The Genetics of Skeletal Muscle Disorders in Horses

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Abstract

Horses are remarkable athletes and a fascinating species in which to study the genetic bases of athletic performance, skeletal muscle biology, and neuromuscular disease. Genetic selection in horses has resulted in many breeds that possess anatomical, physiological, and metabolic variations linked to speed, power, and endurance that are beginning to be defined at the molecular level. Along with the concentration of positive traits, equine breeding programs have also inadvertently concentrated heritable muscle diseases for which mutations impacting electrical conduction, muscle contraction, and energy metabolism within and across breeds have been characterized. The study of heritable muscle diseases in horses has provided exciting insights into the normal structure and function of muscle and important diagnostic tools for veterinarians. Results empower breeders and breed associations to make difficult decisions about how to use this information to improve the overall health and well-being of horses.

INTRODUCTION

Horses are supreme athletes whose beauty and performance depends upon their powerful musculature, which comprises up to 55% of their body mass. Equine breeders have shaped muscle form and function through genetic selection to produce breeds with distinguishing characteristics related to muscle mass, speed, and endurance. The Quarter Horse, for example, arose in the 1940s as a heavily muscled horse adept at racing over short distances of a quarter mile (400 m). By selecting for sprinting speed, Quarter Horse breeders unknowingly concentrated variants in the *MSTN* gene whose product, myostatin, influences muscle development and physiology. Today, approximately 80% of Quarter Horses are homozygous for *MSTN* variants (1, 2) that confer an aptitude for racing over sprint distances (3) as well as a higher proportion of the fastest contracting type 2B(X) muscle fibers in gluteal muscle (**Figure 1**) (2).

The selection of horses for desired physical and performance traits often has the unintended consequence of increasing the frequency of heritable diseases. For example, selection of horses for conspicuous muscle development also led to an increased frequency of a mutation in the α-subunit of the skeletal muscle sodium channel gene (**Figure 2**) (4). The impact of even minor or intermittent perturbations in muscle structure, contraction, and energy metabolism is readily apparent in horses owing to the rigorous expectations of athletic performance. Much of the international equine genome research effort is now devoted to identifying the bases for heritable diseases, many of which affect skeletal muscle, and developing DNA-based diagnostic tests to reduce or eliminate these genes from the populations and provide more customized treatments. Progress in understanding the genetic bases of equine muscle diseases has come from dedicated breed organizations, committed horse owners, collaborative veterinarians, well-documented pedigrees, a reliable means to obtain biopsies of abundant muscle tissue, and the recent development of state-of-the-art resources for genome analysis.

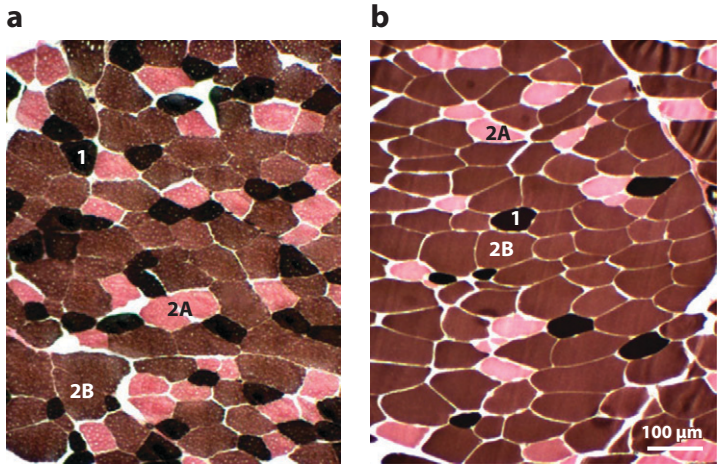

Figure 1

Myosin ATPase stains (pH 4.6) of gluteal muscle biopsies from Quarter Horses without and with a SINE insertion in the *MSTN* gene encoding myostatin. (*a*) Horses homozygous for the wild-type allele have more slow twitch type 1 muscle fibers and fewer fast twitch type 2B muscle fibers than (*b*) horses homozygous for the SINE insertion (2). This SINE insertion and another MSTN variant, a SNP in intron 1, have been associated with aptitude for sprinting ability (3).

This review aims to present the current state of knowledge of the bases for heritable muscular disorders in the horse, including relevant phenotypic descriptions; genetic research methods used; physiological, biochemical, and pathological correlates; and molecular bases, if known. The review groups disorders into those arising from perturbations in muscle cell membrane excitability (myotonia), contraction, and glycogen metabolism. These diseases produce phenotypes that range from intermittent disruption of athletic performance to incompatibility with life.

MYOTONIAS

Myotonias represent a group of muscle diseases that share the feature of delayed relaxation after mechanical stimulation or voluntary contraction. Several clinical descriptions of myotonias in horses exist, but there are two forms for which mutations have been described: hyperkalemic periodic paralysis (HYPP) and myotonia congenita (5–7).

HYPERKALEMIC PERIODIC PARALYSIS

The Clinical Condition

HYPP occurs in American Quarter Horses and related breeds. First recognized in the 1980s (8), HYPP was publicly linked in 2002 to a popular Quarter Horse sire that has over 355,000 descendants. By two to three years of age, horses with HYPP begin to show intermittent signs of muscle twitching (fasciculations). High-potassium diets, fasting, anesthesia, and stress increase the frequency of episodes (9, 10). Episodes begin with a period of facial muscle myotonia, followed by fasciculations in the flanks, neck, and shoulders, and sweating, coincident with elevated serum potassium concentrations. Attacks often cease after 15–20 min but in severe cases progress to weakness and recumbency (**Figure 3**) and occasionally death owing to paralysis

