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Prevalence of clinical signs and factors impacting expression of myosin heavy chain myopathy in Quarter Horse-related breeds with the *MYH1*^{E321G} mutation

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Abstract

Background: The prevalence of clinical signs and factors triggering muscle atrophy and rhabdomyolysis associated with an *MYH1*^{E321G} mutation in Quarter Horses and related breeds (QH) remain poorly understood.

Hypothesis/Objectives: Determine the prevalence and potential triggers of atrophy and stiffness in horses homozygous reference (N/N), heterozygous (My/N), and homozygous (My/My) for the *MYH1*^{E321G} mutation.

Animals: Two-hundred seventy-five N/N, 100 My/N, and 10 My/My QH.

Methods: A retrospective case-control study using a closed-ended questionnaire completed by clients of the Veterinary Genetics Laboratory at the University of California, Davis. History of clinical signs, disease, vaccination and performance were analyzed by genotype using contingency testing.

Results: Atrophy occurred in proportionately more horses with *MYH1*^{E321G} (My) than N/N QH and more frequently in My/My than My/N QH ($P < .001$; My/My 8/10 [80%], My/N 17/100 [17%], N/N 29/275 [11%]). More My/My horses had rapid atrophy ($P < .001$), with recurrence in 50%. Fewer My/My horses recovered versus My/N QH ($P < .001$). Stiffness was common across genotypes ($P = .100$; My/My 4/10 [40%], My/N 18/100 [18%], N/N 48/275 [17%]). Three months before the observed atrophy and stiffness, 47% of *MYH1*^{E321G} QH were vaccinated or had respiratory or gastrointestinal disease. Horses achieving 100% expected performance did

Abbreviations: CI, confidence interval; EEE, Eastern encephalitis; GBED, glycogen branching enzyme deficiency; GYS1, glycogen synthase 1 gene; HYPP, hyperkalemic periodic paralysis; MH malignant, hyperthermia; My/My, homozygous for *MYH1*^{E321G}; My/N, heterozygous for *MYH1*^{E321G}; *MYH1*^{E321G}, glycine to glutamate substitution in myosin heavy chain gene; MYHM, myosin heavy chain myopathy; N/N, normal reference; OR, odds ratio; PSSM1, type 1 polysaccharide storage myopathy; QH, Quarter horse related breeds; VEE, Venezuelan encephalitis; WEE, Western encephalitis.

not differ across genotypes (50% My/My, 71% My/N, 55% N/N), but, only 4/10 My/My QH were competing. My/N horses achieved national or world championships or both.

Conclusion and Clinical Importance: Approximately 20% of My/N QH develop rapid atrophy. Atrophy is more common (80%) in homozygous My/My QH and less likely to resolve. Inciting causes such as vaccination and infection are inapparent in over half of cases.

KEYWORDS
atrophy, equine, muscle, rhabdomyolysis

1 | INTRODUCTION

Immune-mediated myositis in Quarter Horses is characterized by rapid onset of gluteal and epaxial muscle atrophy and the presence of lymphocytic infiltrates in muscle fibers.[1] In 2018, an *MYH1*^{E321G} mutation (chr11:52,993,878T>C, p.321 E>G) in the type 2X myosin heavy chain gene (*MYH1*) was identified as the basis for immune-mediated myositis in Quarter Horses.[2] Genetic testing at the UC Davis Veterinary Genetics Laboratory reports the alternate allele as My and the reference allele as N. Affected horses were both heterozygous (My/N) and homozygous (My/My) for the missense *MYH1*^{E321G}.[2] Subsequent research found that 67% of 111 young horses of Quarter Horse related-breeds (QH) with severe nonexertional rhabdomyolysis were also homozygous or heterozygous for *MYH1*^{E321G}.[3] These horses initially were presented with notable muscle stiffness and markedly increased serum creatine kinase and aspartate transaminase activities with or without muscle atrophy.[3] Lymphocytic infiltrates in muscle fibers were present in <18% of horses with *MYH1*^{E321G} nonexertional rhabdomyolysis.[3] To avoid confusion arising from the 2 different clinical presentations, the term myosin heavy chain myopathy (MYHM) was used to include both immune-mediated myositis and nonexertional rhabdomyolysis phenotypes associated with the *MYH1*^{E321G} variant.[3]

The proportion of My/N and My/My horses that develop MYHM is not well defined. In a study of Quarter Horses housed in the same environment as horses with immune-mediated myositis, 40% of My/N horses were asymptomatic, suggesting variable penetrance. Penetrance may be reliant upon activation of autoimmunity by vaccination or infection, as suggested by studies of clinical MYHM cases.[1,4] The need for more information on penetrance and predisposing factors is emphasized by the fact that 7%-15% of Quarter Horses carry *MYH1*^{E321G}.[5,6] Scientific studies of disease probability, performance limitations, and triggering factors for MYHM would assist owners of *MYH1*^{E321G} horses in making decisions on breeding, prepurchase and vaccination strategies.

