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Pursuing the Genetic Basis for Tying-Up Syndromes in Equine Breeds

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INTRODUCTION

Although many diseases in horses likely have a heritable basis, either due to the effect of one single gene or more commonly several genes, the tools necessary to find genes that cause equine disease or affect performance have not been available until quite recently. A new frontier in equine genetics now has been opened by sequencing the equine genome and by developing genotyping chips (SNP chips) for equine genome mapping. The Equine Neuromuscular Diagnostic Laboratory at the University of Minnesota is now using this technology to identify the genetic basis for forms of tying-up such as polysaccharide storage myopathy (PSSM), recurrent exertional rhabdomyolysis (RER), and disorders of lipid metabolism. The genetic mutations causing four equine muscular disorders have already been identified: hyperkalemic periodic paralysis (HYPP) (Rudolph et al., 1992) and malignant hyperthermia (MH) (Aleman et al., 2004) through research at the University of California, Davis; and glycogen branching enzyme deficiency (GBED) (Ward et al., 2004) and type 1 PSSM (PSSM1) (McCue et al., 2008b) through work performed at the University of Minnesota. This paper will review the current approach used to investigate genetic diseases in horses and summarize the current state of knowledge surrounding the genetic basis for the forms of tying-up involving PSSM1, type 2 PSSM (PSSM2), MH, RER, and a muscular disease of foals, GBED.

Identification of Heritable Traits

Cluster of disease. Both environmental and genetic factors can cause a cluster of disease on a given premise; however, environmental diseases usually affect a greater percentage of the individuals on a premise at one time than genetic disorders. If large numbers of horses suddenly develop signs of tying-up at one time, the first step is usually to investigate changes in feeding and management. Attention is usually focused on potential genetic disease when a specific breed is affected or when several related offspring are affected. The genetic basis for disorders that have a delayed onset of expression or variable penetrance such as forms of tying-up is extremely difficult for breeders and veterinarians to recognize as heritable. For example, clinical signs of PSSM may only occur in PSSM horses with certain dietary conditions (Ribeiro et al., 2004), and the heritable basis for a major form of this disorder went unrecognized for over a thousand years (McCue et al., 2008b). In contrast, a genetic basis for a disease is more readily recognized in young animals, and as such the genetic mutations causing four lethal foal diseases, severe combined immunodeficiency (SCID) (Shin et al., 2000), overo lethal white syndrome OLWS (Santschi et al., 1998), GBED (Ward et al., 2004), and junctional epidermolysis bullosa (Spirito et al., 2002), have already been identified.

Pedigrees. One of the idiosyncrasies of working with equine genetics compared to human genetics is the impact of popular sires. Genetic selection pressure within a breed often results in highly prolific sires predominating in the pedigrees of many individuals. In fact, many horses within a breed often share a common ancestor within five to nine generations. While this concentrates many beneficial traits related to performance or appearance, it may inadvertently spread genetic diseases farther than would be seen in human populations. As an example, there are at least 24 mutations in the sodium channel gene that cause HYPP in thousands of humans, whereas only one shared mutation exists in the hundreds of thousands of horses with HYPP.

Although breeders often want to know whether a genetic disorder runs in a certain line of horses, pedigree analysis alone cannot make this determination, as it only identifies common sire lines and does not provide concrete evidence that a disorder is inherited. As a cautionary tale, in researching PSSM1 we found that two particular Quarter Horse stallions were common on the sire's and dam's sides of pedigrees of 22 PSSM1-affected Quarter Horses and published a paper naming these two stallions as A and B in 1996 (Valberg et al., 1996). Without the certainty of a genetic test, we did not reveal the identity of these stallions, which at times put us at odds with inquiring breeders. When we discovered the genetic basis for PSSM1 in 2008, further research showed that the causative dominant mutation likely originated at least 1,200 years ago and that more than 20 equine breeds are affected by PSSM1, not just the lineage of these two Quarter Horse stallions (McCue et al., 2008a,b). Revealing the names of stallions A and B would have left breeders with a false sense of security in breeding to other stallions, since 10% of Quarter Horses have PSSM1. In addition, this would have implied that breeding to descendants of stallions A and B was inadvisable, yet in fact approximately 50% of stallions A and B's progeny could have been free of the dominantly inherited PSSM1 mutation.

Searching for the Genetic Basis of Equine Diseases

Diagnosis/phenotype. One of the crucial factors in performing genetic studies is to accurately establish criteria to define the specific disease (accurate phenotype), as well as criteria to define controls or unaffected horses. When the only means to diagnose tying-up was measuring muscle enzymes in the bloodstream [creatine kinase (CK) or aspartate transaminase (AST), it was impossible to perform informative genetic studies. However, when diagnostic criteria could be based on more specific muscle histopathology and muscle biochemistry, specific subsets of muscle disorders could be identified that could then be investigated as separate potentially genetic disorders (Valberg, 2009). Thus, separate experimental approaches could be used to find the genetic basis for PSSM1, PSSM2, RER, and GBED.

