

Use of genetic testing and muscle biopsies to identify muscle disease in horses

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Genetic Testing

The selective breeding of animal populations may give rise to a common founder that can disseminate a genetic trait to many thousands of related offspring within a few years. Traits can be controlled by one gene or influenced by many different genes. Single gene disease for which there are validated genetic traits are discussed in this paper. These diseases are inherited as either autosomal dominant or autosomal recessive diseases.

Autosomal dominant traits: Require only one copy of the mutant gene to cause disease. Breeding of an affected heterozygous horse (one copy of the defective gene) to a normal horse results in a 50% chance of producing an affected horse. Breeding two heterozygous affected horses has a 50% chance of producing a heterozygous affected, 25% chance of a homozygous affected (2 copies of the defective gene) and a 25% chance of a homozygous normal being born.

Autosomal recessive traits: Requires two copies of the mutant gene to cause disease. Breeding two affected horses results in a 100% chance of producing an affected horse. Breeding two carriers results in a 25% chance of producing an affected horse, a 25% chance of a normal horse and a 50% chance of a carrier. Breeding an affected and a carrier results in a 50% chance of producing an affected horse and a 50% chance of producing a carrier.

Genetic tests

Not all genetic tests that are commercially offered have been proven to actually be associated with the disease they purportedly are testing for. Genetic testing in horses is not regulated in the US, therefore it is up to animal owners and veterinarians to determine if a genetic test does in fact identify susceptibility to a clinical disease or a specific trait. In other words, users of the test need to critically question if the existence of the genetic variant being tested for has been shown to truly indicate the presence or susceptibility to a disease. This determination should be informed by asking genetic testing laboratories for their peer reviewed publications, looking for publications on websites like Google Scholar and by consulting veterinarians and experts in the field.

The genetic tests discussed here are validated tests that are currently commercially available (August 2018).

What is a scientifically validated genetic test?

Peer reviewed scientific publications are the traditional means by which a genetic test is validated to provide concrete evidence of the involvement of a genetic variant in a specific trait. In the age of social media, some genetic tests are being popularized on Facebook or other media outlets in the absence of validation and verification by scientific publication and peer review.

When veterinary genetic testing has been scientifically validated it means the following steps have been taken:

1. A diagnosis of disease has been carefully established in a group of “affected” horses and confirmed to be absent in a group of “unaffected” (i.e. non-diseased) horses. This diagnosis should be based on the highest standard for testing for a particular disease (i.e. blood test, tissue biopsy, etc.)

2. A change in the genetic sequence (called a genetic variant) has been identified in the diseased horses that passes statistical tests showing it is significantly associated with the presence of the disease.
3. The test of association between the variant and the disease is replicated in additional, separate populations of diseased and healthy horses to ensure the accuracy of the association. The frequency of the variant across breeds is reported.
4. The genetic variant is examined to establish that it changes the function or regulation of a protein or at the very least carefully modeled to show how it alters molecular biology to create the specific disease.
5. Most importantly, as part of the publication process, a careful peer review is conducted by scientists. Peer-review allows the results to be evaluated by other scientists who examine the methods used, results produced, and conclusions reached by the investigators. If accepted for publication, the article identifies and describes the genetic mutation (location of the sequence change and gene involved). Publication also allows others to attempt to replicate the findings. True, disease-causing variants will stand up to this scrutiny.

Equine Genetic Diseases that have commercially available scientifically validated tests

Defect	Breed	Mode of inheritance	Disease
HYPP	QH, Paint, Appaloosa	A-Dominant	Hyperkalemic Periodic Paralysis
GBED	QH, Paint	A-Recessive	Glycogen Branching Enzyme Deficiency
MH	Quarter Horses	A-Dominant	Malignant Hyperthermia
PSSM1	20 breeds: QH, Paints, Morgan, Belgian, Percheron, some Warmbloods.....	A-Dominant	Polysaccharide Storage Myopathy
MYHM	QH, Paint, Appaloosa	A-Dominant	Immune-mediated Myositis, Nonexertional Rhabdomyolysis

HYPERKALEMIC PERIODIC PARALYSIS (HyPP)

Breeds affected: Quarter horse-related bloodlines

Bloodlines: Horses descendant from Impressive.

Prevalence: 3% of the Quarter Horse breed is affected. 60% of halter horses

Age affected: Signs usually begin by 2 to 3 years of age.

