EQUINE

# Evaluation of allele frequencies of inherited disease genes in subgroups of American Quarter Horses

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**Objective**—To estimate allele frequencies of the hyperkalaemic periodic paralysis (HYPP), lethal white foal syndrome (LWFS), glycogen branching enzyme deficiency (GBED), hereditary equine regional dermal asthenia (HERDA), and type 1 polysaccharide storage myopathy (PSSM) genes in elite performance subgroups of American Quarter Horses (AQHs).

**Design**—Prospective genetic survey.

**Animals**—651 elite performance AQHs, 200 control AQHs, and 180 control American Paint Horses (APHs).

**Procedures**—Elite performance AQHs successful in 7 competitive disciplines (barrel racing, cutting, halter, racing, reining, western pleasure, and working cow horse) were genotyped for 5 disease-causing alleles. Age-matched control AQHs and APHs were used to establish comparative whole-breed estimates of allele frequencies.

**Results**—Highest allele frequencies among control AQHs were for type 1 PSSM (0.055) and GBED (0.054), whereas HERDA (0.021) and HYPP (0.008) were less prevalent. Control APHs uniquely harbored LWFS (0.107) and had high prevalence of HYPP (0.025), relative to AQHs. Halter horse subgroups had significantly greater allele frequencies for HYPP (0.299) and PSSM (0.155). Glycogen branching enzyme deficiency, HERDA, and PSSM were found broadly throughout subgroups; cutting subgroups were distinct for HERDA (0.142), and western pleasure subgroups were distinct for GBED (0.132). Racing and barrel racing subgroups had the lowest frequencies of the 5 disease genes.

**Conclusions and Clinical Relevance**—Accurate estimates of disease-causing alleles in AQHs and APHs may guide use of diagnostic genetic testing, aid management of genetic diseases, and help minimize production of affected foals. (*J Am Vet Med Assoc* 2009;234:120–125)

During the past 20 years, the genetic basis of 5 diseases affecting AQHs and the related APHs have been identified. In 1992, a candidate gene approach was used to identify a mutation in the sodium channel gene, SCN4A, that is responsible for potassium-induced paralysis known as HYPP.[1] Three research groups simultaneously explained LWFS after discovering a mutation in the candidate gene endothelin B receptor,[2–4] which had been previously associated with Hirschsprung disease in humans. As genetic mapping resources were developed and became available to the equine genetics community, broader scientific approaches began to accelerate the rate of discovery of disease-causing mutations in AQHs. Glycogen branching enzyme deficiency, a metabolic genetic disease that is fatal in fetal and neonatal stages, was determined to be caused by a defect in the GBE1 gene.[5] A novel mutation in peptidyl-prolyl isomerase B was found to be associated with HERDA, a progressive skin disease that typically develops between 6 months and 2 years of age.[6] One common form of PSSM, responsible for chronic exertional rhabdomyolysis, is caused by a muta-

**Abbreviations**

| | |
|---|---|
| AQH | American Quarter Horse |
| AQHA | American Quarter Horse Association |
| APH | American Paint Horse |
| GBED | Glycogen branching enzyme deficiency |
| HERDA | Hereditary equine regional dermal asthenia |
| HYPP | Hyperkalemic periodic paralysis |
| LWFS | Lethal white foal syndrome |
| PSSM | Polysaccharide storage myopathy |

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tion in glycogen synthase 1.[7] These efforts have led to the development of unambiguous DNA tests that can be used to identify affected individuals and carriers of each respective disease. Genetic testing allows veterinarians to make specific disease diagnoses and allows owners and breeders to make informed breeding decisions.

Two measures commonly used to describe the characteristics of a genetic disease are allele frequency and carrier frequency. Allele frequency is used broadly for both dominant and recessive diseases and is an estimate of the percentage of disease-causing alleles (variants) of a gene among all the alleles in the population. Carrier frequency is used specifically for recessive diseases (LWFS, GBED, and HERDA) in which a single copy of the disease-causing allele does not cause a disease phenotype. These carriers are important in that they have the potential to create diseased animals when bred to other carriers, despite never having a deficiency themselves.