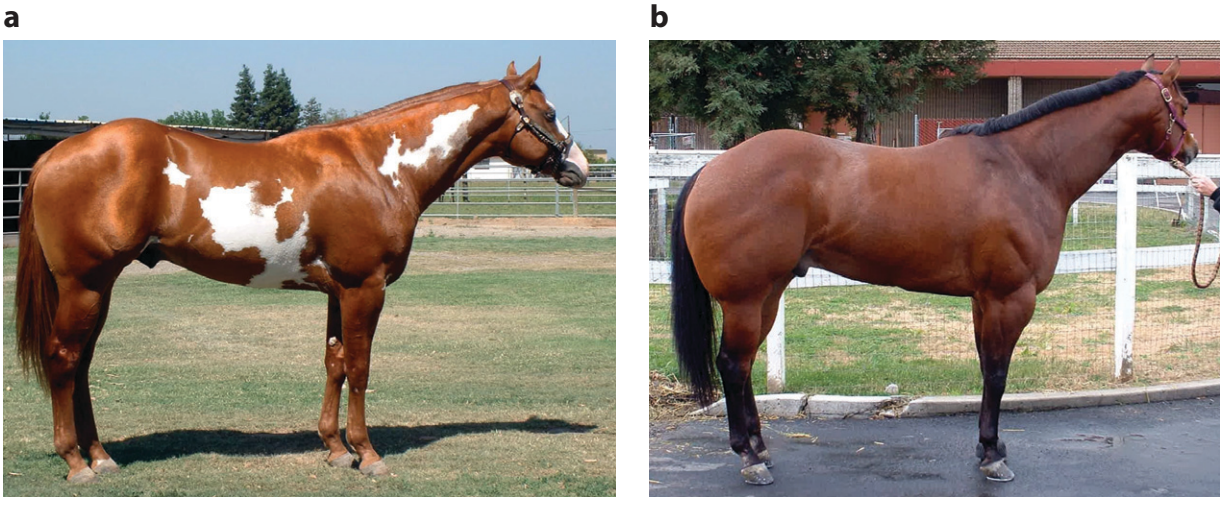

Figure 2

Muscular phenotype in Quarter Horse–related breeds that (*a*) do not have and (*b*) have a dominant mutation in the *SCN4A* gene encoding the skeletal muscle sodium channel that causes hyperkalemic periodic paralysis. Note the more heavily muscled phenotype associated with HYPP (photos courtesy of Dr. Gary Magdesian).

Figure 3

A heavily muscled Quarter Horse homozygous for the *SCN4A* mutation that causes HYPP having an episode of muscle weakness and recumbency that was preceded by muscle fasciculations (photo courtesy of Dr. Sharon Spier).

of upper respiratory muscles. Strict regulation of dietary potassium significantly decreases the frequency of HYPP episodes (11).

Discovery of the *SCN4A* Mutation

HYPP was the first disease in horses for which the molecular defect was determined (4). This ground-breaking achievement resulted from an in-depth clinical investigation, large parallels between equine and human cases of HYPP, and a herd of horses segregating for HYPP. The equine HYPP F1416L mutation occurs in the α-subunit of the skeletal muscle voltage-dependent sodium channel (*SCN4A*) (4). This channel normally functions to allow transient Na^+ entry into muscle cells during the initial phase of the action potential (**Figure 4**). Codon 1416 lies in a highly conserved segment of one of the 24 transmembrane domains of the channel protein. Measurements of channel activity in myotubes from HYPP and control horses, or in *Xenopus* oocytes expressing the rat *SCN4A* containing either a F1416 or L1416 allele, have demonstrated failure of the channel inactivation mechanism. This is observed as increased time to peak current, slowed rates of current decay, shifts in the voltage inactivation curve, increased open probability, and increased mean open channel time (12). Impairment appears to be more pronounced during times of elevated extracellular potassium, such as can occur with diets high in this cation (12). Failure of inactivation in vivo results in membrane depolarization, irritability, and potentially depolarization block and muscular paralysis (13).

Impact of the *SCN4A* Mutation

Heterozygosity for the *SCN4A* mutation is sufficient to cause susceptibility to HYPP. However, a codominant mode of inheritance is indicated as episodes are more severe and more frequent in homozygous horses, with the potential fatal complication of upper airway obstruction (14, 15). To address this issue, the American Quarter Horse Association officially recognized HYPP as a genetic defect or undesirable trait and ruled that, from 2007 forward, foals homozygous for HYPP are not eligible for registration.

Data from 1996 estimated the genotypic disease frequency in Quarter Horses as 4.4% (8), and data reported more than 12 years later suggest that this number has not changed in the breed as

