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Estimated prevalence of the Type 1 Polysaccharide Storage Myopathy mutation in selected North American and European breeds

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Summary

The *GYS1 gene* mutation that is causative of Type 1 Polysaccharide Storage Myopathy (PSSM) has been identified in more than 20 breeds of horses. However, the *GYS1* mutation frequency or Type 1 PSSM prevalence within any given breed is unknown. The purpose of this study was to determine the frequency of the *GYS1* mutation and prevalence of genetic susceptibility to Type 1 PSSM in selected breeds from Europe and North America. The *GYS1* mutation was detected in 11 breeds, including, in order of increasing allele frequency, Shires, Morgans, Appaloosas, Quarter Horses, Paints, Exmoor Ponies, Saxon-Thuringian Coldbloods, South German Coldbloods, Belgians, Rhenish German Coldbloods and Percherons. The prevalence of genetic susceptibility to Type 1 PSSM in these breeds varied from 0.5% to 62.4%. The *GYS1* mutation was not found in the sampled Thoroughbreds, Akhal-Tekes, Connemaras, Clydesdales, Norwegian Fjords, Welsh Ponies, Icelandics, Schleswig Coldbloods or Hanoverians, but failure to detect the mutation does not guarantee its absence. This knowledge will help breed associations determine whether they should screen for the *GYS1* mutation and will alert veterinarians to a possible differential diagnosis for muscle pain, rhabdomyolysis or gait abnormalities.

Keywords glycogen storage disease, glycogen synthase, PSSM.

Introduction

Equine Polysaccharide Storage Myopathy (PSSM) is characterized by increased skeletal muscle glycogen concentration, abnormal polysaccharide accumulation in myofibers (Valberg *et al.* 1992; Firshman *et al.* 2006; McCue *et al.* 2006), and signs of painful muscle cramping, exertional rhabdomyolysis, and/or progressive muscle atrophy (McCue *et al.* 2006). Recently, a mutation in the *GYS1* gene, and the resulting p.Arg309His substitution in the skeletal muscle isoform of glycogen synthase, was identified in 77% of Quarter Horses diagnosed with PSSM (McCue *et al.* 2008b). Heterozygosity for the mutation is sufficient to cause PSSM, however, the penetrance is clearly affected by environmental factors, including diet and exercise, and possibly by breed (McCue *et al.* 2008a,b). The disease resulting from this mutation has been termed Type 1 PSSM, and is associated with accumulation of amylase-resistant polysaccharide and the development of exertional rhabdomyolysis in horses fed diets high in non-structural carbohydrate.

Retrospective analysis demonstrated that the *GYS1* mutation and Type 1 PSSM occur in more than 20 breeds from North America and Europe (McCue *et al.* 2008a; Stanley *et al.* 2009). Type 1 PSSM is a worldwide cause of

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neuromuscular disease in horses, although it is likely that other forms of PSSM exist (McCue *et al.* 2008a,b; Stanley *et al.* 2009). All horses with the *GYS1* mutation share a minimal 350 -kb haplotype, and we hypothesize that the mutation arose many hundreds of years ago in heavy horses and has spread from modern draft breeds to lighter breeds (McCue *et al.* 2008b). However, the frequency of the *GYS1* mutation, and the prevalence of PSSM within individual breeds, is as yet unknown. The purpose of this study was to determine the frequency of the *GYS1* mutation and estimate the prevalence of genetic susceptibility to Type 1 PSSM in a sampling of breeds from Europe and North America, with particular attention directed to those breeds where the *GYS1* mutation has previously been identified.

Materials and methods

Sample collection

Whole blood or hair roots were obtained from horses of selected breeds in North America and Europe. Shires and Clydesdales were sampled in both Europe and North America.

North American horse samples were obtained from the Veterinary Genetics Laboratory (VGL) at the University of California at Davis (Table 1) by systematic random sampling, where every 10th hair root submission for the purpose of breed registration was obtained to ensure even geographical distribution.

DNA samples from European horses were obtained by three different sampling schemes. Simple random sampling was performed to obtain a subset of all foals born to registered Hanoverian breeding mares or stallions in 2001. These studs were distributed throughout all breeding districts in Germany.

Stratified random sampling was used to collect samples from Clydesdales, Saxon-Thuringian Coldblood, Schleswig Coldblood and South German Coldblood horses (Table 1), as well as the Rhenish German Coldblood (n = 12). Clydesdale samples were collected from 10 Scottish breeding yards. Fifty-eight of the horses were derived from six different stallion bloodlines; neither dam nor sire of the remaining 40 horses was significantly over-represented in Scottish

Table 1 *GYS1* allele frequencies in North American and European horse breeds.

