

Two Breed-Specific Bovine MC1-R Alleles in Brown Swiss and Saler Breeds

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Name of Sequence

Bovine Melanocyte-Stimulating-Hormone-Receptor (MC1-R)

EMBL/GenBank Accession Numbers

AJ 297819
Y19103

Species (strains)

Bos taurus (Saler; Brown Swiss)

Origin of the Clone

PCR product

Sequencing Method

Genomic DNA was isolated from blood and semen samples. Fragments of the bovine MC1-R gene were generated by PCR using the following primers (Vanetti et al., 1994):

P1-5'-AATGTA~~AAACGACGGCCAGTCTCCATCTTCT~~ACGCCCTGC-3'

P2-5'-AACAGGAAACAGCTATGACCTCTAGCGGATCCTCTTTGTCAAGGG-3'

P3-5'-AATGTA~~AAACGACGGCCAGTTTGAGCAGGA~~TCCTGAGAGCAAG-3'

P4-5'-AACAGGAAACAGCTATGACCGGGCCAGCATAGCTATGAAGA-3'

The underlined bases are complementary to M13-forward and M13-reverse sequences. Tailing of these primers was done to enable direct sequencing of the PCR products employing the cycle sequencing approach.

All PCR reactions were carried out in a 25 μ l volume, containing 50 mM KCl; 10 mM Tris/HCl (pH 8.8), 1.5 mM MgCl₂; 200 μ M each dNTP; 20 pM each primer; 50 ng of whole genomic DNA, and 1 unit of Taq DNA polymerase. The following PCR conditions were chosen for both primer pairs: 5 min at 94°C, followed by 33 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. The PCR was completed at 72°C for 5 min.

With the primers P1 and P2, a 596-bp fragment was amplified that corresponded to the nucleotides 557 to 1152 of the bovine MC1-R sequence. This fragment included the stop codon. In another PCR with the primers P3 and P4, a 654-bp fragment was amplified that corresponded to the positions 77 and 731 of the bovine MC1-R sequence. This fragment included the start codon. Plus and minus strands were both subsequently subjected to sequence analysis. Sequence analysis was done using an automated sequencer system (LI-COR Corp., Lincoln, NE).

Comments

The coat color of domesticated animals has always been a marker for breed identity. Animal breeders distinguish the respective differences at the DNA level. In simply inherited traits, there is always a good chance to attribute differences in traits to specific alleles. The major phenotypic variation in coat color is dependent on a few genes (Haering et al., 1991). The extension (E)-locus is known to be important. Genetic variants at this locus are responsible either for black (eumelanin) or red (pheomelanin) pigmentation in the melanocytes.

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Recent investigations (Mountjoy et al., 1992; Robbins et al., 1993) demonstrated that the E-locus is encoded by the melanocyte-stimulating hormone receptor (MC1-R). However, several alleles code for this G-protein-coupled receptor, and the majority of phenotypic variation in bovine coat color can be explained by various mutations (Klungland et al., 1995; Kriegesmann et al., 1999).

For breeding purposes, genotyping of the MC1-R has been done in more than 2000 animals that belong mainly to the following breeds: Angus; Holstein; Gallo-way, and crossbred. Some purebred Saler cattle have been shown to have a breed-specific band pattern in single strand conformation polymorphism analysis (Figure 1B).

Sequencing the MC1-R of animals belonging to the Saler (Appendix A) and the Brown Swiss (Appendix B) breeds revealed that two new specific alleles exist. In Salers, an additional single nucleotide polymorphism (C to T) was found at position 410 (Figure 1b), and a frameshift mutation was detected that is also present in other red breeds (Red Holsteins, German Red Cattle). The frameshift mutation occurs at the corresponding position 309 of the MC1-R sequence (Joerg et al., 1996). In contrast to the silent mutation, this mutation causes a nonfunctional receptor and is responsible for red hair color when animals are homozygous for the allele. Based on the analysis of the 21 Salers, the frequency for allele A is 0.79 and for allele B (T at position 410) is 0.21, respectively.

After sequencing animals of the Brown Swiss population, we found a 12-bp nucleotide in-frame duplication at position 670 (Figure 1c). To examine the distribution of that allele, 76 Brown Swiss bulls were screened, and six animals were found to be homozygous. Although the insertion leads to an addition of four amino acids within the third intracellular loop of the receptor, the insertion does not cause any phenotypic differences in hair color. The allele frequencies were 0.32 for the allele with the insertion and 0.68 for the wild-type allele. We conclude that certain alleles are commonly shared among breeds, while others seem to be specific and can be used as molecular markers for breed identification.