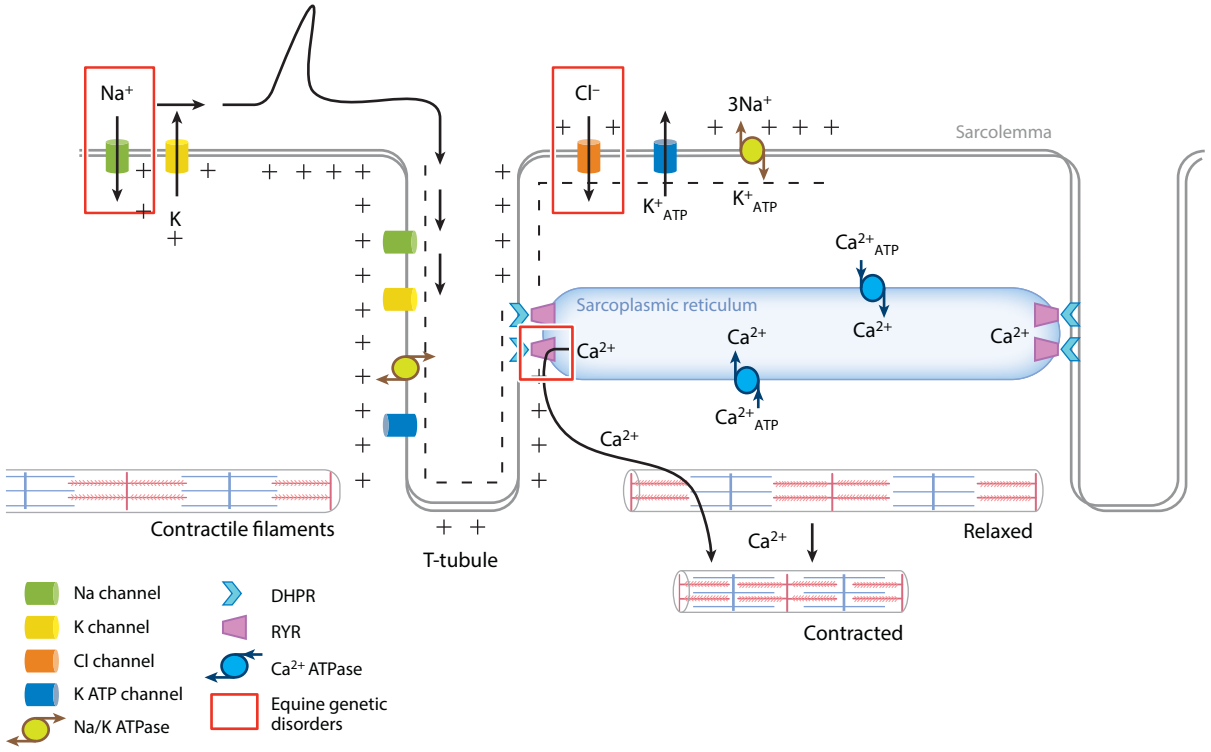

Figure 4

Equine mutations that cause dysfunction in skeletal muscle membrane depolarization and muscle contraction (*red boxes*). Represented are an *SCN4A* mutation (causing hyperkalemic periodic paralysis) that impacts sodium channel function, resulting in a lower threshold for membrane depolarization; a *CLCN1* mutation (causing myotonia congenita) that impacts chloride channel function, causing a prolonged and exaggerated response to muscle cell membrane depolarization; and an *RYR1* mutation (causing malignant hyperthermia) that increases the open probability of the calcium release when triggered by stress, exercise, or general anesthesia, resulting in skeletal muscle contracture, hyperthermia, and lactic acidemia.

a whole (16), with a prevalence as high as 58% in halter horses. Maintenance of this deleterious mutation in the population has been attributed to the fact that halter horses affected with HYPP appear to have been judged to be superior owing to well-developed musculature (17). However, to our knowledge, the physiological basis of increased muscling in these horses is not known. It is possible that continued myotonic discharges could stimulate muscle hypertrophy, but it also remains possible that the genes responsible for increased scoring of halter horses with the *SCN4A* mutation in the show ring are hitchhiking on the ECA11 haplotype, or are on other chromosomes altogether owing to the founder effect. Reports of horses that are negative for the known *SCN4A* mutation but have clinical signs similar to HYPP make it likely that disease phenocopies exist owing to other *SCN4A* mutations or mutations in other genes (18).

MYOTONIA CONGENITA

The Clinical Condition

A congenital nondystrophic form of myotonia termed myotonia congenita occurs in horses as well as in humans and goats (7, 19, 20). Affected horses display conspicuously well-developed

musculature soon after birth and mild to moderate pelvic limb stiffness that improves with exercise. Bilateral bulging of the thigh and rump muscles is often obvious in affected foals, and percussion of affected muscles induces a protracted tight contraction, a subsequent slow relaxation, and a characteristic dimple below the contraction (5, 21). Muscle biopsies from foals with myotonia congenita usually have few histopathological abnormalities (21), and a diagnosis is based on the presence of high-frequency repetitive crescendo-decrescendo electrical bursts in electromyographic examination.

Discovery and Impact of the *CLCN1* Mutation

Although several clinical cases of myotonia congenita have been reported, a genetic mutation has only recently been identified in one New Forest pony (7). A mutation that segregates in an autosomal recessive fashion in a small pedigree was found in the same chloride channel (*CLCN1*) gene that is responsible for myotonia congenita in humans and goats. This D592C mutation occurs in a region highly conserved across species near the C-terminal cytoplasmic domain of the protein (7).

The sarcolemmal chloride channel is essential for setting and restoring the resting membrane potential (**Figure 4**), and it is possible that the D592C *CLCN1* mutation could impact channel function by causing a prolonged and exaggerated response to membrane depolarization (7); however, additional work is necessary to confirm this hypothesis. Whether a *CLCN1* mutation is common to cases of myotonia congenita in other breeds or whether other mutations exist also remains to be determined.

DISORDERS OF MUSCLE CONTRACTION

There are two potential disorders impacting muscle excitation contraction coupling in horses: malignant hyperthermia (MH) and recurrent exertional rhabdomyolysis (RER).

MALIGNANT HYPERTHERMIA

The Clinical Condition

Clinical signs of MH are inconsistently triggered by exposure to halogenated anesthetics, succinylcholine, or in some cases stress or excitement (22, 23). Muscle contracture and extreme rigidity occur during an episode, followed quickly by hyperthermia >40°C, hypercapnia, lactic acidosis, and severe muscle cell damage (rhabdomyolysis) (22). Exercise can also intermittently induce episodes that are characterized by profuse sweating, muscle stiffness, elevated body temperature, and rhabdomyolysis severe enough to warrant euthanasia owing to the inability of the horse to stand (24).

Discovery and Impact of the *RYR1* Mutation

Mutations in the *RYR1* gene encoding the calcium release channel of the skeletal muscle sarcoplasmic reticulum were one of the earliest discoveries in humans and swine of mutations that compromise muscle contractility (25, 26). This channel responds to surface membrane depolarization to release calcium from storage within the sarcoplasmic reticulum into the myoplasm to initiate contraction of the myofilaments (**Figure 4**). A dominant R2454G *RYR1* mutation was discovered in Quarter Horses that developed fatal episodes of MH triggered by halothane anesthesia (22, 27). This region of the protein apparently plays a regulatory role in the control of

channel gating and is one of the hot spots for MH-causing mutations in other species (25). Muscle with *RYR1* mutations often responds to triggering agents by releasing excessive amounts of calcium into the myoplasm. A rise in myoplasmic calcium results in a sustained contraction, accompanied by increased glycogen metabolism, that generates heat, lactic acidosis, increased O_2 consumption, and increased CO_2 production (25).

The prevalence of the R2454G mutation in a random sample of 225 Quarter Horses was 1.3% (28). The incidence of unusual incidents of hyperthermia in Quarter Horses under halothane anesthesia attributable to the *RYR1* mutation has not been determined. Other breeds of horses not reported to have the known *RYR1* mutation can also develop MH under general anesthesia (29–31). Whether there may be other yet-unidentified equine mutations that produce muscle rigidity and hyperthermia triggered by anesthesia or stress is not yet known.

RECURRENT EXERTIONAL RHABDOMYOLYSIS

The Clinical Condition

RER is characterized by the intermittent development of muscle pain, stiffness, sweating, and reluctance to move during or shortly following brief periods of exercise. RER is distinct from sporadic forms of exertional rhabdomyolysis that arise in unfit horses that are overexerted or depleted of electrolytes (32). Horses that develop RER are usually very fit, and episodes are triggered by excitement during exercise, especially in horses fed a high nonstructural carbohydrate diet that have been rested for a few days prior to exercise (33–35). Thoroughbred horses susceptible to RER have also been reported to develop rhabdomyolysis under general anesthesia (36). The nonspecific finding of increased numbers of central nuclei in muscle biopsy samples from horses with active clinical disease is the only common histopathological feature of RER (37).