Knowledge of the frequency of a disease gene allele in a population and the mode of inheritance is critical for determining the impact of a disease in a breed. Because of the large size of the AQH population, approximately 3.24 million as of 2006,[8] and the use of families to identify disease alleles, whole-breed allele frequencies are most accurately estimated with a random sampling of horses representing the entire breed. However, although estimates of allele frequency across the entire breed are important to establish, these values have practical limitations. Because of the wide variety of competitive disciplines, including barrel racing, cutting, halter, racing, reining, western pleasure, and working cow horse, the AQH breed is inherently stratified.

It is well appreciated that diseases are common to particular lines of horses; therefore, related horses have a higher risk of carrying the allele, compared with an AQH chosen at random. In many instances, a horse will have gained notoriety from succeeding in one of many possible competitive disciplines, thereby providing the impetus to breed more heavily to the particular line. When a popular breeding horse, typically a sire, produces a disproportionate number of offspring relative to the typical breeding horse, the disease allele frequency may be amplified within that line if the horse carries a disease-causing mutation. Accordingly, allele frequencies might be several times those in other competitive subgroups because of selection strategies. Thus, whole-breed allele frequencies may grossly underestimate allele frequencies within competitive subgroups.

Although AQHs were originally bred as working ranch horses, they are used for a variety of activities. Competitive events are popular pastimes among the 345,000 members of the AQHA.[8] Because popular breeding horses typically have proven their value by excelling in competitive activities, a sampling of elite AQHs provides a useful approximation of carrier frequencies within distinct subgroups of the breed. In an effort to independently establish and, in some instances, confirm published frequencies of the known disease gene alleles, the purpose of the study reported here was to estimate allele frequencies of the HYPP, LWFS, GBED, HERDA, and type 1 PSSM genes in elite performance subgroups of AQHs.

## Materials and Methods

Subgroups from 7 competitive AQH disciplines were selected and examined, including barrel racing, cutting, halter, racing, reining, western pleasure, and working cow horse. Performance records were acquired from online performance records services.[9–11] Web-based top 100 lists[9] from 2005 and 2006, as defined by money earned, were used for selecting horses for the competitive events of barrel racing, cutting, reining, and working cow horse. The AQHA top 30 lists from 2005 and 2006 halter stallions, geldings, and mares as well as top 30 lists from 2004 to 2006 western pleasure horses were used to define highly competitive halter and western pleasure horses. The top 200 racing horse list from 2005 was downloaded[11] to represent elite racing AQHs. Elite performance horse DNA samples were requested from the AQHA with an agreement of strict confidentiality to ensure sample anonymity. Only subsets of the horses from these lists were genotyped because of limited availability of DNA samples.

A random sampling of 200 age-matched AQHs from which samples had been submitted for parentage testing at the Veterinary Genetics Laboratory, University of California, Davis, was selected as a control population. The state and country of origin of all control horses as well as their parents' and grandparents' names were determined by searching the AQHA members' database. All horses were grouped according to AQHA regions 1 to 10, which represent geographically distinct areas of the United States and Canada; the term international was used to refer to horses residing in all other countries. A set of 180 APHs born in 2002 and 2003 was also selected for analysis and comparison because of the breed's close relation to AQHs and documented cases of each of the 5 diseases within the breed.

Authors (MCTP and SN) provided oversight for genotyping of all samples for HYPP and LWFS in a manner consistent with reported assays.[1,3] All samples for HERDA were genotyped by use of a published assay by authors (RCT, MJH, and DLB) at the University of California, Davis, School of Veterinary Medicine.[6] The GBED genotyping was performed by use of a published assay by authors (MCTP and SN) at the Veterinary Genetics Laboratory (90%) and by authors (MLW, MJH, and JRM) at the University of Minnesota (10%).[5] All PSSM genotypes were evaluated at the University of Minnesota by use of a published assay.[7]