Our first objective was to retrospectively analyze a survey of QH to determine what proportion of N/N, My/N, and My/My horses developed clinical signs of either muscle atrophy or stiffness and whether the prevalence of atrophy or stiffness differed among genotypes. Our second objective was to determine the proportion of clinical and subclinical My/N and My/My QH that experienced potential triggering factors of vaccination, strangles exposure, respiratory disease, and gastrointestinal disease within 3 mo of MYHM disease expression. Our final objective was to determine whether My/N and My/My compared to N/N QH achieved a satisfactory level of performance.

2 | MATERIALS AND METHODS

2.1 | Questionnaire

Horse owners who had submitted individual samples for genetic testing for MYHM or genetic panel tests that included MYHM to the Veterinary Genetics Laboratory at the University of California, Davis were asked if they were willing to participate in research studies. The survey was sent to 1413 owners of 2666 horses who consented by written approval to answer a retrospective questionnaire, with the goal of receiving 400 completed questionnaires. The questionnaire contained closed-ended multiple-choice questions that also allowed for additional written responses (Supporting Information S1). A link to the questionnaire was provided to owners by email and their responses were recorded online. At the end of the open questionnaire period (3 wk), the owners' responses were downloaded into an Excel spreadsheet (Microsoft Corporation) with corresponding genetic data. The questionnaire included the following sections.

2.1.1 | Reasons for testing

Participants were asked the reason for performing the genetic test, which included veterinary recommendation, to screen for disease, breeding decision, pre-purchase or sale requirement, and no particular reason.

2.1.2 | Signalment

Data on the number of horses on the farm, genotype, breed, and sex of each horse was collated.

2.1.3 | Clinical signs of MYHM

The presence of muscle atrophy over the horse's topline was ascertained as well as the date and rate at which atrophy developed (days, weeks, months, years), number of episodes and whether the horse recovered from atrophy. Muscle stiffness not associated with exercise was noted as well as the dates and severity of stiffness (impact on ability to rise), number of episodes and recovery from episodes. In an open written response, participants were given the opportunity to indicate other clinical signs they felt were associated with the *MYH1* mutation and changes they made that improved clinical signs.

2.1.4 | Vaccination and preceding diseases

Vaccination dates and type of vaccines given were specified by participants. Dates of strangles cases on the farm and of respiratory or gastrointestinal disease in the horse also were recorded.

2.1.5 | Performance

Each horse's performance discipline and level were recorded. The highest performance achievement of each horse was ascertained in an open question, along with whether the horse had met the owner's performance expectations (0%, 25%, 50%, 75%, 100% of expectations).

2.2 | Statistical analysis

Quarter Horses, Paint horses, Appaloosa horses or their crosses were retained for analysis as QH.[7] Horses were separated by genotypes into My/My, My/N, and N/N. A binary classification of multiple choice was achieved by division into majority and minority of responses based on *MYH1*^{E321G} horses. Data then was analyzed across all genotypes using 3×2 Chi-squared contingency testing. Where numbers were <5, My/N plus My/My horses were combined, and a Fisher's exact test was employed. Significance was set at $P < .05$ for single tests and $P < .02$ as a Bonferroni correction for multiple testing. Odds ratios (OR) were used for comparisons among the 3 genotypes. Analyses were performed using GraphPad Prism (version 9.2.0) and Excel (Microsoft Corporation).

The derived binary categories included: 1-20 horses on the farm and >20 horses, 0-6 y-of-age and >6 y, and "yes" or "no" for muscle loss on topline, muscle stiffness, or recovered. The number of times the horse experienced muscle atrophy was categorized as 1 or >1 episode and stiffness was classified as 1-3 times and >3 times. The proportion of horses experiencing strangles 6 mo before atrophy and proportion of horses vaccinated or affected by respiratory and gastrointestinal disease was calculated from the dates clinical signs were reported and the dates vaccines or disease were reported. To ensure adequate numbers of cases for analysis, atrophy and stiffness were combined as MYHM clinical signs when evaluating potential inciting factors such as vaccination or infectious disease. Performance type was analyzed as reining versus other, performance expectations were analyzed as 100% of the time and <100%.

