Genetic Testing for Skeletal Atavism

Rebecca Bellone, Ph.D.[1,2] and Liza Gershony, DVM, Ph.D.[1]
[1]UC Davis Veterinary Genetics Laboratory
[2]Department of Population Health and Reproduction
School of Veterinary Medicine UC Davis

Genetic testing is increasingly being utilized as a tool for horse mate selection, identifying horses at risk for disease, and assisting with management decisions. Therefore, it is important to understand which tests are appropriate for your breed and how to utilize testing results. Genetic testing for skeletal atavism became available at the UC Davis Veterinary Genetics Laboratory (VGL) in 2016. Utilizing genetic testing results in mate selection can avoid producing affected animals.

What is skeletal atavism?

Skeletal atavism is a heritable defect of bone development that has been reported to occur in Shetland Ponies. It is characterized by improper bone formation. Specifically, the ulna and fibula grow too long and fail to properly fuse with other bones (radius and tibia, respectively) (see Figure 1). Affecting both front and hind limbs, this disease results in severe angle anomalies, abnormal formation of the knee (carpus) and hock (tarsus), and impaired movement. The angles of the legs and pattern of movement progressively worsen as the foal ages and, in most cases, affected animals are euthanized within six months of life.

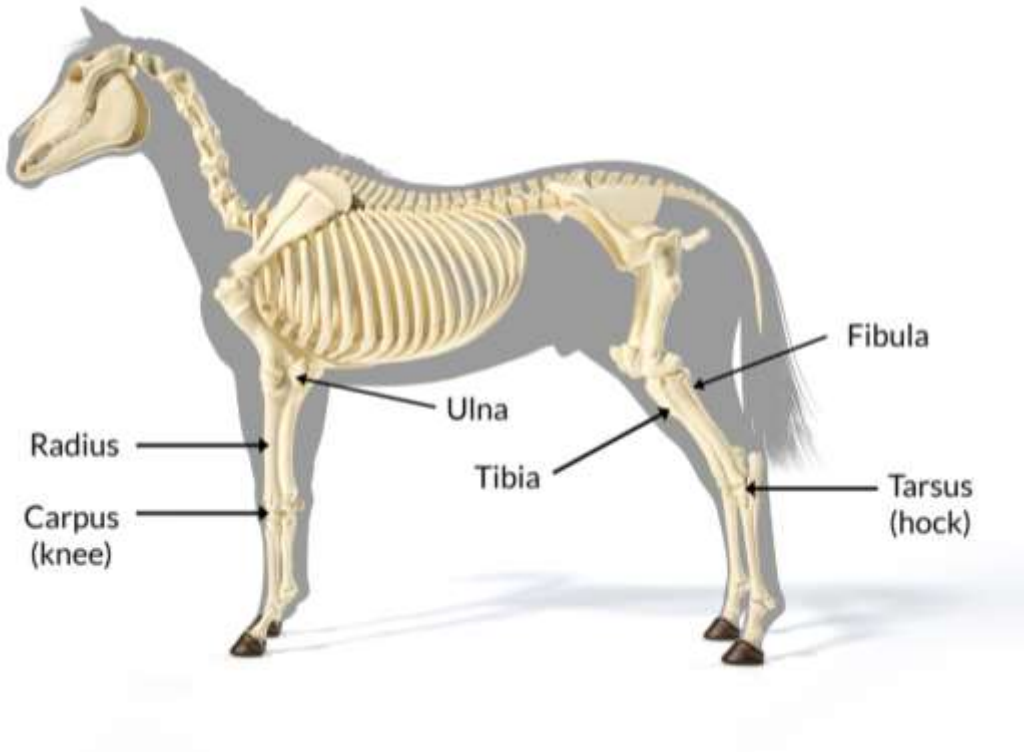

Figure 1: Horse skeleton displaying bones and joints impacted by skeletal atavism.

What causes skeletal atavism?