Allele frequencies of the control AQH population were determined with the maximum likelihood algorithm of Boehnke[12] implemented with public domain software.[13,a] The software takes into consideration parental and grandparental data for all control horses genotyped to correct for any common ancestors within the dataset, thereby providing more accurate allele frequency estimates. Basic allele frequency and carrier frequency calculations were carried out in a spreadsheet program[b] for all AQH subgroups and APHs. Mean estimates of the number of AQHs in the entire population with a specific disease allele were calculated by use of the following equations:

$$n = [2pq] \times [\text{total population}] \text{ for recessive diseases GBED and HERDA}$$

and

$$n = [2pq + q^2] \times [\text{total population}] \text{ for the dominant disease PSSM}$$

where p represents frequency of the wild-type allele, and q represents frequency of the disease allele.[14] Because of the SE inherent in calculations of allele fre-

quency, a range of values was reported with q-SE/p+SE and q+SE/p-SE as lower and upper limits, respectively.

## Results

The sampled control population was intended to represent the genetic diversity of the breed. It was purposefully devoid of half siblings and represented an unbiased sampling in relation to popular sires and mares within 1 generation of the horses tested. On the basis of 2006 population statistics,[8] the AQH control group constituted a reasonable geographic representation of all registered AQHs. Comparison of the percentage of control horses sampled per region to the actual percentage of AQHs residing in the region revealed minimal geographic bias (**Table 1**). Most notably, AQHA region 2, constituting the Northern Rocky Mountains/Plains area, and AQHA region 9, constituting the southern states of Arkansas, Louisiana, Tennessee, Mississippi, and Alabama, were slightly overrepresented (percentage sampled per region minus percentage residing per region, > 3%) in our sampled dataset. Conversely, and to a lesser degree, AQHA region 4, made up of Kentucky, West Virginia, Indiana, Ohio, Michigan, and Ontario; AQHA region 7, constituting the southwestern United States and Hawaii; and AQHA region 10, comprised of the southeastern states of North Carolina, South Carolina, Georgia, and Florida, were slightly underrepresented (percentage residing per region minus percentage sampled per region, > 3%).

Between 45% and 65% of the samples requested from the Veterinary Genetics Laboratory from each of the 7 elite competitive Quarter Horse subgroups were available for genotyping. Of the samples available, 85% were from horses born between 2001 and 2004, illustrating the prevalence of young AQHs in the top ranks of competitive disciplines. To improve the comparison of control AQH disease allele frequencies with those of elite performance horses, all sampled control AQHs were also born between 2001 and 2004.

Data from the control groups confirmed the presence of 4 of the 5 disease alleles in the AQHs tested and all 5 disease alleles in APHs (**Table 2**). The highest allele frequencies among control AQHs were for PSSM (0.055) and GBED (0.054). The HERDA (0.021) and HYPP (0.008) alleles were less abundant in the overall population. Among the control AQHs, no horses homozygous for any of the disease alleles were detected. Lethal white foal syndrome was not detected in the AQH dataset but was highly represented among APHs at an allele frequency of 0.107. Hyperkalaemic periodic paralysis was more prevalent in sampled APHs (0.025 allele frequency), including a horse that was homozygous (H/H), which confirmed a previous report[15] that affected homozygotes continue to be produced in this breed. American Paint horses also had lower allele frequencies for GBED (0.020), PSSM (0.023), and HERDA (0.008), relative to AQHs.

Sampling of elite performance AQH populations confirmed findings from the control group (Table 2).

Table 1—Geographic regional distribution of control AQHs in a study of allele frequencies for 5 diseases.

| Region | Population | Total population (%) | Horses sampled | Sampled population (%) | Difference (%) | Sampling bias (> 3%) |
|---|---|---|---|---|---|---|
| 1 | 212,801 | 6.6 | 9 | 4.5 | 2.1 | — |
| 2 | 523,100 | 16.2 | 51 | 25.5 | –9.3 | Over |
| 3 | 354,524 | 10.9 | 25 | 12.5 | –1.6 | — |
| 4 | 213,761 | 6.6 | 4 | 2.0 | 4.6 | Under |
| 5 | 96,013 | 3.0 | 2 | 1.0 | 2.0 | — |
| 6 | 33,549 | 1.0 | 1 | 0.5 | 0.5 | — |
| 7 | 296,072 | 9.1 | 9 | 4.5 | 4.6 | Under |
| 8 | 931,318 | 28.8 | 62 | 31.0 | –2.2 | — |
| 9 | 286,130 | 8.8 | 29 | 14.5 | –5.7 | Over |
| 10 | 176,148 | 5.4 | 4 | 2.0 | 3.4 | Under |
| International | 114,879 | 3.5 | 4 | 2.0 | 1.5 | — |
| **Total** | **3,238,295** | | **200** | | | |

