative physical map of SSC1 with HSA15 and HSA18. The location of *MC4R* might indicate an additional region of conserved synteny between human chromosome 18 (HSA18) and porcine chromosome 1 (SSC1). The shaded regions summarize results of mono- and bidirectional painting (SSC1 did not produce a paint on any human chromosome; Goureau et al., 1996). Also included are the other genes included in the multipoint linkage map. Portions corresponding to SSC6 and SSC7 are also displayed.

et al., 1993), and mono- and bidirectional painting (human-pig) revealed that the *MC4R* gene was expected to map on SSC1q12–q14 (Goureau et al., 1996). However, our study showed that the *MC4R* gene is located on SSC1q22–q27, a region with expected correspondence to HSA9 and HSA14 (Figure 2). This result indicates that SSC1 might have additional regions of conserved synteny that were not detected by the painting between HSA18 and SSC1. In addition, the gene order of the physical and multipoint linkage analyses showed that several markers assigned from HSA15 to SSC1, including *FGF7* (SSC1q11–q17) and *MEF2A* (SSC1q11–q17) (Larsen et al., 1999), are centromeric to *MC4R*. These results suggest that the location of the *MC4R* gene might define a boundary between regions on SSC1 corresponding to HSA18 and HSA15.

Literature Cited

Archibald, A., J. Brown, S. Couperwhite, H. McQueen, D. Nicholson, C. Haley, W. Coppieters, A. Van de Weghe, A. Strantil, A. Winterø, M. Fredholm, N. Larsen, V. Nielsen, D. Milan, N. Woloszyn, A. Robic, M. Dalens, J. Riquet, J. Gellin, J.-C. Caritez, G. Burgaud, L. Olliver, J.-P. Bidanel, M. Vaiman, C. Renard, H. Geldermann, R. Davolie, D. Ruyter, E. Verstege, M. Groenen, W. Davies, B. Høyheim, A. Keiserud, L. Andersson, H. Ellegren, M. Johannson, J. Miller, L. Marklund, D. Anderson Dear, E. Signer, A. Jeffreys, C. Moran, P. Le Tissier, Muladno, M. Rothschild, C. Tuggle, D. Vaske, J. Helm H.-C. Liu, A. Rahman, T.-P. Yu, R. G. Larson, and C. Schmitz. 1995. The PiGMaP consortium linkage map of the Domestic Pig (*Sus scrofa domestica*). Mamm. Genome 6:157–175.

Gantz, I., H. Miwa, Y. Konda, Y. Shimoto, T. Tashiro, S. J. Watson, J. Delvalle, and T. Yamada. 1993. Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. J. Biol. Chem. 268:15174–15179.

Goureau A., M. Yerle, A. Schmitz, J. Riquet, D. Milan, P. Pinton, G. Frelat, and J. Gellin. 1996. Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. Genomics 36:252–262.

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

Huszar, D., C. Lynch, V. Fairchild-Huntress, J. Dunmore, Q. Fang, L. Berkemeier, W. Gu, R. Kesterson, B. Boston, R. Cone, F. Smith, L. Campfield, P. Burn, and F. Lee. 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131–141.

Larsen, N., S. Marklund, K. Kelly, M. Malek, C. Tuggle, M. Yerle, and M. Rothschild. 1999. New insights into porcine-human synteny conservation. Mamm. Genome 10:488–491.

Yerle, M., G. Echard, A. Robic, A. Mairal, C. Dubut-Fontana, J. Riquet, P. Pinton, D. Milan, Y. Lahbib-Mansais, and J. Gellin. 1996. A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. Cytogenet. Cell Genet. 73:194–202.

Key Words: Melanocyte Stimulating Hormone Receptor, Pigs, Gene Mapping