Breed/origin	Number horses tested	PSSM (A) allele frequency	Confidence interval A allele		Wild-type (G) allele frequency	Confidence interval G allele	
			Lower	Upper		Lower	Upper
North America[1]							
Percheron	149	0.346	0.293	0.401	0.654	0.599	0.707
Belgian	149	0.242	0.195	0.292	0.758	0.708	0.805
Paint	195	0.041	0.024	0.064	0.959	0.936	0.976
Quarter Horse	335	0.034	0.022	0.050	0.966	0.950	0.978
Appaloosa	152	0.030	0.014	0.053	0.970	0.947	0.986
Morgan	214	0.005	0.001	0.014	0.995	0.986	0.999
Shire	195	0.003	0.000	0.011	0.997	0.989	1.000
Thoroughbred	96	0.000	0.000	0.010	1.000	0.990	1.000
Akhal-Teke	50	0.000	0.000	0.019	1.000	0.981	1.000
Connemara	49	0.000	0.000	0.019	1.000	0.981	1.000
Clydesdale	48	0.000	0.000	0.020	1.000	0.980	1.000
Norwegian Fjord	46	0.000	0.000	0.021	1.000	0.979	1.000
Welsh Pony	45	0.000	0.000	0.021	1.000	0.979	1.000
Icelandic	36	0.000	0.000	0.026	1.000	0.974	1.000
Europe							
South German CB[2]	265	0.117	0.091	0.146	0.883	0.854	0.909
Saxon-Thuringian CB[2]	44	0.068	0.028	0.133	0.932	0.867	0.972
Shire[3]	33	0.000	0.000	0.029	1.000	0.971	1.000
Schleswig CB[2]	33	0.000	0.000	0.029	1.000	0.971	1.000
Clydesdale[2]	98	0.000	0.000	0.007	1.000	0.993	1.000
Hanoverian[4]	214	0.000	0.000	0.004	1.000	0.996	1.000

PSSM, Polysaccharide Storage Myopathy.

[1]Samples collected by systematic random sampling.

[2]Samples collected by stratified random sampling.

[3]Samples collected by non-random sampling.

[4]Samples collected by simple random sampling.

Clydesdale pedigrees. Saxon-Thuringian Coldblood, Schleswig Coldblood and South German Coldblood horses were sampled from April 2001 to June 2002. Studs were distributed over the entire breeding district to obtain a representative sample. Additional samples from the progeny of registered South German Coldblood horses were obtained in 2001, 2003 and 2004.

Lastly, non-random sampling was employed to obtain samples from Shires and Exmoor Ponies. All European Shire samples were taken at the Shire Horse Society Annual Show at Peterborough, UK (Table 1). Exmoor pony (n = 12) samples were taken from available horses in Germany.

GYS1 genotyping

Genomic DNA was isolated with commercially available kits (Gentra; Qiagen Ltd, UK; Qiagen, USA) according to the manufacturers' protocols. *GYS1* genotypes for the c.926G>A mutation were obtained by the established PCR-RFLP technique (McCue *et al.* 2008b). Genotypes were recorded as homozygous normal (G/G), heterozygous (G/A) or homozygous affected (A/A).

Statistical analysis

G and A allele frequencies, G/G, G/A and A/A genotype frequencies, and predicted genotypic prevalence of Type 1 PSSM (based on both G/A and A/A genotypes causing susceptibility to PSSM) were calculated within each breed that had >30 samples. Ninety-five per cent confidence intervals for true allele frequency and disease prevalence were calculated using a likelihood-ratio confidence interval method (http://www.r-project.org).

Results

The *GYS1* mutation was detected and mutant allele frequency estimated in samples from nine different breeds across North American and Europe (Table 1). In addition, the mutation was detected in 6 of 12 Rhenish German Coldbloods (1 A/A, 5 G/A) and 1 of 12 Exmoor Ponies (G/A). The mutant allele frequency was the highest in the North American Percheron and Belgian Draft breeds followed by the South German Coldbloods. The mutation was also found at relatively high frequency in Paints, Appaloosas and Quarter Horses, and at low frequency in Shires and Morgans. The *GYS1* mutation was not detected in the Thoroughbred, Akhal-Teke, Connemara, Clydesdale, Norwegian Fjord, Welsh Pony, Icelandic, Schleswig Coldblood and Hannoverian (Table 1).

GYS1 genotypic frequencies, and their resulting estimated Type 1 PSSM prevalences with 95% confidence intervals, are listed in Table 2. The prevalence of genetic susceptibility to Type 1 PSSM (the sum of the A/A and G/A genotype frequencies) ranged from 0.005 and 0.009 in the Shires and Morgans respectively to 0.114, 0.389 and 0.624 in Saxon-Thuringian Coldblood, Belgian and Percheron respectively.

Discussion

The results of this study provide an indication of the frequency of the *GYS1* mutation in a variety of European and North American breeds. This study does not address how frequently horses that possess the *GYS1* mutation develop clinical signs of PSSM. The development of clinical myopathy in horses with the *GYS1* mutation has previously been shown to be affected by diet, management, other genes and perhaps other factors (Ribeiro *et al.* 2004, McCue *et al.* 2009), and thus the true penetrance of the G/A and A/A genotypes is not known. Under certain conditions, however, disease susceptibility appears to have a dominant mode of inheritance and complete penetrance (McCue *et al.* 2008b). Therefore, the upper limit of Type 1 PSSM prevalence in a breed can be determined from the sum of the G/A and A/A genotype frequencies, assuming a dominant mode of inheritance and complete penetrance. By this estimation, the prevalence of genetic susceptibility to Type 1 PSSM varied from 0.00 to 0.624 among the breeds in this study.