Table 2—Disease allele frequencies (estimated value ± SE) of control horse populations and AQH subgroups.

| Population | No. of horses | Disease allele frequencies | | | | |
|---|---|---|---|---|---|---|
| | | HYPP | LWFS | GBED | HERDA | PSSM |
| Control populations | | | | | | |
| AQH[a] | 200 | 0.008 ± 0.004 | NO | 0.054 ± 0.011 | 0.021 ± 0.007 | 0.055 ± 0.012 |
| APH | 180 | 0.025 ± 0.008 | 0.107 ± 0.016 | 0.020 ± 0.007 | 0.008 ± 0.005 | 0.023 ± 0.008 |
| AQH subgroups | | | | | | |
| Halter | 118 | 0.299 ± 0.030 | NO | 0.026 ± 0.010 | 0.004 ± 0.004 | 0.155 ± 0.024 |
| Western pleasure | 39 | 0.013 ± 0.013 | NO | 0.132 ± 0.039 | 0.064 ± 0.028 | 0.043 ± 0.024 |
| Cutting | 113 | NO | NO | 0.068 ± 0.017 | 0.142 ± 0.023 | 0.033 ± 0.012 |
| Working cow horse | 96 | NO | NO | 0.047 ± 0.015 | 0.057 ± 0.017 | 0.028 ± 0.013 |
| Reining | 97 | NO | NO | 0.016 ± 0.009 | 0.046 ± 0.015 | 0.022 ± 0.011 |
| Barrel racing | 82 | 0.006 ± 0.006 | NO | 0.006 ± 0.006 | 0.006 ± 0.006 | 0.007 ± 0.007 |
| Racing | 106 | NO | NO | NO | NO | 0.010 ± 0.007 |

NO = Not observed in the dataset.[a]

In particular, the LWFS allele remained unobserved in all of the elite AQHs tested, whereas the other 4 disease alleles could be found in multiple sampled subgroups. Of the 4 disease alleles detected in AQHs, HYPP was the most narrowly restricted in its distribution in the breed. Hyperkalaemic periodic paralysis had the highest allele frequency (0.299) of any disease within a subgroup. Sixty-two of 117 halter horses genotyped were heterozygous, and 4 were homozygous, corresponding to 56.4% of the halter horses sampled having a genotype that allowed them to transmit the disease allele to future generations (**Table 3**). Of all other elite horses tested, only 1 heterozygous HYPP carrier was detected in the western pleasure subgroup and 1 in the barrel racing subgroup, which indicated an extreme concentration of the HYPP disease allele in halter horses.

The HERDA, PSSM, and GBED alleles were distributed throughout the elite performance subgroups, with some notable concentrations for each disease. The HERDA allele, known to be prominent within cutting horse lines, was found in 32 of 113 (28.3% [Table 3]) elite cutting horses sampled, corresponding to an allele frequency of 0.142. Consistent with the recessive mode of inheritance of HERDA and the serious nature of the disease, no horses homozygous for HERDA were identified. Logically, there was high HERDA allele frequency in reining subgroups (0.046) and working cow horse subgroups (0.057), 2 disciplines that are functionally similar to cutting and often contain horses bred from common lines as judged on the basis of pedigree analysis. The western pleasure subgroup also had high HERDA allele frequency (0.064), although the sampled group was notably smaller (n = 39) and consequently had a higher relative SE than the cutting, reining, and working cow horse groups.