3 | RESULTS

3.1 | All horses in survey

In total, 471 owners provided responses, with 418 (89%) completing all questions. Of these, 33 were of the following breeds: Andalusian, Appendix, American Warmblood, Arabian, Thoroughbred, Gypsy Vanner, Canadian horse, Draft Cross, Mustang, Haflinger, Half Arabian, Half Andalusian, Hanoverian, Oldenburg, Pony of the Americas, Sport Pony, Pinto, Paso Fino, Shire, and Percheron. None of these horses had the *MYH1*^{E321G}. The remaining 385 horses were QH comprising mares, geldings, and stallions (Table 1). There were 27 owners with 1-2 horses on the farm, 170 owners with between 3 and 10 horses and 187 owners with > 10 horses on their farm. One owner did not answer this question. Of the 385 horses, the majority were <6 y of age (Figure 1). The top 3 performance types were reining, with 72/353 responses (19%), pleasure 64/353 (17%), and ranch horses 61/353 (16%). Respondents indicated that the reason for doing genetic testing was to screen for any genetic disease 188 (49%), to make a breeding decision 176 (46%), based on veterinary recommendation 64 (17%), sale or prepurchase requirement 22 (6%), and no particular reason 11 (3%). An individual MYHM test was submitted by 208/385 (54%) owners and a genetic disease panel test was submitted by 177/385 (46%). The genetic disease panel included the muscle diseases MYHM, hyperkalemic periodic paralysis (HYPP), glycogen branching enzyme deficiency (GBED), type 1 polysaccharide storage myopathy (PSSM1) and malignant hyperthermia (MH).

3.2 | Genotypes

Three percent ($N = 10$) of horses in the survey were My/My, 26% ($N = 100$) were My/N and 71% N/N ($N = 275$; Table 1). The reason for performing the genetic test was significantly different among genotypes, with more My/My horses being tested because the participant or their veterinarian suspected the horse had signs of MYHM ($P < .001$; My/My 9/10, 90%; My/N 6/100, 6%; N/N 38/275, 14%).

3.3 | Analysis by genotype

3.3.1 | Signalment

No significant difference was found in the number of farms with ≤20 or >20 horses on the farm across genotypes (≤20 horses, $P = .15$, My/My 9/10, My/N 72/100, NN 181/275). Quarter Horses and Paints were represented in all 3 genotypes. The N/N and My/N groups also included Appaloosas and Quarter Horse crosses in similar

TABLE 1 The number of horses, sex, breed, and performance group of horses included in the study

Genotype	Horses *N* (%)	Mares *N* (%)	Geldings *N* (%)	Stallions *N* (%)	Paint *N* (%)	Quarter Horse *N* (%)	Appaloosa *N* (%)	Crosses *N* (%)	Reining *N* (%)
N/N	275 (71)	163 (59)	40 (15)	72 (26)	54 (20)	198 (72)	17 (6)	6 (2)	39 (14)
My/N	100 (26)	62 (62)	15 (15)	23 (23)	19 (19)	77 (77)	3 (3)	1 (1)	31 (31)
My/My	10 (3)	5 (50)	3 (30)	2 (20)	2 (20)	8 (80)	0	0	2 (29)
P-value 2 × 3 Chi-Square		.76	.41	.78	.99	.60	.33		.001

Note: N/N is homozygous reference.
My/N is heterozygous for the *MYH1* mutation.
My/My is homozygous for the *MYH1* mutation.

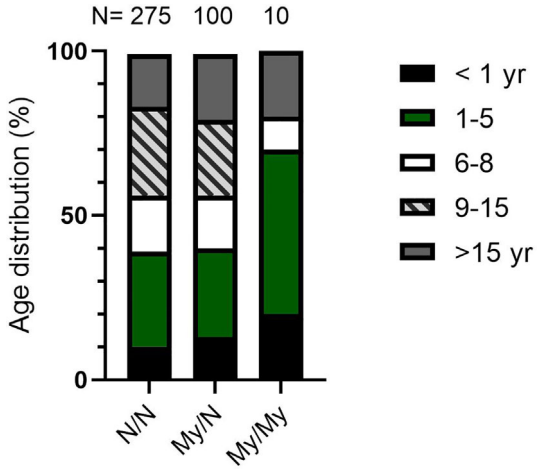

FIGURE 1 The distribution of ages (years) among horses homozygous reference (N/N, $N = 275$), heterozygous (My/N, $N = 100$), and homozygous (My/My, $N = 10$) for the *MYH1*^{E321G} mutation. There was no significant difference in the proportion of horses <6 y of age across genotypes ($P = .165$)

proportions (Table 1). Similar proportions of mares, geldings, and stallions were found across genotypes (Table 1). The proportion of horses <6 y of age was not significantly different among genotypes ($P = .17$; Figure 1).

