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Genetic testing for polysaccharide storage myopathy in horses

Whilst certain neuromuscular diseases of horses require muscle biopsy for accurate classification, a handful can be diagnosed by genetic testing. This is because these diseases are caused by a specific change in the horse's DNA, called a mutation, that leads to the specific disease in question. Examples of horse muscle diseases with causative genetic mutations include hyperkalaemic periodic paralysis (HYPP), myotonia congenita, malignant hyperthermia (MH) and type 1 polysaccharide storage myopathy (PSSM1). Whilst mutations can arise spontaneously, often they are passed down from parents to offspring: as such, some mutations and diseases occur more commonly in some breeds, or lines of horses than in others.

Validating a genetic test

Peer review is used when scientists submit a scientific paper for publication; this process occurs when other independent scientists check that an appropriate process has been followed and the science is robust and valid. This is crucial for the advancement of science and veterinary medicine but is especially important when a new test might significantly influence the animal's future athletic use, breeding potential or a decision for euthanasia.

In the case of a genetic test, this validation process would typically involve recruiting a large group of affected horses, that have the disease, and a large group of unaffected horses, that are healthy (and definitely don't have the disease). Scientists then use statistical tests to see if there is a mutation that is much more likely to be found in affected horses than unaffected horses, rather than simply being there by chance. They would then test a different group of affected and unaffected horses, to see if the same result was found again. Additional experiments can then be used to prove that the specific DNA change (the mutation) has an effect that causes the disease (called functional testing).

It is important that the full process of identifying a mutation and proving it causes a disease is peer reviewed and then published for other scientists, vets and owners to read (and if appropriate, critique), so that the test is considered by the scientific community as valid. Basically, this process is fundamental to scientific advancement: it allows us to know if test results can be trusted.

PSSM Type 1 (PSSM1)

Polysaccharide storage myopathy (PSSM) is a genetic muscle disease that causes horses to have episodes of exertional rhabdomyolysis (or tying up) and in some animals causes muscle weakness. PSSM was first diagnosed by muscle biopsy: affected horses accumulate excessive deposits of glycogen (an energy rich storage molecule) in muscle and with time, a substance known as polyglucosan (or polysaccharide) which fills muscle cells [1]. In 2008, a team of scientists in the US identified a genetic mutation in the equine *GYS1* gene, and published the results of their scientific validation process proving that the mutation was associated with PSSM [2]. Working in collaboration, we then went on to prove the mechanism by which the mutation causes the disease [3]. Unusually, the mutation increases activity of a key enzyme (glycogen synthase) in muscle that is responsible for storage of glycogen; further, the enzyme (in affected horses), is permanently switched on in muscle cells – so that it continues generating new glycogen even when it is not needed. However, not every horse with microscopic features of PSSM tests positive for the *GYS1* mutation. The ones that do, are

referred to as having PSSM Type 1 (PSSM1), whilst the ones that test negative are sometimes referred to as having PSSM Type 2 (PSSM2) [4; 5].

Genetic testing for PSSM1 has become very helpful not only for diagnosis, but for breeders making informed and ethical decisions about which horses to breed from. By testing DNA (from a blood sample or hair roots) muscle biopsy can be avoided. Over time, it has turned out that many different breeds of horse carry this same gene mutation across the world, strongly suggesting they share a common ancestor; however, some of these horses display no apparent signs of disease (they are 'sub-clinical') whereas others have prominent or intermittently severe signs. Likely, the way these horses are managed plays a significant role in the disorder's clinical severity but there are probably other aspects that influence the disease (such as breed or fitness). It is important to recognise that some horses with PSSM1 can be very effectively managed and have successful careers. We are working on identifying reasons for differences in clinical severity and specific treatments for this condition.

PSSM Type 2 (PSSM2)

There is currently no scientifically validated genetic test for PSSM2, and scientists still don't know what causes this disease. Indeed, it remains unclear whether this is a specific disease at all (with a single cause) or whether the term PSSM2 refers to a whole gamut of muscle disorders in horses - some with possible genetic causes but others caused by environmental or other problems. Currently, the optimal way to identify horses as having PSSM2 is via muscle biopsy, albeit with the caveats outlined above. However, because we don't know what causes PSSM2, or indeed whether it is a specific disease at all, making recommendations for treatments, management or prognosis are speculative at best. Many groups, including our own, are working on these issues.

There are companies in Europe and the USA offering a panel of genetic tests commercially for diagnosis of equine muscle diseases including PSSM2. We do not recommend or use these tests for diagnosis of PSSM2 or other myopathies as the results offered have not to our knowledge been scientifically validated in any peer reviewed literature or shared in other form with the scientific community. In short, there is no evidence that the offered tests identify mutations that actually cause muscle disease or indeed, are associated with disease in any way at all. Just like in humans, different horses have millions of differences in their genetic code, but without the scientific validation outlined above, it is impossible to know which (if any) are disease-associated. Furthermore, a group in the US has published two papers [6; 7] testing some of these mutations in different horse breeds that had been examined by muscle biopsy: they found no association between PSSM2 (or any other muscle disease) and the genetic variants offered by these companies.

It can be very tempting when your horse has signs that might suggest a muscle problem to seek any answer you can find, particularly when the problem is proving challenging to diagnose and manage. It might be even more tempting to believe an unsubstantiated genetic result when it 'fits' with what you have suspected. However, we encourage people to use an evidence basis to decision making when diagnosing these conditions and take into account the information presented above. Until results of genetic testing for PSSM2 and other myopathies in horses are presented in peer reviewed, validated scientific literature that is accepted by the scientific community (as has been the case for PSSM1), we do not recommend their use and certainly do not support veterinary or life decisions being made based on their results.

For help or advice relating to your own horse, please always discuss the issues with your own vet.

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July 2022

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