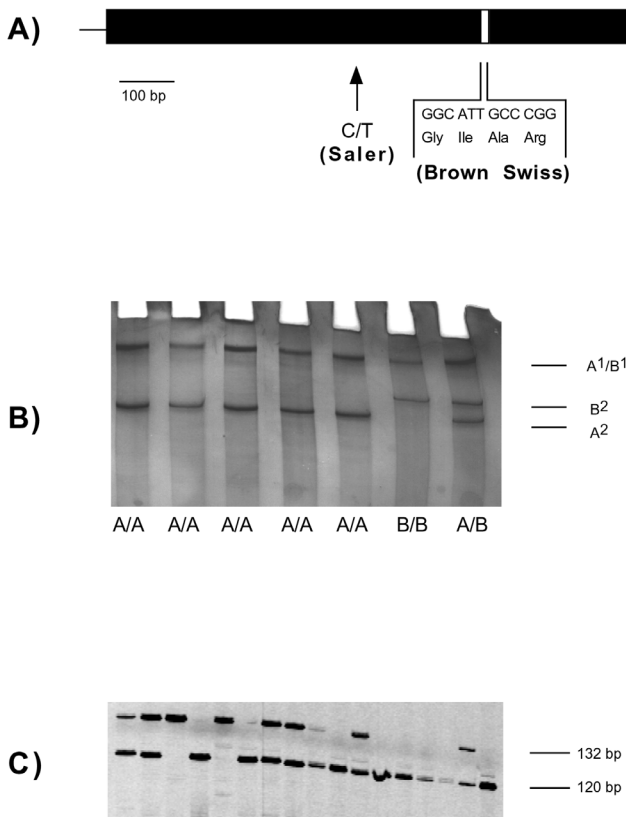


Figure 1. (A) Position of the mutations in the coding sequence of the MC1-R. The C/T mutation found in Salers and the 12-bp insert present in Brown Swiss are indicated. (B) Single strand conformation polymorphism (SSCP) analysis of the bovine MC1-R displaying the C/T polymorphism in Saler cattle after silver staining of the gel. The PCR conditions (5'-CATGTACTACTTTATCTGCTGCC-3', rev-5'-AAGAGTTGAAGTTCTTGAAGATG-3') were as follows: 5 min at 94°C, followed by 33 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The PCR was completed at 72°C for 5 min. To perform the SSCP analysis, a 5- μ l aliquot of the amplified 633-bp fragment was mixed with stop solution (98% formamide; 10 mM EDTA; 0.05% bromophenol blue), denatured at 95°C for 10 min, chilled on ice, and loaded on a 10% (37.5:1; 1X TBE) polyacrylamide gel. Electrophoresis was run for 12 h at 120 V at room temperature. The gels were stained using a standard silver staining procedure. (C) The length polymorphism of Brown Swiss cattle was displayed by sequence analysis using an automated sequencer gel. A primer pair (for 5'-TCATAGC-TATGCTGGCCC-3'; rev-5'-CCAAAGCCCTGATGAATG-3') was used to amplify a 120-bp fragment corresponding to the position 588 to 720 of the MC1-R. The following PCR conditions were chosen 5 min at 94°C, 33 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The PCR was completed at 72°C for 5 min. Genotype analysis was done using an automated sequencer (LI-COR Corp., Lincoln, NE).

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APPENDIX

a) Saler-MC1-R

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60      atgcctgcac ttggctccca gaggcggtg ctgggttccc ttaactgcac gccccagcc
120      accctcccct tcaccctggc cccaaccgg acggggcccc agtgcctgga ggtgtccatc
180      cctgacgggc tctttctcag cctggggctg gtgagtctcg tggagaacgt gctggtagtg
240      gctgccattg ccaagaaccg caacctgcac tccccatgt actactttat ctgctgcttg
300      gctgtgtctg acttgctggt gacgctcagc aacgtgctgg agacggcagt catgctgctg
360      ctggaggcgg tgtcctggcc acccaggcgg ccgtggtgca gcagctggac aatgtcatcg
420      acgtgctcat ctgcggatcc atgggtgtcca gcctctgctt cctgggtgct attgctgtgg
480      accgctacat ctccatcttc tacgccttgc ggtaccacag tgttgtgaca ctgccccgag
540      cgtggaggat cattgcggcc atctgggtgg ccagcatcct caccagcctg ctcttcatca
600      cctactacaa ccacaaggtc atcctgctgt gcctcgttgg cctcttcata gctatgctgg
660      ccctgatggc cgtcctctac gtccacatgc tggccccggc ctgccagcat gcccggggca
720      ttgcccggtc ccagaagagg cagcgcccca tcatcaggg ctttggcctc aagggcgctg
780      ccacctcac catcctgctg ggcgtcttct tctctgctg gggccccttc ttctgcacc
840      tctcgtcat cgtcctctgc cccagcacc ccacctgtgg ctgcatcttc aagaacttca
900      acctttcct ggcctcatc atttgcaacg ccattgtgga cccctcatc tatgccttcc
953      gcagccagga gctccggaag acgctccaag aggtgctgca gtgctcctgg tga

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b) Brown Swiss-MC1-R

atgectgcac ttggctccca gaggcggctg ctgggttccc ttaactgcac gccccagcc
60
accctcccct tcaccctggc cccaaccgg acggggcccc agtgctgga ggtgtccatc
120
cctgacgggc tctttctcag cctggggctg gtgagtctcg tggagaacgt gctggtagtg
180
gctgccattg ccaagaaccg caacctgcac tccccatgt actactttat ctgctgcctg
240
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300
ctggaggccg gtgtcctggc caccagcgg gccgtggtgc agcagctgga caatgtcatc
360
gacgtgctca tctgcggatc catgggtgcc agcctctgct tctgggtgc cattgctgtg
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acctactaca accacaaggt catcctgctg tgccctggtg gcctcttcat agctatgctg
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660
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960
tggtga
966