Polysaccharide storage myopathy allele frequency was greatest in the halter subgroup. Of 110 halter horses that were genotyped for this marker, 28 were heterozygous and 3 were homozygous, corresponding to 28.2% of halter horses tested being capable of producing affected offspring (Table 3). All other subgroups carried the PSSM allele at values less than those detected in the control population. In regards to the racing subgroup, PSSM was the only disease allele detected in the 106 horses sampled; 2 horses each had a single copy of the defective gene.

The GBED allele was most commonly found in western pleasure horses (allele frequency, 0.132) and cutting horses (allele frequency, 0.068). Glycogen branching enzyme deficiency is a recessive lethal mutation, and as expected, only heterozygous horses were identified in the dataset. Although the GBED disease allele was detected in all other subgroups except racing horses, the allele frequency as determined for each subgroup was lower than that observed in the control population.

Although the majority (259/299 [86.6%]) of horses with a disease-causing allele in this dataset only had a single known defective gene, 40 horses had > 1 disease allele. Among the control populations, 5 AQHs were heterozygous for 2 disease-causing alleles (2 with HYPP/PSSM, 2 with HERDA/GBED, and 1 with PSSM/GBED) and 8 APHs carried 2 disease alleles (4 with HYPP/LWFS, 3 with LWFS/PSSM, and 1 with LWFS/GBED). The halter subgroup was notable because 20% (22/110) of horses that were successfully genotyped at all 5 genetic loci had > 1 disease-causing allele. Twenty-one halter horses had 2 disease alleles (16 with HYPP/PSSM, 2 with HYPP/GBED, 2 with PSSM/GBED, and 1 with HERDA/PSSM), and 1 halter horse had 3 disease alleles (HYPP/PSSM/GBED). Four cutting horses (2 with HERDA/GBED and 2 with HERDA/PSSM) and a single western pleasure horse (HERDA/GBED) were heterozygous for multiple disease-causing alleles.

Whole-breed estimates of allele frequencies and an appreciation of the distribution of these alleles throughout the AQH population can be used to estimate the number of horses with known disease alleles throughout the breed. Glycogen branching enzyme deficiency, HERDA, and PSSM were readily observed in the control AQH population and in at least 6 of the 7 subgroups tested, suggesting a broad dispersal of these disease alleles throughout the breed. By use of whole-breed allele frequency estimates and a population of 3.24 million AQHs worldwide, we estimated that there are between 278,640 and 421,200 horses carrying the GBED allele, between 90,720 and 181,440 horses carrying the HERDA allele, and between 272,650 and 419,600 horses carrying the type 1 PSSM allele. Estimating the number of HYPP horses in the AQH breed was not practical on the basis of this dataset because of the low prevalence of disease within the control AQH group, the concentration of HYPP alleles within the halter subgroup, and virtual absence of the allele in other sampled subgroups.

Table 3—Observed percentages of horses carrying a disease-causing allele for whole breeds (AQH and APH) and elite competitive subgroups.

| | Affected dominant (%) | | Carrying recessive (%) | | |
|---|---|---|---|---|---|
| **Population** | **HYPP** | **PSSM** | **GBED** | **HERDA** | **LWFS** |
| AQH | 1.5 | 11.3 | 11.0 | 3.5 | NO |
| Control APH | 4.5 | 4.5 | 3.9 | 1.7 | 21.3 |
| Halter | 56.4 | 28.2 | 5.1 | 0.8 | NO |
| Western pleasure | 1.1 | 8.6 | 26.3 | 12.8 | NO |
| Cutting | NO | 6.7 | 13.6 | 28.3 | NO |
| Reining | NO | 4.3 | 3.1 | 9.3 | NO |
| Working cow horse | NO | 5.7 | 9.5 | 11.5 | NO |
| Barrel racing | 1.2 | 1.4 | 1.2 | 1.2 | NO |
| Racing | NO | 2.0 | NO | NO | NO |

*See* Table 2 for key.