Scientists from Sweden investigated the full **DNA** sequence (known as the **genome**) of six affected ponies and compared those data to genomes of 21 healthy animals. They identified two overlapping variants thought to cause this disease and confirmed this finding in eight additional affected animals.

The first variant, known has **Del1**, is approximately 160,000 units of DNA deleted from a region on the X and Y chromosomes known as the **pseudoautosomal** region. This is the most common variant causing skeletal atavism. The second variant, known as **Del2**, is characterized by roughly 60-80,000 units of DNA deleted from approximately the same region.

These **deletions** are thought to impact the gene known as *short stature homeobox* or *SHOX* for short. This gene plays an important role in the cells that form the bone. Humans with genetic variants in this gene also have skeletal defects. Del1 encompasses the entire functional component of the *SHOX* gene whereas Del2 is thought to encompass important regions of DNA that tell the cells to make the *SHOX* gene product. Horses with two copies of either deletion (**Del1/Del1** or **Del2/Del2**) or with one copy of each deletion (**Del1/Del2**) are affected.

What do genetic test results mean?

Del1 and Del2 are both deletions of DNA located in a region of the X and Y chromosomes, known as the pseudoautosomal region**.** The X and Y chromosomes determine sex and most regions of these sex chromosomes are unique (only found on X or only found on Y), except for the pseudoautosomal region, which is common to both. Since Del1 and Del2 fall in a region common to both X and Y, this disease follows a **recessive** mode of inheritance. This means that affected horses must have two copies of a deletion to be affected.

Affected horses: Del1/Del1, Del2/Del2, or Del1/Del2

Affected animals will have inherited one deleted version from each parent, either Del1 or Del2 (Figure 2-4).

Carrier horses: N/Del1, N/Del2

Ponies with only one copy of a deletion are said to be **carriers**; in other words, they carry one copy of a deletion variant in their DNA but they are not affected. Because they carry a genetic variant that causes disease, they can produce affected ponies.

Normal/Clear horses: N/N

Ponies that do not have any copies of either deletion are said to be normal (N=normal) and are reported as clear with regards to skeletal atavism.

How can genetic testing results for skeletal atavism be utilized in mate selection?

It is suspected that 12 Shetland ponies out of 100 will carry one of the deletion variants that cause disease. Therefore, genetic testing can be a powerful tool to avoid producing affected ponies.

Crossing two carriers (N/Del1 or N/Del2) to each other has a **25% chance of producing a pony with skeletal atavism -** every time you perform this cross regardless of previous outcomes. Thus, crossing carriers should be avoided.