3.3.2 | Other genetic mutations

In the full genetic panel performed for 2 My/My horses, 1 horse also had the *GYS1* mutation responsible for PSSM1.[8] The prevalence of other genetic mutations in My/N horses was PSSM1 6/34 (18%), GBED 4/34 (12%), HYPP 2/34 horses (6%), and MH 1/34 (3%). For N/N, the distribution was PSSM1 14/141 (12%), GBED 14/141 (12%), HYPP 6/141 (4%), and MH 3/141 (2%).

3.3.3 | Muscle atrophy

Muscle atrophy affected both *MYH1*^{E321G} and N/N (29/275, 11%) horses (Figure 2). Proportionately more My/My QH (8/10) developed atrophy than N/N and My/N (17/100) QH (Figure 2). The OR for developing atrophy was significantly higher for My/My compared to N/N (OR, 33.9; 95% confidence interval [CI], 7.50 to 160.90; Table 2).

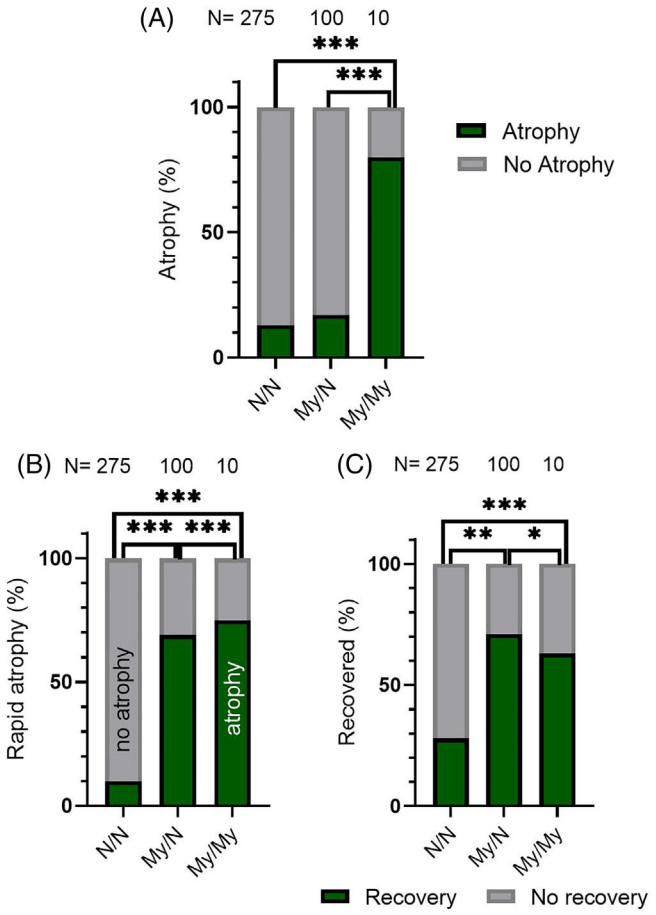

FIGURE 2 (A) The proportion of horses that developed atrophy of epaxial and gluteal muscles among 275 N/N, 100 My/N, and 10 My/My horses. Atrophy was most prevalent in My/My horses. (B) The proportion of horses with atrophy (N/N, $N = 29$, My/N, $N = 17$, My/My, $N = 8$) that had a rapid onset of atrophy across genotypes. Rapid atrophy was significantly more prevalent in My/N and My/My than N/N horses. (C) The proportion of horses with atrophy that recovered from atrophy. Recovery was more common in horses with My/N or My/My genotypes than N/N. *$P < .05$, **$P < .01$, ***$P < .001$

TABLE 2 The odds ratio, 95% confidence interval (CI) and *P* value for comparison of clinical signs of atrophy and stiffness across genotypes

	My/My vs My/N	My/My vs N/N	MY/N vs N/N
Atrophy			
Odds ratio	19.5	33.9	1.7
95% CI	3.94 to 93.95	7.50 to 160.90	.90 to 3.26
P	<.001	<.001	.11
Rapid atrophy			
Odds ratio	13.5	136	10.1
95% CI	3.568 to 46.16	25.20 to 574.6	2.862 to 34.51
P	.001	<.001	<.001
Moderate/severe stiffness			
Odds ratio	8.0	6.6	1.2
95% CI	2.039 to 35.19	1.515 to 31.33	0.4558 to 3.028
P	.02	.04	.80

Note: Significance $P < .025$, N/N is homozygous reference.
My/N is heterozygous for the *MYH1* mutation.
My/My is homozygous for the *MYH1* mutation.