RER is the most common muscular disorder in Thoroughbred and Standardbred breeds (34, 38–40). Five to ten percent of Thoroughbred and Standardbred horses develop exercise-induced rhabdomyolysis at some point during a racing season. Recurrence is so frequent in 17% of affected Thoroughbred horses that they do not race again that season (34). RER is more prevalent in female horses and horses with a nervous temperament (34, 38, 39). Inadvertent selection for the RER trait is suggested by the finding that RER Standardbred racehorses had a higher winning percentage than control racehorses in the 683 horses evaluated (38).

Physiological and Biochemical Alterations

Muscle contracture testing uses dissected bundles of muscle fibers that are tethered at one end, attached to a force transducer on the other, kept in an oxygenated bath, and electrically stimulated. Increasing concentrations of MH-triggering agents are added to the bath, and effects on muscle tension are assessed. Early studies (31, 41) used excised semimembranous muscle bundles and found that the threshold for inducing contracture with caffeine was lower in Thoroughbreds and Standardbreds susceptible to RER than in breed-matched controls. Subsequent studies of intact tendon-to-tendon external intercostal muscle fiber bundles also identified a lower threshold for the development of potassium-, caffeine-, or halothane-induced contractures compared with controls; all three treatments stimulate the release of calcium from the sarcoplasmic reticulum via the calcium release channel (36). These intact RER muscle preparations also demonstrated a significantly faster rate of relaxation from a twitch compared with controls. An intrinsic defect in muscle contraction was further supported by the observation that muscle cells grown in cell culture as myotubes displayed higher myoplasmic Ca^{2+} concentrations in response to caffeine (42).

Increased sensitivity of contractile activity in RER muscle cells could potentially be due to an altered sensitivity of the contractile apparatus to activation by Ca^{2+}, increased activity of the sarcoplasmic reticulum Ca^{2+} release channel, or decreased activity of the sarcoplasmic reticulum Ca^{2+}-ATPase. However, biochemical investigations using isolated myofibril (43) or sarcoplasmic reticulum membrane preparations (44) from RER and control horses found no support for an alteration in these processes. The precise defect impacting muscle contraction in RER horses has yet to be identified, and it remains to be seen whether or not the abnormal contracture testing of RER horses is a specific or a nonspecific indicator of a defect in muscle cell contraction or calcium regulation.

Episodes of exertional rhabdomyolysis in susceptible horses can be decreased by providing regular daily exercise and by avoiding high nonstructural carbohydrate diets, which are known to increase excitability (33, 35). In practice, dantrolene is also used to reduce episodes of rhabdomyolysis. When given 60–90 min before exercise, dantrolene significantly reduces muscle damage with exercise in RER horses (as indicated by serum creatine kinase activity) (45, 46). Dantrolene reduces the release of calcium into the myoplasm via the calcium release channel and, in so doing, increases the caffeine contracture threshold for muscle bundles from RER and control horses in vitro.

Genetic Studies

Multiple lines of evidence suggest that RER susceptibility has an underlying genetic basis. A Markov chain Monte Carlo method was used to analyze 62 RER and 34 control Thoroughbred horses from an extended pedigree for the conditional probability of foundation genotypes (47). All affected horses shared a common ancestor, and this ancestor and five other stallions had a conditional probability of 1.00 for being affected. Subsequently, a multiyear prospective breeding trial produced a three-generation pedigree from which 23 foals derived from RER × RER or RER × control matings were assessed with the in vitro contracture test (48). A diagnosis of RER in the offspring was based on increased sensitivity to halothane or caffeine-induced contractures. Results of the segregation analysis were consistent with an autosomal dominant pattern and inconsistent with a recessive or an X-linked pattern of inheritance. A study in Japan estimated the heritability of RER in a cohort of 6,538 Thoroughbreds (501 deemed affected) using Bayesian analysis with Gibbs sampling based on the threshold model for a binary trait. An analysis using three or four generations resulted in a heritability estimate of approximately 0.42 (49).

A genetic linkage study using over 400 microsatellite markers was performed in four Thoroughbred pedigrees containing a total of 96 horses (35 affected). This study did not identify regions of the genome significantly linked to RER susceptibility loci, and the genes encoding the sarcoplasmic reticulum Ca^{2+} release channel (*RYR1*) and Ca^{2+}-ATPase (*ATP2A1*), as well as the transverse tubule voltage sensor (*CACNA1S*), were excluded (50). Another study used a multipoint linkage analysis in five half-sib families (51 RER and 277 controls, 117 microsatellites), combined with an association analysis (144 cases and 144 controls, 766 microsatellites), to identify potential RER loci. Both the linkage analysis and genome-wide association study (GWAS) identified a locus on ECA12 and the GWAS also identified a region on ECA20 as candidate regions containing RER loci (51). That RER appears to be a more genetically complex disease than first appreciated calls into question the validity of previous genetic linkage studies that assumed Mendelian inheritance.

A GWAS with 48,282 SNP markers on 48 RER cases and 37 Thoroughbred controls has recently been employed (52). The most significant SNPs spanned approximately 13 Mb on ECA16, and the p-value of the most significant SNP after correcting for population structure was 8.0 ×

10^{-6}. This region on ECA16 was further evaluated by genotyping 247 SNPs in both the initial population and a second population of 34 cases and 98 control Thoroughbreds; several SNPs across the 13-Mb region on ECA16 showed significance in this validation experiment (52). The above loci must be further validated with additional horses and higher-density SNP arrays to increase the likelihood of detecting loci contributing to RER susceptibility. Future studies of Standardbred horses could also determine if RER susceptibility loci are shared with Thoroughbreds and if there is any indication of association of RER loci with performance.