## Discussion

The AQH and closely related APH breeds harbor 5 genetic diseases that have been characterized down to the level of basic, likely causative DNA polymorphisms. The present study provided estimates of whole-breed allele frequencies by use of the standard, published, commercially available DNA tests.[1–7] The AQH breed as a whole had high allele frequencies for type 1 PSSM (0.055) and GBED (0.054), with a lower, yet important, prevalence of HERDA (0.021) and HYPP (0.008). American Paint Horses were notable for a high allele frequency for LWFS (0.107) and a higher risk of HYPP (0.025 allele frequency), relative to AQHs. Because of the strong selection pressures used throughout the AQH industry, 7 subgroups representing elite competitive AQHs were anonymously genotyped to estimate subgroup frequencies. Compared with the AQH controls, halter subgroups had substantially greater allele frequencies for HYPP and PSSM, whereas cutting, working cow horse, and western pleasure subgroups had higher allele frequencies for HERDA and GBED.

The AQH breed has a relatively low frequency of the disease allele for HYPP. Because carrying a single copy of the dominant HYPP allele imparts a visible phenotype, breeders have a simple way to select against the disease, explaining the low allele frequencies in elite competitive nonhalter subgroups. The type 1 form of PSSM also has a dominant mode of inheritance and requires only a single copy of the PSSM allele to confer susceptibility to disease. Before the advent of a genetic test, selection strategies against this disease had been complicated by the fact that both environmental and management factors contribute to expression of the phenotype. High-fat, low-starch diets and controlled daily exercise ameliorate clinical signs in 75% of horses with PSSM,[16] and when breeding horses are housed at pasture and not fed concentrates, the manifestations of PSSM are typically subclinical.[17] Accordingly, horses that carry the genetic defect responsible for type 1 PSSM may have been bred heavily in the absence of indications of disease. Glycogen branching enzyme deficiency and HERDA are recessive diseases and presumably do not have a selectable phenotype associated with carrying a single copy of the defective gene. Although production of a foal affected by these diseases implies that the parents are carriers, these disease alleles are more difficult to monitor because affected foals from such matings are produced only 25% of the time and repeat matings are rare. Thus, given the number of disease alleles relevant to the breed and their different modes of inheritance, it is difficult to clearly define the overall importance of a disease or, better yet, eliminate it without the use of diagnostic DNA tests. Our data clearly indicated that HERDA (0.021), GBED (0.054), and type 1 PSSM (0.055) alleles are more prevalent in the AQH breed than the HYPP allele, and more diligent efforts to control production of affected foals will be required.

Estimates of allele frequency in the control dataset corresponded well to published reports. The overall HERDA carrier frequency of 3.5% (7/200) in the AQH control group agreed well with previous estimates of carrier frequency of 3.65% based on 1,041 unaffected horses.[6] The GBED allele frequencies of control populations (0.054 and 0.020) were comparable to an earlier report of 0.041 and 0.036 in AQHs and APHs, respectively.[18] Previous estimates of PSSM prevalence rates in the AQH breed based solely on histologic examination of muscle are between 6% and 12%,[17] and results of the present study indicated that the type 1 PSSM allele was present at a frequency of 0.055. Although results of the present study were in general agreement with those published rates, there is evidence that PSSM may have at least 2 genetic causes.[7] Therefore, the present study captures only a subset of the total genetic load responsible for this phenotype. The causative allele for LWFS was not detected in any of the AQH samples and was predominantly found in APHs, a closely related breed of horse. On the basis of the overo white-spotting phenotype associated with carrying a single copy of the disease allele, it is logical that the APH population has developed a higher frequency of the disease allele, compared with AQHs. However, evidence of multiple registered AQH mares bred to APHs producing a lethal white foal[3,19] indicates that the LWFS allele can be found in the registered AQH population, but that a larger sample population would be required to detect this low LWFS allele frequency.