Our results clearly show that the prevalence of the *GYS1* mutation and homozygosity for the mutation was high in the North American draft and some of the German Coldblood breeds, similar to previous results for Cob Normands (Herszberg *et al.* 2009). This may be because of the dramatic reduction in population sizes after modernization of the agricultural industry (Druml *et al.* 2008; Kavar & Dovc 2008), accompanied by founder effects and subsequent inbreeding. The low or zero prevalence of the *GYS1* mutation in Shires, Clydesdales and Schleswig Coldbloods may be reflective of breed origin and/or genetic divergence. Shires and Clydesdales are of British and Scottish origin and may be more genetically distant to the other draft breeds of continental European origin. Furthermore, the Schleswig Coldblood is the most genetically divergent of the German Coldblood breeds (Aberle *et al.* 2004). It is also possible that the European draft horse sampling schemes may have introduced bias because of relatedness of individuals, population stratification or selection of healthy individuals at a performance event.

The prevalence of the *GYS1* mutation in North American Quarter Horses and Paint horses of 0.066 and 0.077 respectively is similar to those found previously by Tryon *et al.* In that study, the prevalence of type 1 PSSM was estimated to be 0.113 in a stratified population of elite competitive Quarter Horses and 0.045 in a random population of Paint horses (Tryon *et al.* 2009).

The *GYS1* mutation was not identified in the North American Thoroughbreds, Akhal-Tekes, Connemaras, Clydesdales, Norwegian Fjords, Icelandic horses, Welsh Ponies, Schleswig Coldbloods, or the European Clydesdales, Shires, or Hanoverians. However, sample sizes were low for

Table 2 Estimated Polysaccharide Storage Myopathy (PSSM) prevalence in North American and European horse breeds.

Breed/origin	Number horses with A alleles	A/A frequency	G/A frequency	PSSM prevalence	Confidence interval PSSM prevalence	
					Lower	Upper
North America[1]						
Percheron	93/149	0.067	0.557	0.624	0.545	0.699
Belgian	58/149	0.094	0.295	0.389	0.313	0.469
Paint	15/195	0.005	0.072	0.077	0.045	0.120
Quarter Horse	22/335	0.003	0.063	0.066	0.042	0.096
Appaloosa	9/152	0	0.059	0.059	0.029	0.104
Morgan	2/214	0	0.009	0.009	0.002	0.029
Shire	1/195	0	0.005	0.005	0.000	0.022
Thoroughbred	0/96	0	0	0.000	0.000	0.020
Akhal-Teke	0/50	0	0	0.000	0.000	0.038
Connemara	0/49	0	0	0.000	0.000	0.038
Clydesdale	0/48	0	0	0.000	0.000	0.039
Norwegian Fjord	0/46	0	0	0.000	0.000	0.041
Welsh Pony	0/45	0	0	0.000	0.000	0.042
Icelandic	0/36	0	0	0.000	0.000	0.052
Europe						
South German CB[2]	54/265	0.030	0.174	0.204	0.158	0.255
Saxon-Thuringian CB[2]	5/44	0.023	0.909	0.114	0.042	0.229
Shire[3]	0/33	0.00	0.00	0.000	0.000	0.057
Schleswig CB[2]	0/33	0.00	0.00	0.000	0.000	0.057
Clydesdale[2]	0/98	0.00	0.00	0.000	0.000	0.014
Hanoverian[4]	0/214	0.00	0.00	0.000	0.000	0.009

[1]Samples collected by systematic random sampling.
[2]Samples collected by stratified random sampling.
[3]Samples collected by non-random sampling.
[4]Samples collected by simple random sampling.

some of these breeds, and genotyping a minimum of 300 individuals from each breed would be necessary to have a 95% probability of detecting the *GYS1* mutation if the true frequency is less than 0.01 (Gregorius 1980). Even with less than optimum sample sizes, the upper limit of the confidence interval for mutation frequency was less than 0.03 in all breeds where the mutation was not identified, suggesting that if the mutation segregates in these breeds it is at a low frequency. The mutation was not detected in Hanoverian samples in this study, but it has previously been detected in Hanoverian horses (McCue *et al.* 2008a; Stanley *et al.* 2009), suggesting that it does segregate at low frequency in this breed.

In conclusion, the highest *GYS1* mutation frequencies were found in several draft breeds originating from continental Europe. It is also prevalent in breeds with Quarter Horse influence. We report for the first time the presence of the *GYS1* mutation in Exmoor Ponies, and Rhenish German Coldbloods. The *GYS1* mutation is rare to nonexistent in some draft breeds, as well as some light horse breeds, including Thoroughbreds, among others. In these breeds, the most applicable first diagnostic procedure to determine the cause of a myopathy remains the traditional histopathological evaluation via muscle biopsy (McCue *et al.* 2008a). Despite some study design limitations, the *GYS1* mutation frequency estimates provided in this paper provide clinically useful guidelines for veterinarians, breeders and breed associations when making genetic testing decisions.

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Conflicts of interest

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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