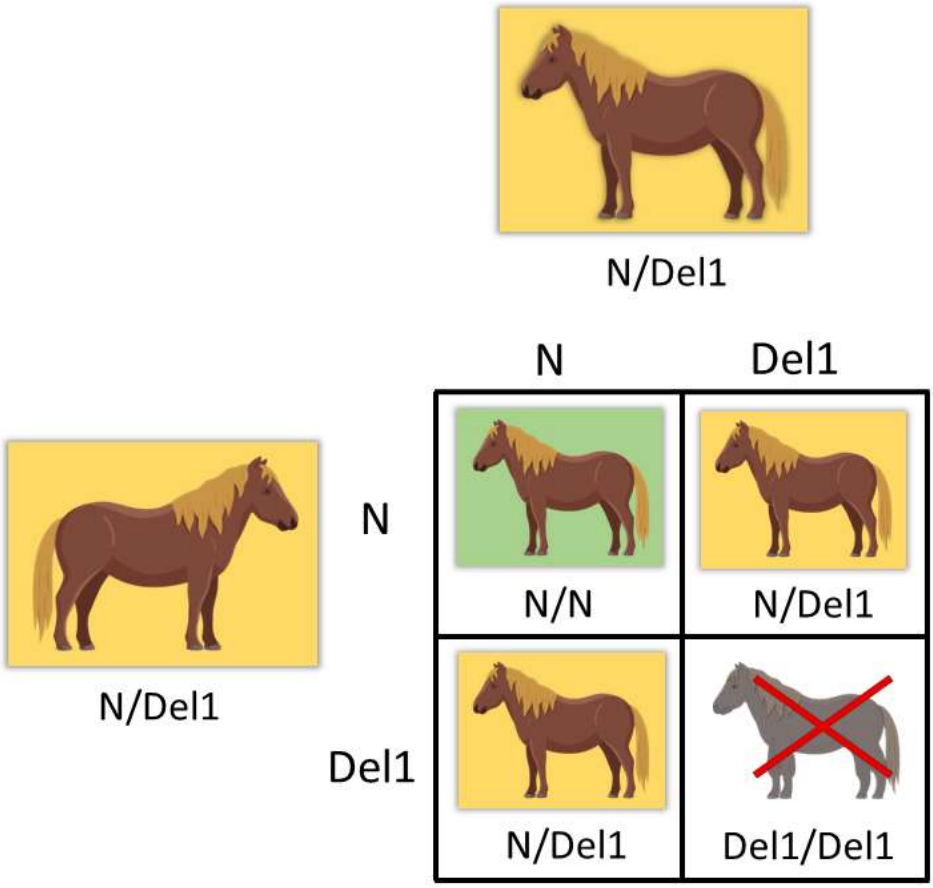

Figure 2: Possible breeding outcomes of crossing two ponies that are carriers for the Del1 variant. This cross results in a 25% chance of producing a pony with skeletal atavism. Ponies with this disease are typically euthanized by six months of age, which is denoted by the red cross in Figure 2-4. When crossing two carriers, there is also a 25% chance of producing a clear pony (no copies of the skeletal atavism variants). In Figures 2-5 clear horses are denoted with a green background. The cross shown here also has a 50% chance (2 out of 4 possibilities) of producing a carrier pony. In Figures 2-5 carrier ponies are denoted in yellow to demonstrate that breeding carriers to carriers should be avoided to not produce affected animals.

In other words, it is recommended to not mate a pony with N/Del1 results to one with either N/Del1 (Figure 2) or N/Del2 (Figure 4) results. Similarly, it is recommended to avoid crossing N/Del2 ponies to either N/Del2 (Figure 3) or N/Del1 (Figure 4).

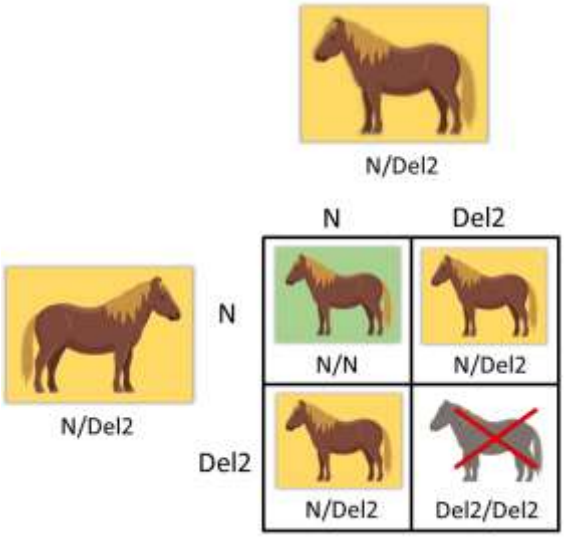

Figure 3: Possible breeding outcomes of crossing two ponies that are both carriers for the Del2 variant. This cross results in a 25% chance of producing a pony with skeletal atavism.