Comparative medicine and candidate genes. Prior to the development of equine genome maps, the only means to identify genetic diseases in horses was to find a similar condition in other species for which the genetic basis was known. This comparative "candidate gene" approach assumed that the similar diseases involved the same gene and prior to 2008 typically required time-consuming screening of cDNA libraries or concluding that the equine gene's DNA sequence was close enough to the human/mouse sequence that PCR primers could be designed to sequence the equine gene. This initial approach was used successfully for HYPP, OLWS, SCID, and JEB. However, a delay of several years

occurred before the genetic mutation in the *GBE1* gene that causes GBED was identified because portions of the equine gene were so different from human and mouse gene that the equine gene could not be completely sequenced. Large-scale sequencing of the entire horse genome in 2008 (Wade et al., 2009) has largely overcome this problem and sequencing of equine genes that may contain a mutation is more routine (although not without problems!).

Genome mapping. For some equine diseases such as PSSM and RER, extensive research into human and other animal disorders fails to identify a direct correlate or an obvious candidate gene. In such cases, a genome mapping approach can be used to narrow down the search for the causative gene to a specific region on one or more equine chromosomes. To perform genome mapping, genomic DNA from a number of horses that have the specific disease phenotype and a number of horses certain to be free of the disease is required. The number of cases and controls required varies between breeds and varies depending on whether a single gene or multiple genes are believed to affect the disease phenotype. Breeds with a higher degree of similarity in their genome (and a resultant sharing of segments of identical DNA sequence known as haplotypes), such as Thoroughbreds, may require fewer horses than breeds with more genetic diversity such as Quarter Horses and many Warmblood breeds. Multiple gene traits will require a larger sample size than single gene traits. In addition, it is important to balance the degree of relatedness among cases and controls in selecting horses for genome mapping. This ensures that a chromosomal region identified by a genome scan as likely to contain the disease gene is indeed more likely due to the actual disease state rather than due to shared genetic markers present because of a higher degree of relatedness in cases vs controls.

To identify the genetic basis for PSSM1, microsatellite genetic markers located on all equine chromosomes (excluding X and Y) were typed to determine if alleles (one of a series of different forms of a gene) of a specific marker occurred more frequently in PSSM1 horses than controls, such as would be expected if the genomic segment containing the PSSM1 gene was derived from a common founder (McCue et al., 2008b). A genetic marker on a region of equine chromosome 10 could then be found to be highly associated with PSSM1. The human genome sequence was consulted to determine if potential genes that could cause excessive polysaccharide storage existed in this homologous region. The glycogen synthase 1 gene (*GYS1*), encoding the enzyme that synthesizes glycogen in skeletal muscle, was found in the region of interest. Sequencing of the *GYS1* gene in PSSM and control horses revealed a single base pair substitution in PSSM horses that changed the genetic code and thereby the amino acid sequence of the glycogen synthase enzyme. Assays of the activity of glycogen synthase in PSSM horse muscle showed that the enzyme was more active with or without stimulation by glucose 6 phosphate compared to healthy horses.

Genome mapping in horses has been greatly assisted by the recent development of equine SNP gene chips, which provides a far greater density of genetic markers (~54,000) and a more rapid mechanized means to scan the genome than previously possible. In addition, the complete sequencing of the horse genome (Wade et al., 2009) performed at the Broad Institute under the auspices of the National Human Genome Research Institute greatly enhances our ability to locate and sequence equine genes. SNP chips are currently being used to map the genes contributing to RER in Thoroughbred horses and type 2 PSSM in Quarter Horses.

Genetic Testing

Once a genetic mutation is identified, simplified assay such as restriction fragment length polymorphisms (RFLP) or higher throughput Real Time PCR can often be used to test populations of animals for the mutation. Such tests can be used commercially to identify affected individuals. In addition, they can be used to screen random samples from large populations to determine the prevalence of the mutation in various breeds.

In some instances, horses may possess a genetic mutation, but it may not show clinical signs of disease, likely as a result of variable or incomplete penetrance. For example, some horses that have the mutation for PSSM1 still lack any clinical signs of tying-up. The lack of penetrance of signs of tying-up in such cases may be due to a subtle effect of this mutation on the glycogen synthase activity, as well as environmental management or effects of other genes. For example, PSSM1 horses that are symptom-free are often kept outside or in regular work and consume only sparse high-fiber, low-starch/sugar grasses. In contrast, clinical signs of tying-up can be worse in PSSM1 horses if they also possess the genetic mutation causing MH (McCue et al., 2009b).