Clinical signs: Range from asymptomatic to intermittent muscle tremors and weakness. Horses homozygous for HyPP may present with difficulty swallowing or respiratory distress.

Mode of inheritance: Autosomal dominant.

Mutation: A point mutation that results in a phenylalanine/leucine substitution in a key part of the voltage-dependent skeletal muscle sodium channel alpha subunit that controls channel activity (*SCN4A*).

Testing: Veterinary Genetics Laboratory at the University of California, Davis on mane or tail hair roots.

GLYCOGEN BRANCHING ENZYME DEFICIENCY (GBED)

Breeds affected: Quarter horse-related bloodlines

Bloodlines: Horses descendant from Zantanon and King

Prevalence: 8% of the Quarter Horse breed. And 28% of Western pleasure are carriers

Age affected: Signs usually present in utero or at birth

Clinical signs: Abortion or stillbirth, may be born alive and are weak at birth. With supportive care may live to up to 18 weeks of age. Death may be sudden when exercised on pasture, associated with weak respiratory muscles or the result of euthanasia due to persistent recumbency. Treatable flexural deformities of all limbs and recurrent hypoglycemia (low blood sugar) and seizures occur in some affected foals.

Mode of inheritance: Autosomal recessive.

Mutation: A point mutation in exon 1 changes a tyrosine to a premature stop codon in the glycogen branching enzyme gene (*GBE1*) that is expressed in numerous tissues.

Testing: Histopathological tissue samples (muscle and heart) stained for Periodic acid Schiff's (PAS) show a variable amount of abnormal PAS positive globular and crystalline intracellular inclusions. Genetic testing is done by Veterinary Genetics Laboratory at the University of California, Davis or Vetgen in Michigan on mane or tail hair roots or Animal Genetics, or Progressive Molecular Diagnostics

Type 1 POLYSACCHARIDE STORAGE MYOPATHY

Two forms of PSSM appear to exist type 1 and type 2 PSSM. We have found the mutation for the type 1 in the *GYS1* gene, the cause or causes of type 2 PSSM are under investigation but not yet known. There are no scientifically validated tests for PSSM2.

Type 1 PSSM

Breeds affected: Quarter horse-related bloodlines, Belgians, Percherons, Morgans, Mustangs and some Warmblood breeds.

Bloodlines: Present in founders of QHs and therefore widespread in all types of QHs with highest prevalence in halter and pleasure horses.

Prevalence: 36-50% of Belgians and Percherons, 8% of the Quarter Horse related breeds, 30% of halter horses

Age affected: Signs usually begin by 2 to 3 years of age but may occur in Weanlings. Some horses are subclinical.

Clinical signs: Firm painful muscles, stiffness, skin twitching, sweating, weakness and reluctance to move with light exercise. Sometimes gait abnormalities, mild colic and muscle wasting. Serum CK and AST activity elevated except in Drafts.

Mode of inheritance: Autosomal dominant.

Mutation: Point mutation that results in an arginine to histidine substitution in the *GYS1* gene that codes for the skeletal muscle form of the glycogen synthase enzyme.

Testing: Muscle biopsy samples evaluated for presence of amylase-resistant polysaccharide.

Genetic testing on mane or tail hair roots at the University of Minnesota Veterinary Diagnostic Laboratory or Animal Genetics.

MALIGNANT HYPERTHERMIA

Breeds affected: Quarter horse-related bloodlines

Bloodlines: High frequency in one or two QH families and often co-exists with PSSM

Prevalence: 0.1% of the Quarter Horse breed is affected.

Age affected: Adults
Clinical signs: High temperature, metabolic failure and death under anesthesia. Tying up and fever, Signs of PSSM are more severe when both mutations are present. Sudden death
Mode of inheritance: Autosomal dominant.
Mutation: Point mutation that results in an arginine to glycine substitution in the *RYR1* gene
Testing: Genetic testing at Neuromuscular Diagnostic Laboratory at the University of Minnesota or the Veterinary Genetics Laboratory at UC Davis.

MYOSIN HEAVY CHAIN 1 MYOPATHY

Breeds affected: Quarter horse-related bloodlines
Bloodlines: High frequency in reining horses, working cow horses, halter horses
Prevalence: 8% of the Quarter Horse breed is affected.
Age affected: Weanlings to adults
Clinical signs: Either severe nonexertional rhabdomyolysis with very high CK and AST or rapid onset muscle atrophy especially involving the epaxial and gluteal muscles.
Mode of inheritance: Autosomal Codominant, with homozygotes more severely affected.
Mutation: Point mutation that results in a glutamic acid to glycine substitution in the *MYH1* gene
Testing: Genetic testing at Veterinary Genetics Diagnostic Laboratory, UC Davis

SUMMARY TABLE: Observed percentages of horses carrying a disease-causing allele for whole breeds (QH and APH paints) and for elite competitive subgroups.