GLYCOGEN STORAGE DISORDERS

Glycogen is a highly branched glucose polymer consisting of α-1,4-glycosidic linkages produced by glycogen synthase and numerous α-1,6 linkages formed by glycogen branching enzyme (**Figure 5**). Whereas in humans most glycogen storage disorders are caused by defects in enzymes of glycogen metabolism, both mutations now known to cause glycogen storage disorders in horses are due to defects in glycogen synthesis: type 1 polysaccharide storage myopathy (PSSM1) and

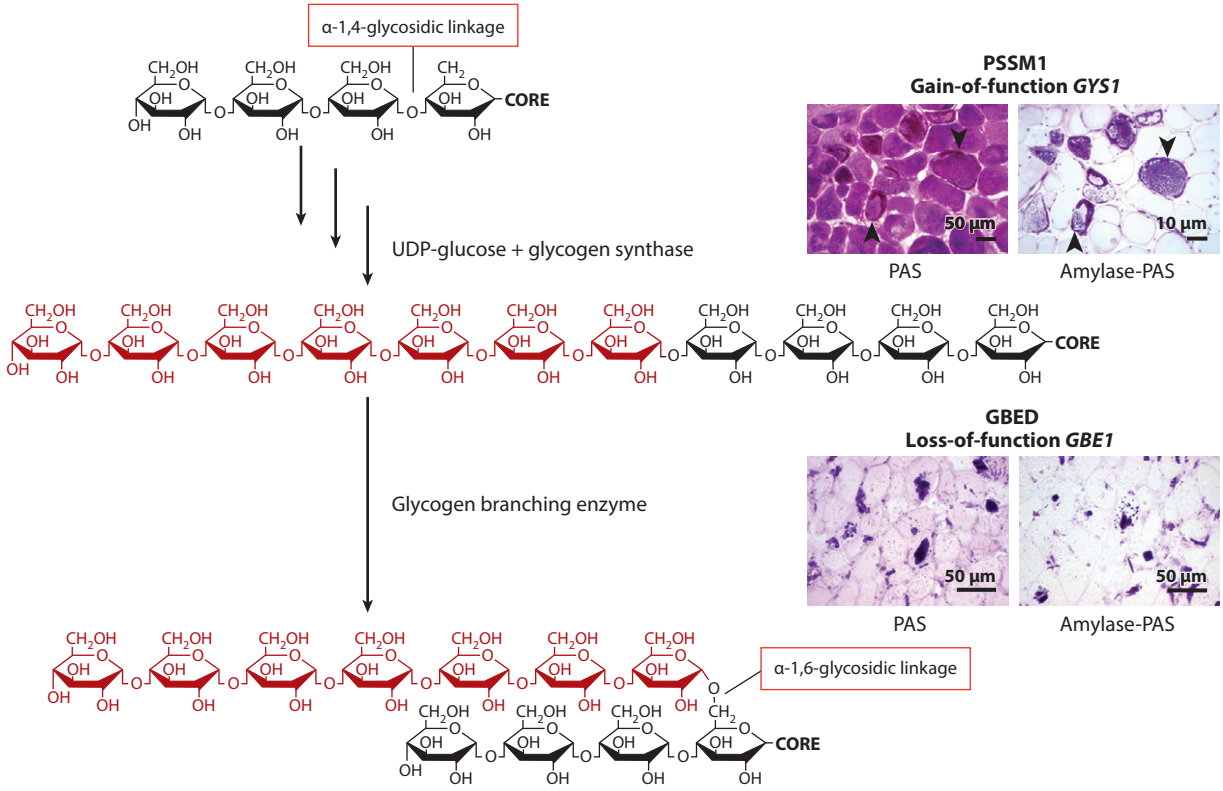

Figure 5

Equine mutations that impact the synthesis of glycogen in skeletal muscle. Represented are a *GYS1* mutation (polysaccharide storage myopathy) that results in higher muscle glycogen concentrations (PAS stain) and the production of more α-1,4- and fewer α-1,6-glycosidic linkages, which result in the formation of an amylase-resistant polysaccharide in some muscle fibers, and a *GBE1* mutation (glycogen branching enzyme deficiency) that results in a loss of function of glycogen branching enzyme, a decrease in muscle glycogen concentrations (PAS stain), and the accumulation of an amylase-resistant polysaccharide with largely straight α-1,4-glycosidic linkages.

glycogen branching enzyme deficiency (GBED) (53, 54). There are other suspected glycogen storage disorders in horses that do not possess either the PSSM1 or GBED mutations but also have an apparently abnormal deposition of glycogen in histological sections of skeletal muscle. These cases are at present termed type 2 PSSM (PSSM2).

POLYSACCHARIDE STORAGE MYOPATHY

The Clinical Condition

During their use for transportation and agriculture in the nineteenth century, draft horses often suffered from exertional rhabdomyolysis, and excessive muscle glycogen storage was described as a feature of this condition in Swedish Ardenner draft horses (55). The term azoturia was used to describe the disorder in draft breeds for many decades. The discovery of PSSM as a specific myopathy came in 1992 based on the abnormal amylase-resistant polysaccharide and excessive glycogen found in muscle biopsies of Quarter Horses that developed exertional rhabdomyolysis (**Figure 5**) (56). Abnormal polysaccharide accumulation and excessive glycogen were also documented in the muscle of draft breeds, indicating that this was likely the same disorder as the earlier described azoturia (57, 58).

Unexercised horses with PSSM1 usually appear normal, but clinical signs of a short stride, firm musculature, stiffness, pain, sweating, and reluctance to move forward develop with light exercise. Signs of PSSM1 usually become apparent when horses start enforced exercise at the age of two or three years; however, by one month of age, foals with PSSM1 can have evidence of subclinical muscle damage (59). If foals become ill with a respiratory or gastrointestinal infection, severe, life-threatening rhabdomyolysis can develop without inciting exercise (60).

Skeletal muscle glycogen concentrations in horses with PSSM1 are typically two to four times the levels in normal horses (56, 61). Abnormal amylase-resistant polysaccharide accumulates in PSSM1 skeletal muscle by 16 months of age in as few as one or two fibers to as many as 30% of the type 2 skeletal muscle fibers (56, 59, 62). The abnormal polysaccharide consists of both β glycogen particles and filamentous material and has a less branched structure than normal glycogen (56, 63, 64). Large aggregates of abnormal polysaccharide are often associated with autophagic rimmed vacuoles and are tagged for degradation by ubiquitin (62, 65). Although abnormal polysaccharide is occasionally found in cardiac muscle fibers of severely affected horses, cardiac dysfunction is not a feature of PSSM1 (57, 64, 66).