Known Genes Contributing to Tying-Up in Horses

Polysaccharide storage myopathy (PSSM). Two forms appear to exist, type 1 and type 2. The mutation for PSSM1 has now been identified but not all forms of PSSM are due to the *GYS1* mutation.

Type 1 PSSM

Breeds affected:	At least 20 breeds. Quarter Horse-related bloodlines, Belgians, Percherons, Morgans, Mustangs, Tennessee Walkers, and some Warmblood breeds.
Bloodlines:	Widespread in many breeds; in the Quarter Horse it is most common in halter horses.
Prevalence:	36-50% of Belgians and Percherons, 8% of the Quarter Horse-related breeds. Rare in Clydesdales and Shires.
Age affected:	Signs usually begin by 2 to 3 years of age but may occur in weanlings. May be 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.
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 crystalline polysaccharide. Genetic testing on mane or tail hair roots, or unclotted blood samples at the Neuromuscular Laboratory at the University of Minnesota.

Type 2 PSSM (McCue et al., 2009a)

Breeds affected:	Quarter Horse-related breeds, a few Arabians and possibly other light breeds.
Age affected:	Signs usually begin by 2 to 3 years of age but may occur in weanlings. Some horses are subclinical.
Clinical signs:	Rhabdomyolysis with or without exercise.
Inheritance:	Unknown.
Mutation:	Unknown. We are currently mapping PSSM2 with funding from the AQHA.
Testing:	Muscle biopsy samples evaluated for presence of abnormal polysaccharide at the Neuromuscular Laboratory at the University of Minnesota.

Malignant hyperthermia (MH)

Breeds affected:	Quarter Horse-related bloodlines.
Bloodlines:	Present at a very high frequency in two Quarter Horse bloodlines. Often coexists with PSSM.
Prevalence:	<1% of the Quarter Horse breed is affected.
Age affected:	Adults.
Clinical signs:	High temperature, metabolic failure and death under anesthesia. Exertional rhabdomyolysis especially if present with the *GYS1* PSSM1 mutation.
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.

Recurrent exertional rhabdomyolysis (RER)

Breeds affected:	Thoroughbreds and possibly Standardbreds and Arabians.
Bloodlines:	Unknown, possibly more common in those selected for nervousness and speed combined.
Prevalence:	5-10% of Thoroughbreds.
Age affected:	Signs usually present when horses are fit, fed >5 lb of high-starch concentrate and when excited. More common in young fillies than geldings.
Clinical signs:	Firm painful muscles, lameness, stiffness, sweating, short stride, and reluctance to move after moderate exercise.
Inheritance:	Suggested to be autosomal dominant based on pedigree analysis and breeding trial. May be multigenic.
Mutation:	Unknown. *RYR1*, *CACNA1S*, and *ATP2A1* genes ruled out by linkage analysis. (Dranchak et al., 2006) Genome mapping underway.
Testing:	Based on breed, clinical signs, lack of histopathological evidence of PSSM in muscle biopsy samples.

Glycogen branching enzyme deficiency (GBED)

Breeds affected:	Quarter Horse-related bloodlines.
Bloodlines:	Horses descended from Zantanon and King.
Prevalence:	8% of the Quarter Horse breed 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.
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.

Summary Table for Quarter Horses

Much more is known about genetic disorders in Quarter Horses because the AQHA supports research into genetic diseases and because there are millions more horses of this breed than other equine breeds. Table 1 is modified from Tryon et al. (2009). This table provides estimates of the percentage of horses within performance Quarter Horse types that are affected by known genetic muscle diseases. Note, for example, that 56% of halter horses have genetic susceptibility to HYPP, 28% of halter horses have genetic susceptibility to PSSM, and 26% of western pleasure horses carry GBED. This study also showed that a significant percentage of horses have two or more of these genetic mutations.

Table 1. Observed percentages of horses carrying a disease-causing allele for Quarter Horses (QH) and Paints (APH) as well as for elite competitive subgroups.

	Affected dominant (%)		Carrier recessive (%)
Population	HYPP	PSSM1	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.

Conclusions

Genetic disorders are common in horses in part as a result of popular breeding practices. The development of genetic tests provides owners with the ability to make informed choices in their breeding programs. In addition, such tests can be used by veterinarians to diagnose genetic diseases and also to use when performing prepurchase examinations. The development of new equine genetic tools may well produce a rapid expansion in the number of genetic tests available in the near future.

Conflict of interest statement: Drs. Valberg, Mickelson and McCue own the license for PSSM testing and receive sales income from its use. Their financial and business interests have been reviewed and managed by the University of Minnesota in accordance with its conflict of interest policies.

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