The OR for developing atrophy in My/N QH versus N/N was 1.7 (95% CI, 0.90 to 3.26; Table 2). Of those that developed atrophy, rapid onset of atrophy occurred in proportionately more My/My (75%) and My/N (59%) compared to N/N QH ($P < .001$; Figure 2). The OR for My/My versus N/N to develop atrophy that was rapid in onset was 136.0 (95% CI, 25.20 to 574.6) and for My/N versus N/N the OR was 10.1 (95% CI, 2.862 to 34.51; Table 2). In N/N QH, atrophy was more likely to develop slowly over months (15/29, 52%), as compared to My/N (2/17, 12%) and My/My QH (1/8, 13%, $P = .02$).

Recovery from muscle atrophy occurred in significantly more My/My (5/8, 63%; $P < .001$) and My/N (12/17, 71%; $P < .001$) QH than in N/N QH (8/29, 28%; Figure 2). Proportionately more ($P = .01$) My/N QH recovered from atrophy than My/My horses (Figure 2). The prevalence of muscle atrophy (>1 episode) did not differ between My/My (5/8, 63%; $P = .70$) as compared to N/N (12/29, 41%) or My/N QH (5/17, 29%; $P = .53$).

3.3.4 | Non-exertional rhabdomyolysis

Muscle stiffness was present in a similar ($P = .19$) proportion of horses across genotypes (My/My, 4/10, 40%; My/N, 18/100, 18%; N/N, 48/275, 17%). The proportion of horses with stiffness reported severe enough to impact ability to rise was not different ($P = .41$) among genotypes (My/My, 3/4, 75%; My/N, 6/18, 33%; N/N, 14/48, 29%). The OR for stiffness to be moderate to severe was significantly higher for My/My vs My/N at 8.0 (95% CI, 2.039 to 35.19) and tended to be higher when compared to N/N ($P = .04$; Table 2). Of the horses developing stiffness, a similar proportion ($P = .80$) experienced complete recovery across genotypes (My/My, 1/4, 75%; N/N, 23/48, 48%; My/N, 9/18, 50%). Among the horses with stiffness, 1 My/My, 5 My/N, and 28 N/N had a genetic panel performed. The single My/My horse also had PSSM1, 1/5 My/N QH carried GBED with no other mutations in this group. For N/N horses, 1/28 had HYPP, 3/28 carried GBED, and 5/28 had PSSM1, 1 of which also had MH.

3.3.5 | Other clinical signs

In the write-in section, poor performance was reported by 3 My/My and 11 My/N owners and some of these owners also reported lethargy and change in appetite (My/My, 3/3; My/N, 5/11). Four owners of My/My horses reported muscle stiffness, tightness and tremors as other clinical signs associated with MYHM. Four owners of My/N horses reported swelling at vaccination sites and 3 reported muscle spasms and soreness as additional clinical signs of MYHM.

3.3.6 | Vaccination

None of the My/My (0/8) and 29% of My/N (7/24, 29%) horses with reported atrophy or stiffness had been vaccinated within 3 mo of clinical signs, which was not significantly different from N/N horses (11/62, 18%; $P = 1.00$). Vaccines used in the atrophied or stiff My/N horses included IM influenza/herpes virus (6/7), West Nile (4/7), Eastern, Western, Venezuelan encephalitis (EEE/WEE/VEE) (3/6), tetanus (3/7), rabies (2/7), and Potomac Horse Fever (1/7). The proportion of horses with no apparent association between clinical signs and a vaccination in the preceding 3 mo was 100% for My/My (8/8) and 67% (16/24) for My/N QH.

3.3.7 | Respiratory and gastrointestinal diseases

Two of the My/My (2/8) and My/N (3/24) horses that had atrophy or stiffness had signs of a respiratory disease in the 3 mos preceding clinical signs, which was fewer than N/N horses (5/62, 8%; $P = 0.3$). In

horses that developed clinical signs, exposure to strangles occurred in more ($P = .04$) My/N QH (My/My, 0/8; My/N, 3/24, [13%]) than N/N horses (0/62). None of the My/My and 4% of My/N (1/24) horses that experienced atrophy or stiffness had signs of a gastrointestinal disease 3 mo preceding clinical signs which was not significantly different from N/N horses (2/62, 3%; $P = 1.00$).

3.3.8 | Combined potential triggering factors

Twenty-five percent (2/8) of My/My, 46% (11/24) of My/N and 31% (19/62) of N/N experienced potential triggering factors of combined vaccination, respiratory or gastrointestinal disease. Thus, potential triggering factors were apparent in 25% of *MYH1*^{E231G} homoyzotes and 46% of heterozygotes that developed clinical signs of MYHM.

The 2 My/My QH that did not develop atrophy or stiffness were not reported to have had a respiratory or gastrointestinal disease and did receive routine vaccinations (EEE/WEE/VEE, West Nile, Influenza/Herpes virus, tetanus, rabies). Of the 76 My/N horses that did not develop atrophy or stiffness, 6 horses experienced respiratory or gastrointestinal disease and 86% (65/76) were reported to be vaccinated. Thus, combined among *MYH*^{E321G} horses that did not develop clinical signs, 79% were exposed to potential triggering factors such as vaccines or infectious disease.

