2009

Red Angus Osteopetrosis Sequence Information

Tim Smith

Follow this and additional works at: http://digitalcommons.unl.edu/usdaarsfacpub

Part of the Agricultural Science Commons

http://digitalcommons.unl.edu/usdaarsfacpub/717

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Red Angus Osteopetrosis Sequence Information

The recessive genetic defect of osteopetrosis (marble bone disease) in Red Angus cattle has been defined by a mutation in the bovine SLC4A2 gene located on Bos Taurus chromosome 4. The mutation was discovered in a joint effort by the Red Angus Association of America (RAAA), University of Illinois, University of Nebraska-Lincoln, University of Wyoming, and the Agricultural Research Service of the United States Department of Agriculture (USDA/ARS). The information is available for public use without restriction, taking into account the disclaimers and caveats described below.

The causative mutation is a deletion of ~2721 bp of the SLC4A2 gene. This deletion encompasses the entirety of exon 2 and the 5' portion of exon 3. A diagnostic assay that has been used for screening several hundred Red Angus animals is based on the amplification of a normal amplicon and a deletion-only amplicon using a trio of PCR primers (see below). Screening for other sequence variation along this portion of the gene among cattle populations has not been completed, so the potential for alleles that do not produce amplification products due to SNP under the primers exists. This likelihood is reduced for the allele containing the deletion (i.e. lower chance of false negatives) since they share a common haplotype, but the precise likelihood of false negative is not known at present. Conversely, the probability of SNP under the primers in SLC4A2 alleles with normal function is higher, such that the appearance of apparently homozygous mutant but unaffected animals is not negligible due to failure of the normal functioning allele to amplify. However, we have not observed any of this class in the approximately 1000 animals in the test populations.

In summary, the validation conducted so far indicates that the primers shown below successfully detect the presence of the deletion discovered segregating in the affected Red Angus pedigrees in the study population. The utility of this assay in Angus is not yet known but may be reduced, and the possibility of other mutations in SLC4A2 or in the other seven genes known to cause osteopetrosis in other mammalian species and segregating in Red Angus or any other cattle breed was not fully assessed. As a result, it should be noted that a negative test result does not guarantee that a given animal is not a carrier for recessive trait leading to osteopetrosis in calves, only that it is not carrying the specific mutation that has been causing the problems identified in some sire lines of the current Red Angus breed.

Annotation Key

green text - exon sequences for bovine SLC4A2
orange text - assay primer sequences
grey highlight - deletion mutation
green highlight - splice junction consensus

http://www.ars.usda.gov/sp2UserFiles/Place/54380510/Publications/RedAngusOsteopetrosisSequenceInformation.pdf
ACCTACAGACCCCCGTCACGAGTCTGGCCCTTACAGCTGGCAGGTGGTACCCCGCTCAACAGACAGAAGGACAGACAGAAGCTACACTTGAAGCGGCCTTGACCTCATCCCTTAAGGAGTTGATTATAGATTTATCTTGTGGCTTCTCTCTAATTGTCTCATGATTGCATTCTTGAGGGCTGTGAACTGGATGAAAACTGCCCTCAAACAGCACTCCTCGGCGCCGCCGGAACCGACGTAGGGGCTTAAACAGTTTGTTAATTAACTCGTTTATGTCTGAACGTTTTCTGCGTACACAATGCTGTCCTTCCCTCGGCTGGCAGACTCTCAGAAGCGAAACATAGATTCGCTTATCTGGAAGAATTTATAATTTAAGAAAACAAGAATCTTTAAATGACAGGTGATGACTATGAGTAGGGTTCGAGAAAGGAGGTCATGGCAGGGAATCTCTCCGAGGCAGAAGTCCCCCGGTTCGGAGACTTAGCACAGAGCAAGGGGTATCCAACCCGCGCCGCCATCACCGCTCTTCGACCCCACCCCCGTCCTCCTGGCAAAGATAAGGGCTTTCTTGGAGGCACTGGGGACTACAAAGGGTGGAAGGCTGGGGCTGCCTGCGGGCGCTGAGAGATTCTTGACCCCCGACTGCATTGATATTTCAATTTCCTCCGAATTGCCTAATAGCTTGGTGCCTCTTTTTTTTTTTTTTTTTTTCCCCCCTGCCTGGGATTCCAGGATGAGGCGGGCTTCCTTTCCGCCCAGAAGGGCCAAATGAAGCTAGGAGACGGCGTCTCCAGGCACCCGAGCTCGTTGCG