Discovery and Impact of a *GYS1* Mutation

Analyses of five- to six-generation pedigrees that evaluated founder contributions, the contribution of specific founders to inbreeding, and the coefficient of inbreeding between Quarter Horses or horses from Quarter Horse–related breeds diagnosed with PSSM found a high probability that a select few founder stallions were responsible for transmitting the condition (67). A GWAS was used to identify loci that conferred susceptibility to PSSM1 using Quarter Horse cases that descended from a founder stallion within seven generations to increase the extent of shared chromosomal segments surrounding a PSSM1 mutation. Microsatellite markers in an approximately 2.7-Mb interval on ECA10 showed association with PSSM (54), and the region was confirmed in an independent sample of PSSM and control Quarter Horses. The skeletal muscle glycogen synthase gene (*GYS1*) was the most plausible positional candidate gene.

GYS1 cDNA sequencing revealed a polymorphism in exon 6 that changes the normal arginine residue at position 309 to a histidine (R309H). Conservation of this residue and the flanking amino

acid sequence in sequenced vertebrate *GYS1* genes suggested an essential role in glycogen synthase function, as well as the plausibility that this variant was the causative PSSM1 mutation (54). Heterozygosity for the H309 allele was shown to be sufficient for horses to be PSSM1 cases, but 22% of PSSM cases were homozygous wild type and 5% of normal horses were heterozygous. This nonconcordance suggested that the *GYS1* mutation is not a fully penetrant dominant allele and that one or more phenocopies are present.

The *GYS1* mutation has been identified in a large number of breeds in both North America and Europe (16, 68–71). Approximately 6–8% of Quarter Horses and Paint Horses are either heterozygous or homozygous for the *GYS1* disease (H309) allele (70). The most sedentary Quarter Horse performance type, the halter horse, has a much higher percentage of horses with the H309 allele (28%) compared with barrel racing Quarter Horses (2%) (16). In North America, 33% of Belgian draft horses and >50% of Percheron draft horses are either heterozygous or homozygous for the H309 allele (70). Many draft breeds originating in continental Europe have an even higher *H309* allele frequency, particularly breeds related to the original Belgian draft, such as the Trekpaard, Comtois, and Breton (68). Severity of clinical and histological profiles in Belgian and Percheron horses with PSSM1 is influenced by genotype, with homozygosity associated with higher serum muscle enzyme levels (indicative of muscle damage) and increased subsarcolemmal vacuolation and cytoplasmic polysaccharide inclusions (72). The prevalence of the *GYS1* mutation is very low in athletic light breeds, such as Arabians, Thoroughbreds, and Standardbreds (73).

Genotype interactions clearly alter the impact of the *GYS1* mutation. In a controlled exercise trial, horses with both *GYS1* and *RYR1* mutations suffered from more severe exercise intolerance and muscle damage than did horses with the *GYS1* mutation alone (74). In addition, 14% of Quarter Horses of the halter performance type have mutations in both *SCNA4* (HYPP) and *GYS1* (16). In some horses, the effect of both of these mutations is severe, life-threatening rhabdomyolysis that develops when horses become recumbent during an HYPP attack.

Effects on Glycogen Synthase, Glycogen, and Energy Metabolism

Quarter Horses with PSSM1 have higher resting muscle glycogen levels than control horses do. Although these glycogen stores are depleted to similar extents during an exercise bout in both PSSM1 and control horses, repletion of muscle glycogen stores occurs more quickly in horses with PSSM1 in comparison with controls (**Figure 6**), consistent with an altered regulation/activity of glycogen synthase and glycogen storage (61, 75). This possibility was evaluated in muscle biopsy extracts, where glycogen synthase activity was significantly (~1.7-times) greater in PSSM1 muscle than in control muscle in both the presence and absence of the allosteric regulator glucose-6-phosphate (**Figure 7**) (54). Several possibilities exist as to how an R309H substitution results in a dominant gain of function of glycogen synthase and excessive glycogen accumulation. One possibility is that the mutant enzyme is resistant to negative regulation, or more sensitive to positive regulation. Altered phosphorylation/dephosphorylation by a multitude of protein kinases and phosphatases, altered affinity for its substrates (UDP-glucose and glycogen), or altered allosteric regulation by glucose-6-phosphate are also possible. The gain-of-function mutation in the equine *GYS1* contrasts with the human glycogen storage disease (GSD) type 0 associated with *GYS1* polymorphisms. GSD 0 results in a deficiency of glycogen synthase activity, depletion of glycogen, cardiomyopathy, and weakness (76, 77).

One of the most puzzling aspects of PSSM1 is the mechanism by which a gain of function in *GYS1* and increased glycogen storage result in muscle cell damage. PSSM1 horses struggle to achieve maximal speeds and fatigue quickly at their maximal speed but have less muscle damage with this form of exercise than with submaximal exercise (61, 75). A deficit in energy generation in

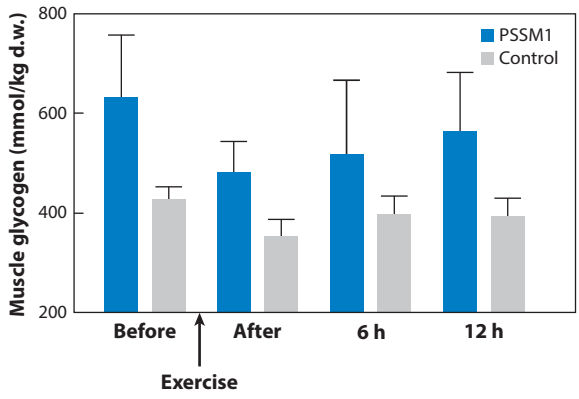

Figure 6

Mean muscle glycogen concentrations in five PSSM1 and five healthy control horses before and after a near-maximal standardized exercise test and 6 and 12 h after exercise. Note the significantly higher muscle glycogen concentrations before exercise and the rapid repletion of glycogen in PSSM1 compared with control horses. Adapted from Reference 99. Abbreviation: d.w., dry weight.

PSSM1 horses during submaximal exercise is supported by metabolic studies (78). During near-maximal exercise, PSSM1 horses have lower maximal oxygen uptake and higher lactate concentrations than control horses do, indicating decreased flux through oxidative energy metabolism. Further, adenosine monophosphate (AMP) concentrations declined, and inosine monophosphate (IMP) concentrations increased, in muscle fibers of PSSM1 horses relative to control horses performing 20 min of submaximal exercise (78). When adenosine triphosphate (ATP) levels in muscle cannot be effectively restored by metabolic pathways during exercise, the myokinase reaction increasingly produces ATP and AMP from adenosine diphosphate. AMP is then increasingly degraded to IMP by AMP deaminase. Thus, premature degradation of adenine nucleotides to IMP in PSSM1 horses appears to indicate abnormal regulation of the flux of substrates in aerobic metabolism; such an energy deficit might cause segmental damage to muscle fibers during exercise.