3.3.9 | Performance

Significantly more ($P < .001$) My/My and My/N horses were reining horses than N/N horses (Table 1). Of the horses >2 y of age that were competing, 50% (2/4) of My/My and 71% (25/35) of My/N QH achieved 100% of performance expectations, which was significantly less ($P = .240$) than for N/N QH (49/85, 58%). The 2 successfully competing My/My horses were a western pleasure horse (American Paint Horse Association World Show) and a cutting horse (National Ranch and Stock Horse Alliance Show). The highest levels achieved by My/N horses were World Champion, National Champion, >$200 000 winnings. The N/N horses had achieved levels such as World Champion, European Ranch Champion, World American Quarter Horse Association Show top 3. Thus, horses of all genotypes were capable of high achievement, but for the small number of My/My horses in the study, only 2 My/My horses were competing successfully.

4 | DISCUSSION

Our results show that muscle atrophy and stiffness are common in QH and that, in a subset of these horses, they can be attributed to the *MYH1*^{E321G} mutation. The term MYHM is used to describe the clinical disease that can develop in horses with this mutation. We describe here, for the first time, the actual prevalence of atrophy and stiffness in horses homozygous and heterozygous for *MYH1*^{E321G} using a relatively large cohort of QH that had samples submitted for genetic testing for a variety of reasons. In addition, we evaluated the presence of factors previously implicated as triggers for developing clinical signs. Our results provide further support for a codominant pattern of inheritance for *MYH1*^{E321G}, with homozygotes having a higher prevalence of clinical signs than My/N horses. Potential predisposing factors were apparent in 25% of *MYH1*^{E231G} homoyzotes and 46% of heterozygotes.

Overall, the prevalence of *MYH1*^{E321G} in our study population was 29%, much higher than the prevalence in random samples of QH at 7%-15%.[5,6] Reining and ranch horses had the highest prevalence among performance groups at 21%-22% in agreement with other studies.[5,6] The high prevalence of *MYH1*^{E321G} QH in our study likely was influenced by the fact that 63% of participating owners performed genetic testing because their veterinarians recommended it or to screen for genetic disease, as well as by the preponderance of reining and ranch horses in our sample. The benefit of the bias toward horses with *MYH1*^{E321G} in our study, however, was that it provided 10 My/My horses to evaluate. The *MYH1*^{E321G} homozygotes were not present in random samples of QH with unknown phenotypes in previous studies and only 1 My/My horse was found in random sampling of elite performance horses, validating this approach to identify additional My/My horses essential for investigation of environmental triggers and other factors.[5,6]

Although there were more My/My horses in our study than found in random sampling studies, the number of homozygous horses was still relatively small. Nevertheless, we can conclude that clinical signs of MYHM are common in My/My horses, with 8 of 9 developing atrophy and 3 of 10 moderate to severe stiffness. Only 2/10 homozygotes in our study did not have clinical signs of MYHM. The OR for developing atrophy had wide CI influenced by the small number of homozygotes, with a 136-fold higher OR for developing rapid muscle atrophy and 6-fold higher OR for developing moderate to severe muscle stiffness in My/My compared to N/N horses. In addition, My/My horses were less likely to recover from atrophy than N/N horses. The high prevalence of atrophy in homozygotes is in agreement with 2 previous studies.[2,3] In the first study, selection of horses based on muscle atrophy and lymphocytic infiltrates resulted in inclusion of 39 My/My QH (56% of total *MYH1*^{E321G} horses in that study).[2] Selection of horses based on nonexertional rhabdomyolysis in the subsequent study resulted in inclusion of 83 My/My horses (75% of total *MYH1*^{E321G} horses).[3] Thus, although homozygosity for the *MYH1*^{E321G} mutation appears to be rare, it confers very high risk for developing clinical signs of MYHM, which may not remit.