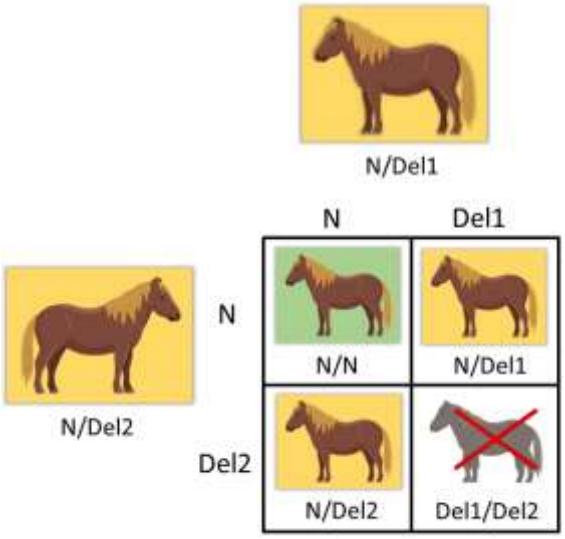

Figure 4: Possible breeding outcomes of crossing Del1 and Del2 carriers. This cross results in a 25% chance of producing a pony with skeletal atavism.

Mating carrier ponies, either N/Del1 or N/Del2, to normal horses (N/N) will yield a 50/50 chance of producing either a clear pony or a carrier pony. Ponies with skeletal atavism caused by Del1 or Del2 cannot be produced by this cross (Figure 5).