The present study also clearly identified the unequal distribution of disease alleles in the subgroups of AQHs. In particular, most of the HYPP-affected horses in the breed appeared to be contained in the halter subgroup. Important frequencies of PSSM alleles were also notable in halter subgroups, although unlike HYPP, the PSSM allele is commonly found in most other subgroups tested. The cutting, reining, and working cow horse subgroups appeared to have HERDA, PSSM, and GBED disease alleles circulating through their breeding animals at various frequencies. Therefore, multiple genetic tests are relevant for breeders of these subgroups. Owners of cutting horses should be most concerned with HERDA because of the 28.3% carrier frequency among elite horses that were successful on the competitive circuit. Although this high carrier frequency was undoubtedly a function of the prominent use of popular sires, it has been hypothesized that a heterozygous genotype for the HERDA allele may confer some type of competitive advantage for cutting.[6] Proving such a hypothesis will be difficult without a firmer understanding of the genetic etiology of HERDA. Western pleasure horses constitute a less definitive subgroup of the breed, and perhaps because of this less focused quality, they have all 4 of the disease alleles at moderate frequencies. Although the GBED allele frequency was highest in this sampled subgroup, additional testing for HYPP, HERDA, and PSSM should be considered to reduce the prevalence of these diseases.

The racing and barrel racing subgroups were at the least risk of carrying the genetic diseases tested in this study. Only the type 1 PSSM allele was detected in the 106 racing horses tested. Within the barrel racing subgroup, only 4 horses were found to have disease-causing alleles. One instance of each of the commonly detected AQH disease alleles (1 with HYPP, 1 with GBED, 1 with HERDA, and 1 with PSSM) was detected among

those 4 horses. It is unclear why the racing groups had a lower frequency of disease alleles, particularly because heterozygous carriers of the autosomal recessive conditions do not presumably have a phenotype associated with them. Less intensive inbreeding strategies coupled with the use of different sire lines, compared with other subgroups, may have limited the amplification of allele frequencies in those lines. Thoroughbreds continue to be bred into the AQH lineage through the use of appendix AQHs, and consequently, it is reasonable that racing AQH subgroups may typically have more Thoroughbred genes in their lines, compared with other subgroups.

The frequency of horses with a disease-causing allele provides a convenient way for breeders and veterinarians to consider the risk associated with breeding AQHs (Table 3). For recessive diseases such as LWFS, GBED, and HERDA, these figures are simply double those of the allele frequency and are equivalent to the carrier frequency. Breeding 2 carriers will result in affected offspring 25% of the time. For dominant diseases such as HYPP and PSSM, a single allele is enough to cause disease, although horses that were homozygous for a mutation could be found in the population for both diseases. Determination of heterozygous or homozygous status for dominant diseases will inform breeders whether affected offspring are likely (50%) or guaranteed (100%), respectively, when bred to an unaffected wild-type AQH.

Mandatory testing for genetic diseases is not the standard among registries overseeing AQH and APH breeds. In 1998, the AQHA began advocating the use of DNA testing for HYPP to attempt to better inform owners regarding the carrier status of the horses they owned that were related to a popular sire associated with the disease. Despite these efforts, HYPP carriers are still allowed to breed and pass on their disease alleles to future generations, albeit with a greater amount of disclosure for all parties involved. In 2007, the rules were altered to require related horses to be tested for HYPP as well as the exclusion of homozygous (H/H) horses from registration with AQHA because those horses are more severely affected and are guaranteed to pass on the HYPP allele if bred. Given that HERDA, GBED, and PSSM are more prevalent than HYPP in the breed at large, efforts to control expansion of these allele frequencies will be even more challenging.

In the past, many breeders would conduct DNA testing only if there was a known carrier related to the horses being used for breeding. However, testing only lines or subgroups for which there are a notable number of affected horses already being produced is not an entirely logical strategy. Our data confirmed that nearly all AQH subgroups carry these disease alleles. The use of superovulation, artificial insemination, and, most recently, cloning changes the dynamics of breeding. With the availability of these techniques, only a few years are needed to pass many copies of a disease allele to hundreds or thousands of horses from a popular sire or mare. The continued use of popular breeding horses without appropriate genetic screening may therefore lead to future increases in affected foals. Thorough screening of popular horses for disease alleles is highly recommended.

Presently, most equine genetic tests are less expensive than standard CBCs routinely conducted for an ill animal. From this perspective, a relatively small amount of money and time invested allows for a definitive disease diagnosis and may pay dividends by allowing informed breeding decisions that ensure a healthier foal.

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