A microarray containing ~750 oligonucleotide probes was used to measure gene expression in skeletal muscle of PSSM1 and control Cob Normand draft horses (79). The analysis revealed 16 genes upregulated over 1.5-fold and 37 genes downregulated over 1.5-fold. Protein synthesis, apoptosis, cellular movement, growth, and proliferation were the main cellular functions significantly associated with the modulated genes. The authors concluded that PSSM mitochondrial dysfunction, glycogenesis inhibition, and chronic hypoxia may contribute to the PSSM1 disease process (79).

Phenotypic Variability

Considerable phenotypic variability exists with PSSM1 among individuals and across breeds. Clinical signs of PSSM occurred in 66% of horses with the *GYS1* mutation that were first- or second-generation offspring of a Warmblood stallion with the *GYS1* mutation (80). In that family, the mutation conferred a sevenfold higher risk of developing signs of exertional rhabdomyolysis. Why some horses remain asymptomatic whereas other horses suffer from daily episodes of muscle pain and stiffness is not known. Temperament, body type, and gender do not impact expression of the disease.

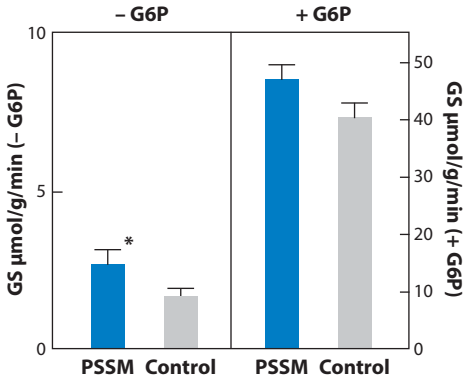

Figure 7

Mean glycogen synthase (GS) activity in the presence and absence of its allosteric activator, glucose 6-phosphate (G6P). Note the significantly greater activity of GS with or without G6P. Used with permission from Reference 54.

Clinical signs of PSSM1 are, however, impacted by diet (81–83). If horses with PSSM1 are fed a high nonstructural carbohydrate diet, they develop more consistent and more severe rhabdomyolysis with exercise than when they are fed a low nonstructural carbohydrate diet that is supplemented with fat. The detrimental impact of a high nonstructural carbohydrate diet on exercise intolerance in PSSM1 horses may be due to its ability to increase serum insulin, a known activator of glycogen synthase (81, 83, 84). Gradually increasing daily exercise decreases clinical signs in PSSM1 horses, and this could arise from the training effect necessary to improve oxidative energy metabolism (81, 82).

Approximately 80% of Warmbloods, 28% of Quarter Horses, and 20% of draft horses with abnormal polysaccharide in muscle biopsies and clinical signs of exercise intolerance do not possess the *GYS1* mutation (**Table 1**) (63, 71). Abnormal polysaccharide in most of these cases is sensitive to amylase digestion rather than resistant, as in PSSM1 (63). The term type 2 PSSM (PSSM2) is commonly used to refer to horses with clinical signs of exercise intolerance that lack the *GYS1* mutation but have histochemical evidence of abnormal glycogen in muscle biopsies (63). It may well be that many different disorders can result in the production of glycogen with an abnormal histological appearance in equine skeletal muscle (85–87), and not all horses diagnosed as PSSM2 will have the same cellular or molecular basis for their disease.

Historical Selection for the *GYS1* Mutation

It is necessary to consider why a mutation associated with a deleterious muscle disease could have such high allele frequencies in a variety of breeds. Additional SNP discovery flanking the mutation, followed by genotyping of 279 horses, revealed a paucity of haplotypes carrying the *GYS1* disease allele when compared with haplotypes containing the wild-type allele (88). Additionally, increased linkage disequilibrium, measured by relative extended haplotype homozygosity, exists in haplotypes carrying the mutation compared with haplotypes carrying the wild-type allele. Coalescent simulations of Belgian horse populations demonstrated that the high frequency and extended haplotype associated with the *GYS1* mutation in this breed were unlikely to have arisen under neutrality or owing to population demography. These data suggest that the *GYS1* mutation underwent historical selection in the Belgian and, by inference, in many related draft breeds. In

Table 1 *GYS1* **genotyping results for various breeds of horses diagnosed with PSSM[1]**

	H/H		R/H		R/R		Total	PSSM1	PSSM2
Breed	N=	%	N=	%	N=	%	N=	% + *GYS1*	% − *GYS1*
Quarter Horse–related	13	4.5	197	67.5	182	28.1	292	71.9	28.1
Draft	13	12.7	76	74.5	13	12.7	102	87.3	22.7
Warmblood	0	0	5	17.9	23	82.1	28	17.9	82.1
Mixed	2	5.3	27	71.1	9	23.7	38	76.3	23.7
Other*	0	0	6	24.0	19	76.0	25	24.0	76.0
Unknown	0	0	8	80.0	2	20.0	10	80.0	20.0
Total	**283**	**5.7**	**319**	**64.4**	**148**	**29.9**	**358**	**70.1**	**29.9**

[1]Values are based on the accumulation of abnormal periodic acid Schiff's positive inclusions in muscle biopsies that were submitted to the Neuromuscular Diagnostic Laboratory at the University of Minnesota because the horses were suspected of having an exertional myopathy. Polysaccharide storage myopathy (PSSM)-affected horses with the H/H and R/H genotypes are considered to have PSSM1, and affected horses with the R/R genotype are considered to have PSSM2.

*Other breeds include Andalusian, Arabian, Icelandic Horse, Lipizzaner, Morgan, Paso Fino, Rocky Mountain Horse, Tennessee Walking Horse, Thoroughbred, and Welsh Cob.

contrast, in Quarter Horses, elevated relative extended haplotype homozygosity was associated with multiple haplotypes containing the *GYS1* disease allele and may be the result of recent population expansion or a popular sire effect in this breed (88).

The *GYS1* disease allele with its associated high muscle glycogen content may be an example of a gene that was once considered beneficial in horses when high-energy feeds were scarce and long hours of daily work were a routine. This mutation has since been carried on into many of the modern breeds, where it can now be detrimental under modern management conditions of high nonstructural carbohydrate feeds and irregular daily exercise.