Among the 100 My/N horses in our study, 17% were reported to have developed atrophy, a proportion not significantly different from 11% for N/N horses. Thus, it appears that most My/N horses do not develop clinical signs of muscle atrophy. This finding is in agreement with the study that identified the *MYH1*^{E321G} mutation, in which 40% of horses with no apparent clinical signs that were housed on farms with clinical cases (at-risk cohort) were My/N.[2] Instead, differences were observed in the rate of muscle atrophy in My/N and N/N horses that developed muscle atrophy. In N/N horses, atrophy took months to develop, a common feature of several disorders including lower

motor neuron disease, nutritional deficiencies and systemic disease.[9] In contrast, in affected My/N horses, atrophy largely occurred within days, with the OR for rapid muscle atrophy being approximately 10-fold higher for My/N than for N/N horses. Rapid onset muscle atrophy is a known hallmark of MYHM, with horses losing 30%-40% of muscle mass within days.[1,4] The development of clinical disease in some *MYH1*^{E321G} heterozygotes is supported by clinical studies in which heterozygotes comprised 34% of the *MYH1*^{E321G} horses that developed atrophy and 25% of *MYH1*^{E321G} horses that developed nonexertional rhabdomyolysis.[2,3] Thus, a principal finding in our study is that approximately 17% of My/N horses developed muscle atrophy and that the index of suspicion of MYHM should be particularly high when rapid onset of atrophy occurs. Chronic muscle atrophy in horses also can arise from malnutrition, cachexia, equine motor neuron disease, vitamin E-responsive myopathy, pituitary pars intermedia dysfunction and old age, and it is suspected that N/N horses likely had other clinical reasons for atrophy unrelated to immune-mediated myositis or nonexertional rhabdomyolysis.[9]

It was difficult to discern the proportion of horses that developed nonexertional rhabdomyolysis associated with *MYH1*^{E321G} in our study. Stiffness was reported to impact 40% of My/My, 18% of My/N and 17% of N/N horses with 30% of My/My, 9% of My/N and 5% of N/N horses having moderate to severe stiffness. This finding could have been impacted by the reason many owners performed genetic testing (ie, to screen for genetic diseases). In retrospect, the specific question used to evaluate nonexertional rhabdomyolysis may not have been adequately formulated. Our question queried the severity of muscle stiffness with moderate to severe indicating that the horse had difficulty in rising from recumbency, a feature of nonexertional rhabdomyolysis in young MYHM horses.[3] Many other conditions, including other myopathies and arthritis, could have resulted in an affirmative answer by owners. In fact, in horses with genetic panel test results and signs of stiffness reported, 18% of N/N horses had PSSM1 compared to the 5%-11% prevalence of PSSM1 reported in random QH samples.[10,11] The small number of My/My horses in our study also likely limited our ability to assess this clinical sign. Nevertheless, our results do show that only a small proportion of My/N horses develop moderate to severe muscle stiffness, a feature known to be associated with MYHM.[1,3,4]

Another goal of our study was to identify potential factors that trigger signs of MYHM. Two previous studies documented the presence of respiratory or gastrointestinal disease in horses with active immune-mediated myositis.[1,4] These 2 studies reported that 42% and 28% of horses with MYHM, respectively, had a respiratory infection. In the study that was based on hospitalized horses, 78% of cases had some type of infectious disease that included pathogens such as *S. equi equip*, *S. equi zooepidemicus*, *C. pseudotuberculosis*, *Anaplasma phagocytophilum*, equine herpes virus-1, and equine influenza virus.[4] In our study, 25% of My/My and 21% of My/N horses had respiratory or gastrointestinal disease before developing clinical signs. Hospitalized horses likely had more severe disease than horses in our study, and a second exposure to an infectious agent potentially also could trigger an autoimmune episode without clinical signs of infectious disease being apparent. Thus, assessing infectious triggering factors is problematic in MYHM. Infection is postulated to trigger MYHM because of shared epitopes between the altered *MYH1*^{E321G} myosin and bacteria, such as the M proteins of group A *Streptococcus* spp that initiate an adaptive immune response against type 2X fibers.[2] The ability of mimic peptides derived from different infectious agents to trigger a particular autoimmune disease in humans is postulated to vary depending on the ability of the infected individual to present various epitopes in the context of their human lymphocyte antigen molecules.[12] Thus, individual horses with *MYH1*^{E321G} may have variable susceptibility for developing MYHM depending on their individual equine lymphocyte antigen types.

Vaccines including strangles, influenza and herpes virus-1 also have been implicated as triggering factors for MYHM.[1,4] Post-vaccination idiopathic inflammatory myopathies occur rarely in human medicine and, in a small number of susceptible individuals, are believed to be related to autoimmune responses.[13] In our study, none of the My/My and 29% of My/N horses were vaccinated 3 mo before developing clinical signs. The most common vaccine administered before clinical signs developed was IM influenza and herpes virus-1, in agreement with previous findings.[4] A small number of horses may develop local swelling and muscle degeneration from adjuvants in vaccines, which could expose the altered *MYH1*^{E321G} myosin to the innate immune system. Vaccination, however, did not trigger an immune reaction in >70% of My/N horses. None of the subclinical My/My horses were vaccinated. When all potential trigger factors were combined, an inciting agent was not apparent in 54% of My/My and 75% of My/N QH. From a practical perspective, it may be prudent for owners of horses with *MYH1*^{E321G} to initially provide a minimum combination of vaccinations spaced out over 3-6 wk and carefully observe horses for local swelling, malaise, whole body stiffness and muscle atrophy. In this manner, owners can discern which if any of the vaccines are triggers for MYHM in their individual horses. In the future, an alternative vaccine or elimination of an individual vaccine may be necessary for reactive horses. If horses do show signs of MYHM, early treatment with corticosteroids at the onset of atrophy and use of dantrolene in horses with moderate to severely increased serum creatine kinase activity are recommended for horses with MYHM.[4]

