

# Osteopetrosis Update: Researchers find Causal Mutation

**A collaborative effort of scientists at USDA MARC, USDA BARC, University of Illinois, University of Nebraska-Lincoln, University of Maryland and University of Wyoming has identified a mutation causing Osteopetrosis (OS) in Red Angus cattle.** They used the recently developed Bovine SNP50 or "50K" chip that was important to the success and speed of the project. The disease is caused by a mutation in a gene necessary for bone remodeling during development, known as SLC4A2. Mutations in this gene have also been shown to cause OS in a mouse model, reported in the February issue of the Proceedings of the National Academy of Science.

Using breeder reports on OS affected calves as well as DNA samples obtained from these calves and many of their parents, the team was able to determine that part of the SLC4A2 gene is missing in both copies of bovine chromosome number 4 in the OS calves.

A DNA test capable of identifying carriers of this defect has been developed and will become commercially available in the next 30 to 45 days. The test has undergone several optimizations that have led us to believe that it is highly reproducible and accurate. However, as with any task requiring human intervention, errors can occur. Of course, we do our best to limit any

errors that occur by automating portions of the test and appropriate incorporation of testing controls. Although we cannot guarantee perfection, we estimate that the DNA test may have an error rate of around 1 in 10,000.

As part of test development, we have been able to provide the genotypes and OS status of many AI sires. These sires were used as an essential part of our research for three purposes. First, a broad cross section of genetics representing the Red Angus breed is necessary to assess the validity of any diagnostic test that is developed. This assessment is based on the principle that because OS is a lethal abnormality with an assumed recessive mode of inheritance, there should not be any living animals that are homozygous for the mutation. Second, if our assessment is correct and we have identified the mutation that causes OS, then the results of testing would provide genotype information for all of the sires used in this analysis. This allows breeders to assess the genetic risk of their breeding programs and prepare for implementation of future testing programs. Third, this sampling provides an overall assessment of the frequency of the mutation within the entire Red Angus population.

The naming scheme that was chosen to use for this test identifies animals as having one of three possible genotypes. If an animal is homozygous for the normal vari-

ant (called an allele) we refer to them as **OS-Free (OSF)** indicating that they have been tested for the causative mutation and been found to be "free" of the mutation. Therefore these animals are unable to transmit it to any of their offspring. If an individual is tested and found to be heterozygous or "carrier" for the mutation, meaning that they possess one normal allele and one mutant allele, they are referred to as **OS-Carrier (OSC)**. These animals pass the mutation on to approximately half of their offspring. Although affected calves are rarely tested, they would be homozygous for the mutation and referred to as **OS-Affected (OSA)**. This nomenclature is based on several concepts. First, the term used should have relevance to the more technical aspects of the disease, particularly for veterinary professionals who might encounter the condition as part of their practice. In these instances, if they are unfamiliar with the condition it is more likely that they will investigate it using the medical descriptors and not common terminology. Second, there is a precedent for describing these recessives that has scientific merit and is already in place within other organizations (<http://www.whff.info/index.php?content=recessives&>).

In regard to the accuracy of the test, there are two distinct components that contribute to how the DNA test performs. The first component involves the scientific data that underlie the test. The second

## Osteopetrosis Update: Researchers find Causal Mutation

component is the design and execution of the diagnostic assay performed as part of the testing procedure. From a scientific standpoint, the OS test is based on the presence of a specific change or mutation in the DNA sequence of specific genes of an animal. For OS, this change is deletion of a segment of DNA that includes portions of a gene (a gene is a sequence in the DNA that encodes a protein) that has been shown to be involved in bone development. This mutation results in no protein being produced from this gene and therefore it is unable to carry out its normal function, referred to as a loss-of-function mutation. After identifying the specific mutation,

experiments were conducted to validate the relationship between the mutation and OS. Based on these experiments, we believe that the scientific basis of the test is accurate; in other words, testing for this specific mutation will lead to correct classification of Red Angus animals. However, it remains unclear as to the utility of this diagnostic in Black Angus animals even given the ancestral relationship between the Red Angus and Black Angus breeds.

**Disclaimer:** It is important to realize that OS has been linked to mutations in four other genes in humans, which means that there may be other mutations that could

also lead to the disease in cattle. The current test can identify carriers of a specific mutation that we are confident is causing the disease in Red Angus animals, and therefore a positive test result identifies animals carrying the disease. However, a negative test result cannot guarantee that an animal is not carrying any mutations capable of causing OS. If a mating of animals that test free of the mutation results in an OS calf, DNA from the calf and his parents would be a valuable research tool to identify other potential mutations so we can work to eliminate all genetic causes of the disease. ■