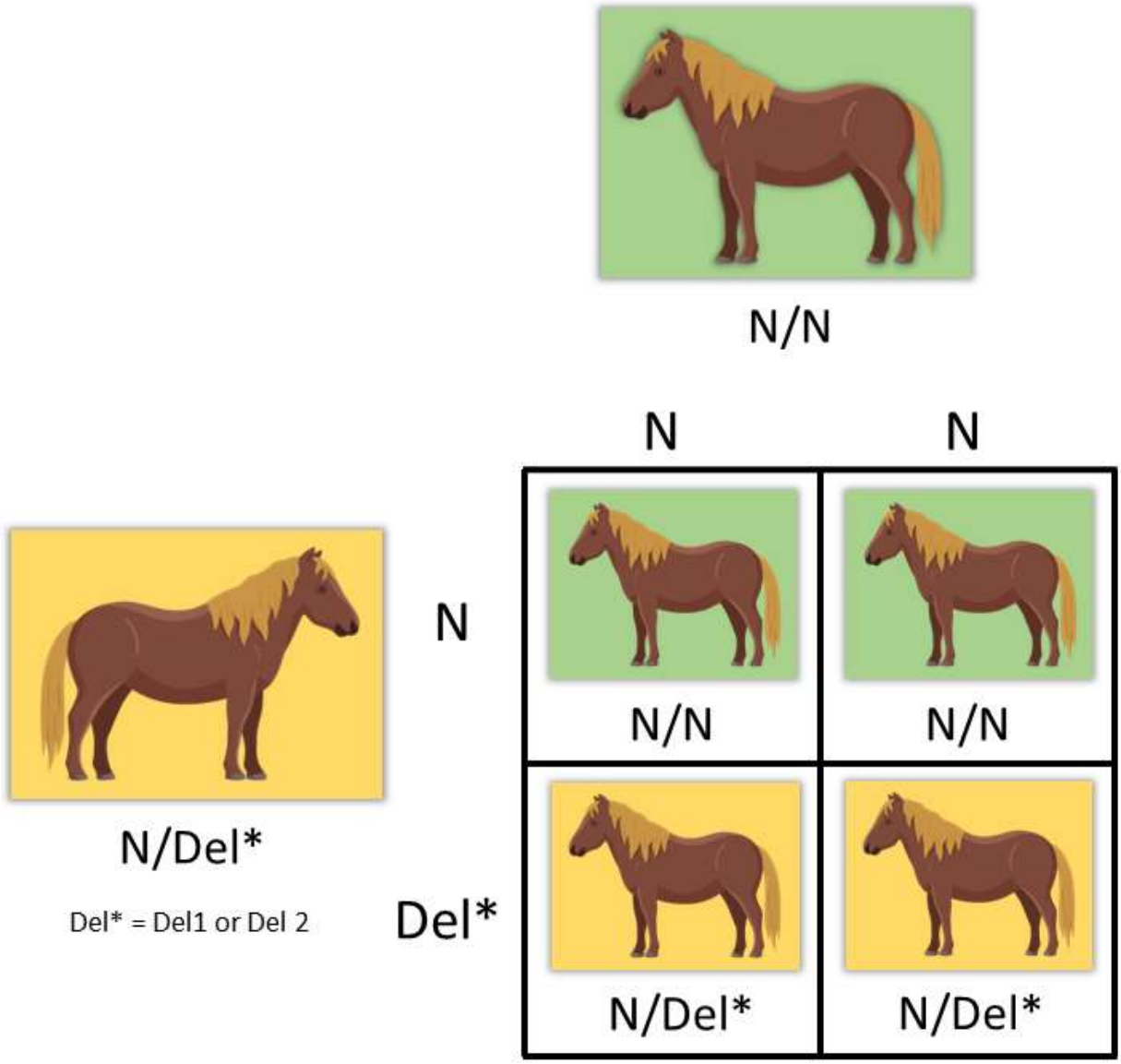

Figure 5: Possible breeding outcomes of crossing a Del1 or Del2 carrier to normal horses (N/N). This type of cross results in a 50% chance of producing a clear pony and a 50% chance of producing a carrier pony. The * in this figure represents that this could be Del1 or Del2 and the results would be the same.

How are the mutations tested for and is the test accurate:

The UC Davis VGL skeletal atavism test evaluates DNA from the animal being tested for the two deletions (Del1 and Del2) thought to cause this disease. In other words, we test directly for the variants described above and not for linked markers, making these tests highly accurate. Accuracy of testing is the UC Davis VGL's top priority. Therefore, we build in many quality control steps into our testing methods to ensure the accuracy of results.

Is the test for skeletal atavism patented?

When a genetic variant contributing to a disease is discovered, the scientist/s that made the discovery may patent the invention. In cases where these variants have been patented, to test for them, laboratories need to obtain a license from the patent holder. The genetic variants causing skeletal atavism are patented and the VGL tests for them under a license agreement with the patent holder Capilet Genetics.

For more information:

https://vgl.ucdavis.edu/test/skeletal-atavism

Rafati, N., *et al.* (2016). Large Deletions at the SHOX Locus in the Pseudoautosomal Region Are Associated with Skeletal Atavism in Shetland Ponies. *G3: Genes|Genomes|Genetics* (Bethesda), 6(7), 2213-2223. https://doi.org/10.1534/g3.116.029645.

Genetic Glossary:

DNA: Deoxyribonucleic Acid, a complex organic molecule that contains genetic information. DNA is the material of heredity and is found in all known living organisms.

Genome: An organism's complete DNA sequence found in one set of its chromosomes. This includes the DNA that makes up the 20,000+ genes of mammals as well as all non-coding regions of DNA.

Deletion: A type of mutation in which part of the DNA sequence is lost; the loss can be as small as a single unit or as much as an entire section of a chromosome.

Pseudoautosomal region (PAR for short): are regions of similar sequence on both the sex chromosomes (X and Y). Since these same sequences are found on both the X and Y chromosomes, inheritance of variants in these regions behave similarly to those in non sex chromosomes.

Recessive: A trait is recessive when two copies of a variant are needed to produce (express) the phenotype.

Del1: First deletion detected to cause skeletal atavism. Approximately 160,000 -180,000 units of DNA deleted from a region on the X and Y chromosomes that causes skeletal atavism.

Del2: Second deletion detected to cause skeletal atavism. Approximately 60,000-80,000 units of DNA deleted from a region on the X and Y chromosomes that causes skeletal atavism.

Carrier: An organism possessing one copy (heterozygous) of a recessive variant. The term "carrier" is often used in the context of disease-associated or otherwise deleterious variants. Because carriers are heterozygous, they do not express the phenotype of the recessive variant.

However, carriers can transmit the recessive allele to their offspring and can thus produce affected offspring if their mate also contributes a recessive allele.