Population	Affected dominant (%)		Carrier recessive (%)
	HYPP	PSSM	GBED
QH	1.5	11.3	11.0
APH	4.5	4.5	3.9
Halter	56.4	28.2	5.1
Western pleasure	1.1	8.6	26.3
Cutting	NO	6.7	13.6
Reining	NO	4.3	3.1
Working cow horse	NO	5.7	9.5
Barrel racing	1.2	1.4	1.2
Racing	NO	2.0	NO

NO = Not observed in the dataset.

from “Tryon RC, Penedo MCT, McCue, ME, Valberg SJ, Mickelson JR, Famula TR, Wagner M, Jackson M, Hamilton M, Noteboon S, Bannasch DL”. **Allele frequencies of inherited disease genes in subpopulations of American Quarter Horses.** JAVMA2009 Jan 1;234(1):120-5

Muscle Biopsy

For diseases where genetic testing is not available, muscle histopathology can reveal the underlying basis for disorders characterized by weakness, atrophy, rhabdomyolysis, and exercise

intolerance. Muscle biopsy is often not effective in identifying the underlying basis for myopathies primarily characterized by muscle fasciculations and electromyography should be considered in these cases. Considerations for muscle biopsy include

1. The most appropriate muscle is selected for biopsy based on the differential diagnoses.
 - a. Exertional rhabdomyolysis: open biopsies of the semimembranosus muscle or percutaneous 6-mm needle biopsies of the middle gluteal muscle if submitted on-sight at the Neuromuscular Diagnostic Lab.
 - b. For suspected immune-mediated myositis, a gluteal or lumbar muscle biopsy obtained temporal to the onset of atrophy
 - c. For focal atrophy a sample of that specific muscle group is indicated.
 - d. Equine motor neuron disease or vitamin E deficient myopathy are optimally diagnosed through biopsy of the sacrocaudalis dorsalis medialis muscle.
 - e. Samples of the diaphragm or deep postural muscles are most likely to reveal excessive lipid storage in hypoglycin A myopathy.
 - f. Deep pelvic muscles such as the iliopsoas should be obtained postmortem when examining a horse with recumbency from severe rhabdomyolysis.
2. An adequate amount of muscle is obtained
 - a. $\frac{1}{2}$ by $\frac{1}{2}$ by $\frac{1}{4}$ inch cube before open excision
3. The specimen is handled appropriately
 - a. Sample is not squished with forceps
 - b. Sample is wrapped in damp but not soaking wet gauze (dampened with saline)
 - c. Sample is placed in a hard container with lightly packed gauze to prevent bouncing around during shipment
 - d. Hard container is placed on icepacks and shipped next day priority overnight
4. The specimen is in proper fixation
 - a. Fresh samples wrapped in damp gauze on icepacks
 - b. A smaller portion placed in formalin as a back up
5. The most appropriate tinctorial, histochemical, and immunohistochemical stains are used to obtain a diagnosis.

A limited amount of information restricted to cellular infiltrates and fiber sizes is obtained from standard hematoxylin and eosin stains of formalin fixed paraffin embedded skeletal muscle. Fresh samples frozen in a particular fashion in isopentane suspended in liquid nitrogen can be stained with a battery of tinctorial and histochemical stains to fully characterize a neuromuscular disorder. Such stains will highlight muscle fiber sizes, shapes, fiber type distribution, mitochondrial distribution, polysaccharide and lipid staining pattern, vacuolar content, neuromuscular junctions, nerve branches, connective tissue, and blood vessels. In addition, frozen samples can be used for immunohistochemistry and biochemical analysis of substrate concentrations and enzyme activities, as well as DNA isolation.

Conflict of interest statement: Drs. Valberg and colleagues own the license for PSSM testing and receive sales income from its use. Dr. Valberg, Michigan State University, Dr Finno and the University of California Davis have a patent pending for the genetic test for MYHM. Their financial and business interests have been reviewed and managed by the Universities in accordance with its conflict of interest policies.

References available at

Website: <https://cvm.msu.edu/research/faculty-research/valberg-laboratory>