GGGAAGCGCTGGGCGGCCGCCTCCCGCCGCGCGGAGGGTCAAGCCAGGGGCGGCCTCCTTGGCTCTGGGATCCGGGCGCCGCGCAGTGCTTGGCCGCGCGCCTCCGCCCCCTGGGTCTCGGCCCCACTCCCCGCCTCCAGGCCCGCCGCGGGGAACGCGCACGGTGCGCCGGGGCGCGCGCACGTAGGGGGCTGGCCTGCCCGCGAAGGGGGAAAGTTGAGTTGGGAGAAGTTGGGAGCGGCGGGGGCGCGCCCCGAGGTGGGCCCGGGGGAAGCCGTCGGGAGCGTGCGAGGGCTCCAGAGCCGGCGGGGAGAGAGACCCCCGAGAAGCCCCACCGAGGGTGCCCGGGGCTTTGGCCAAGGGTGAGGGTGTGTGTGTGTGTGTGAGTGAGAGACCGCCACTGGGGTTCTGGGCGAGTGAGGGTCTGTGTCAAACGGGTGTGAGTGTGTGCGTGTGCGCGTGTGAAGAAGCCCGAGAGGGGAGCCTGGATGGACTTGGGAAGGGCATTGCCATGCCTCTCCTTGCACGGGCCACCGGGTCTGGGACAGGTACTGGTGGTGGCGGTCAGATGGCTTCCAGCCCTGGGGAGGGCCTTGCGGCTCTGTTCCCTGAAAAGCCCTGGGAGGTTTATTCTCCTCAGCGGGAGAGGAGGAACTTTGGGAATCCTGGGTCTCTAACCGATGACAGCCCCCTCCCGCCCTGGGTCTTGGAGGCTGTGCCTGCCGGGCCTGCTGCAAGGGGCTTCTGGGCAGACCGCCCCGCCCCCCGGCCCCACGGCCACCCAGGCCCGGCCCCTCCCGGCCCCCACGTGGCCGCCGCCCCACGTGTCCCCCTTCCAGTGTTCCTGGGAAGGGCAATGATTAACCCCAGCGCCGGCATCATCCGCGCCTCATTGGTCACTTGGGCCCACGGGGCACGTGACTGGGGCGAGGTGCCGGCCAGTCCCCTCCCCCTCCGCCCCCAGTTCACCCCAGCCCGCAGCCATGGACTTCCTCCTGCGGCCTCAGGTGCGAGGGGCTCTCTGGCCCTCCTCCCCCCACCCCGGGCTAGCCCCCTTCTGCTCGCTGGCTTCAGCCGCCCCGCTCTACCGCCTTCCTCCGGTGCTCTTTCTGGTGAGAGTGGGGCCCCGTGCCTGGGGGTAGTTGGCGGAGAAGCCGGGCTCGAGCGGCAGAGATGGGCTGACCAGGGTTGGGGCGGGGGGTGCAAGCTTCTGGTCCTGGCCTCTAGTATCCAGTGTGTCCCCCCTCCCTGCCCCACCCAGCGGCTCCTGACCACCCCTCCCAGGCTCTGCAAGGCCCGCCCTCCTTCCCAGCCCACCTCCCTCCGCTCTTTCTTGCTCGGGTACCCCTGCCCTTGAGATCTTGATGTCCTTCAAGTCCAGAAGCTCTTGCTTGAGCAAATATTTGGTGGTGCCTGGAGCTGAGACCAGCCGGCAGGG

GGTGGTGGTGGTTGGGGGGGCGCTGGACCAAAGCTGCAGAGTGGTGGCTGCCTGCACTGCTGGGCTGAGCTCTCCGAAGCGCC

Your User Name: 3/21/09 9:16 AM

Comment: Note: the primer used is the reverse complement of this sequence
 ACCACGGTCTGGCCTCCGACAGTACTCAGTCGGGCGGCGGGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

AGGCCACATTCACTGGGCGTGGAGCAGCAGGGACTTCTGCTGAGGGGCGGCGGGGCGGGGCGGGGCGGGGCGGGGCGGGG
GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG

GGCCCAAAACAGGCTGACGAGGGCGGCGGGGCGGGGCGGGGCGGGGCGGGGCGGGGCGGGGCGGGGCGGGGCGGGGCGGGG

OST_normal amplicon, 470 bp

>OST_NORMAL_F
GGGAAGGGGAGCAGACCTAAGCT

>OST_NORMAL_R
GGGAGAGGTGGTGGAGAT

Comment: Note: the primer used is the reverse complement of this sequence

ASSAY_PRIMERS

OST_BREAK_F
GGGAAGGGGAGCAGACCTAAGCT

OST_BREAK_RD
GGTGGATGTGATGGGAAGACT

OST_BREAK_RN
TGGAGAGACAGCAGCAGAGAT

95°C for 5 min

31 cycles of:

94°C for 45 sec

63°C for 45 sec

72°C for 45 sec

followed by:

72°C for 5 min
10°C for 5 min

Products can be separated on a 1.6% agarose, 0.5X TBE gel with a 4 cm well-to-well distance (see below).