GLYCOGEN BRANCHING ENZYME DEFICIENCY

The Clinical Condition

GBED is a lethal condition in the Quarter Horse and Paint Horse breeds. GBED was discovered through observation of abnormal globular polysaccharide inclusions in tissues from foals that died in utero or were weak at birth and either died or were euthanized within 18 weeks of age because of muscular weakness, respiratory failure, hypoglycemic seizures, and cardiac arrest (89, 90). Periodic acid–Schiff stains of biopsies demonstrated abnormal globular polysaccharide and crystalline material in brain, liver, skeletal muscle, cardiac muscle, and cardiac Purkinje fibers, with little or no normal-appearing glycogen (**Figure 5**). These amylase-resistant inclusions resembled those present in humans with glycogen storage disease type IV, which is due to deficiency in the glycogen branching enzyme. Subsequently little or no branching enzyme activity or immune-detectable branching enzyme protein could be detected in affected foal tissues, and a highly unbranched nature of isolated polysaccharides was demonstrated with iodine absorption spectra analysis (89).

Discovery and Impact of the *GBE1* Mutation

Genetic association between microsatellite markers on ECA26 and the disease phenotype in 9 cases and 55 controls confirmed the candidacy of the *GBE1* gene (91). *GBE1* sequencing revealed a mutation in exon 1 that resulted in a premature stop codon (Y34×) in the 699–amino acid protein (53). GBED was then demonstrated to be a fatal autosomal recessive disease, currently known to affect only Quarter Horses and Paint Horses, with no abnormal phenotype yet observed in carriers.

Glycogen, an energy-dense, highly branched molecule, is a required energy source in the rapidly growing fetus and neonate. The production of straight chains of α-1,4-glycosidic linkages without branch points drastically decreases the number of nonreducing ends within the glycogen molecule, thereby limiting the rates of both synthesis and degradation. The inability to mobilize glycogen in highly glucose-dependent tissues, such as skeletal muscle, cardiac muscle, and Purkinje fibers, likely results in catastrophic energy deficits in foals with GBED.

Carrier frequency estimates of 7.1% and 8.3% have been reported in the Paint and Quarter Horse breeds, respectively, with rates as high as 26% in Western Pleasure horses (92). The high carrier rate and popularity of carrier stallions suggested that many GBED foals should be born every year; however, the clinical condition appeared infrequently in neonatal foals. Genotyping of 190 Quarter Horse foals that were aborted, stillborn, or died at full or near term owing to unknown causes demonstrated that approximately 4% were homozygous for the *GBE1* mutation, with most being aborted within the second trimester (89, 92, 93). Thus, death in utero may be the primary clinical presentation for GBED, with a rare few homozygotes surviving through gestation. This fact likely explains why this disease went unrecognized for decades.

CONCLUDING REMARKS

Focused research efforts over the past two decades have defined specific forms of heritable equine muscle disease through careful phenotyping of patient characteristics, physiological responses, muscle histopathology, biochemistry, and genetic analysis. The result in many cases is commercially available diagnostic DNA testing and nutrigenomic management strategies for muscle diseases that are in use in the equine population. Our understanding of the genetic basis of more equine muscle disorders still must grow to stop unknowing transmission of diseases to future generations and allow us to develop more targeted evidence-based treatments. Although the majority of muscle diseases in this review have been described in Quarter Horses, this is likely the result of the large number of Quarter Horses in the world (over four million registered horses) relative to other breeds, the strong support of the American Quarter Horse Association for equine research, and the selection pressure for specific performance types within the Quarter Horse breed.

SUMMARY POINTS

1. The currently known autosomal dominant equine muscle disorders, HYPP *(SCN4A)*, MH (*RYR1*), and PSSM1 (*GYS1*), intermittently negatively impact muscle function in adult horses.
2. The currently known autosomal recessive equine muscle disorders, GBED (*GBE1*) and myotonia (*CLCN1*), have a profound and persistent impact on muscle function in horses from a young age.

3. Because horses are unsurpassed athletes that are exercised daily, the effects of genetic mutations that impact their muscle mass, muscle membrane excitability, muscle contraction, or energy metabolism are readily apparent.
4. The popularity of certain sires means that one genetic mutation is often responsible for hundreds of thousands of cases of the same muscle disease (e.g., HYPP, PSSM1).
5. A mutation in *SCNA4* may have inadvertently been selected for along with heavily muscled phenotypes.
6. Because of its potential beneficial effect of increased muscle energy storage, a gain-of-function mutation in *GYS1* may have inadvertently been selected for when horses worked harder and feed was sparse. Today, energy-dense diets and irregular exercise schedules may explain why the mutation now has a detrimental effect on exercise performance.
7. Nutrigenomics plays an important role in managing muscle disorders in horses, and the frequency of clinical signs of disease can be significantly reduced by decreasing dietary potassium (HYPP) and dietary nonstructural carbohydrates (PSSM1 and RER) and adding a fat supplement (PSSM1 and RER).

FUTURE ISSUES

1. Many muscle disorders present with similar clinical signs of muscle pain and cramping with exercise. The ability to distinguish specific subsets of muscle diseases is critical to being able to perform accurate genetic analysis. Detailed physiological, muscle histopathological, and biochemical studies that differentiate these subsets of exertional myopathies will be crucial to future studies of the genetic basis for a variety of equine muscle disorders.
2. Owing to the close relationships within many breeds, current research clearly shows that many horses have mutations in more than one disease-causing gene. In the future, how will breed organizations take advantage of available DNA testing to decrease the frequency of undesirable muscle diseases?
3. Genetic bottlenecks and inbreeding in some small breeds will make it difficult to eliminate undesirable genetic traits while preserving desirable traits in these breeds. What approaches can be instituted to help these breeds deal with existing genetic disease without concentrating other known and yet-to-be-known genetic diseases?
4. Unlike humans, horses will eat the same diet every day, so nutritional intake can be readily controlled. Can additional nutrigenomic strategies be developed to manage heritable muscle disorders in horses?
5. Studies of large cohorts as well as the use of high-density SNP arrays and next-generation sequencing technologies will be necessary to identify mutations contributing to more complex heritable disorders that impact muscular performance.

DISCLOSURE STATEMENT

Drs. Mickelson and Valberg are owners of the patent for the PSSM genetic test and receive sales income from its use. Their financial interests have been reviewed and managed by the University of Minnesota in accordance with its conflict of interest policies. The University of Minnesota is the owner of the patent for the GBED genetic test and receives royalty from the licensing of this test to service laboratories.

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