The final goal of our study was to determine if a horse's performance was affected by the *MYH1*^{E321G} mutation. Four My/My horses >2 y of age were actively competing and 2 of these horses met all of their owners' expectations. Seventy-one percent of the My/N horses >2 y of age that were competing achieved all of their owners' expectations, which was not significantly lower than the 58% for N/N horses. Thus, our results indicate that My/N horses can be very successful, achieving national and world championships. Although only 2 My/My horses met owners' expectations for performance, only 4 of 10 My/My horses could be evaluated, and more research is needed to fully gauge if homozygosity more severely impacts performance compared to My/N and N/N.

In conclusion, My/My QH were relatively rare in our study population, but it was clear that horses with this genotype commonly develop rapid muscle atrophy that may not completely resolve, and

they may not reach their owners' performance expectations. Atrophy is less common in My/N horses, affecting 17% of My/N horses and the majority meet their owners' performance expectations. Inciting factors for MYHM such as vaccination or infectious disease that commonly precede clinical signs in hospitalized horses were not apparent in approximately 75% of My/My and 54% of My/N horses that developed atrophy or stiffness in our study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

The authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

An IACUC and Animal Use Form exemption for archived samples was in place for this study. An Institutional Research Board exemption was provided for the surveys administered in this study and written client consent was obtained from all participants.

HUMAN ETHICS APPROVAL DECLARATION

The authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Lewis SS, Valberg SJ, Nielsen IL. Suspected immune-mediated myositis in horses. *J Vet Intern Med*. 2007;21:495-503.
2. Finno CJ, Gianino G, Perumbakkam S, et al. A missense mutation in MYH1 is associated with susceptibility to immune-mediated myositis in quarter horses. *Skelet Muscle*. 2018;8:7.
3. Valberg SJ, Henry ML, Perumbakkam S, Gardner KL, Finno CJ. An E321G MYH1 mutation is strongly associated with nonexertional rhabdomyolysis in Quarter Horses. *J Vet Intern Med*. 2018;32:1718-1725.
4. Hunyadi L, Sundman EA, Kass PH, Williams DC, Aleman M. Clinical implications and hospital outcome of immune-mediated myositis in horses. *J Vet Intern Med*. 2017;31:170-175.
5. Gianino GM, Valberg SJ, Perumbakkam S, et al. Prevalence of the E321G MYH1 variant for immune-mediated myositis and nonexertional rhabdomyolysis in performance subgroups of American Quarter Horses. *J Vet Intern Med*. 2019;33:897-901.
6. Albuquerque ALH, Delfiol DJZ, Andrade DGA, et al. Prevalence of the E321G MYH1 variant in Brazilian Quarter Horses. *Equine Vet J*. 2021. Oct 4. doi: 10.1111/evj.13521. Epub ahead of print. PMID: 34606642
7. Petersen JL, Mickelson JR, Valberg SJ, McCue ME. Genome-wide SNP data show little differentiation between the appaloosa and other American stock horse breeds. *Anim Genet*. 2015;46:585-586.
8. McCue ME, Valberg SJ, Miller MB, et al. Glycogen synthase (GYS1) mutation causes a novel skeletal muscle glycogenosis. *Genomics*. 2008;91:458-466.
9. Valberg SJ. Muscle conditions affecting sport horses. *Vet Clin North Am Equine Pract*. 2018;34:253-276.
10. Tryon RC, Penedo MC, McCue ME, et al. Evaluation of allele frequencies of inherited disease genes in subgroups of American Quarter Horses. *J Am Vet Med Assoc*. 2009;234:120-125.
11. McCue ME, Valberg SJ, Lucio M, et al. Glycogen synthase 1 (GYS1) mutation in diverse breeds with polysaccharide storage myopathy. *J Vet Intern Med*. 2008;22:1228-1233.
12. Ercolini AM, Miller SD. The role of infections in autoimmune disease. *Clin Exp Immunol*. 2009;155:1-15.
13. Stübgen J-P. A review on the association between inflammatory myopathies and vaccination. *Autoimmun Rev*. 2014;13:31-39.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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