Polysaccharide storage myopathy- the story so far

R.J. Naylor

Comparative Neuromuscular Diseases Laboratory, Veterinary Clinical Sciences, The Royal Veterinary College, Royal College Street, London, UK.

Email: rnaylor@rvc.ac.uk

Telephone: 00 44 7815 849162

Fax: 00 44 1707 666304

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Abstract

Polysaccharide storage myopathy (PSSM) was first described in 1992 in Quarter horse, Appaloosa and Paint related breeds with clinical signs of exertional rhabdomyolysis. The disease is characterized by the accumulation of excessive glycogen and diastase resistant amylopectin polysaccharide inclusions within skeletal muscle fibres. The discovery of a mutation in the glycogen synthase 1 (*GYS1*) gene in some but not all horses with the disease suggested that PSSM represents a group of diseases with similar pathology but different aetiologies, and that the pathogenesis is more complex than initially thought. Type 1 PSSM (PSSM1) refers to horses with the *GYS1* mutation and has subsequently been identified in a large number of breeds found in Europe and the North America. Clinical presentations associated with PSSM1 can vary and increased muscle enzyme activity at rest or following exercise often accompanies PSSM1, however such changes may not be present in all cases. A diagnosis of PSSM is made on the basis of histopathology or specifically PSSM1 is diagnosed by genotyping horses

for the *GYS1* mutation. Cases usually respond well to management changes, in particular a diet low in starch and high in fat when it is accompanied by regular exercise.

Introduction

Exertional rhabdomyolysis is a syndrome of muscle damage that is usually precipitated by exercise. Once considered a single disease entity, it is now understood to represents a common clinical presentation of several very distinct disease processes (Valberg et al. 1999).

First described by Valberg et al. in 1992 in Quarter horse and Appaloosa related breeds with exertional rhabdomyolysis, PSSM has subsequently been identified in a number of different breeds found in Europe and the United States (Valentine et al. 2000; McCue et al. 2006).

Polysaccharide storage myopathy is characterized by the accumulation of excessive glycogen and diastase resistant amylopectin polysaccharide inclusions within skeletal muscle fibres (Valberg et al. 1992) (Figure 1). Unlike normal glycogen stored in muscle fibres these polysaccharide inclusions are resistant to digestion with diastase and therefore are not broken down in the normal manner. The discovery of a mutation in the glycogen synthase 1 (*GYS1*) gene in some but not all horses with the disease (McCue et al. 2008) suggested that PSSM is in fact a group of diseases of different aetiologies, and that the pathogenesis is more complex than initially thought. As not all horses with PSSM possess the *GYS1* mutation (McCue al. 2008); this led to the disease being re-classified as type 1 PSSM (PSSM1) referring to individuals that possess the gene mutation, and type 2 PSSM (PSSM2) for individuals that have the characteristic histopathology in their skeletal muscle but do not possess the mutant allele. Many of the earlier studies of horses with PSSM performed by Dr Valberg and co-workers are now understood

to have involved horses with the *GYS1* mutation and therefore refer to PSSM1 (Valberg-personal communication). It remains possible that the polysaccharide inclusions in horses with PSSM2 may be a common end-point of several different pathological processes. As more is understood about this subset of horses without the *GYS1* mutation several abnormalities of glycogen metabolism may be identified and this group subdivided further, as with human glycogen storage diseases. This paper reviews our current understanding of type 1 PSSM (PSSM1; horses with the *GYS1* mutation) for which a definitive test is currently available.

Skeletal muscle pathology

Histopathology of muscle from horses with PSSM1 reveals excessive glycogen alongside abnormal non-lysosomal bound polyglucosan bodies containing less highly branched glycogen with protein aggregates (Valentine et al. 2001; Annandale et al. 2004; McCue et al. 2009). The presence of subsarcolemmal vacuoles, predominantly in type 2 muscle fibres (those with a propensity for glycolytic metabolism) are also a common finding (Valberg et al. 1992) (Figure 1). No disruption of the important membrane associated protein dystrophin has been identified in affected horses (Naylor et al. 2012). Non-specific chronic myopathic changes such as internalised nuclei and variation in muscle fibre size are consistent with previous muscle damage and regeneration. Affected horses also have a shift in muscle fibre type from type 2x to type 2a fast twitch fibres (Naylor et al. 2012). As the disease affects type 2 muscle fibres, muscles that contain a high proportion of these fibres such as the semimembranosis or gluteal muscle are usually selected for biopsy. The severity of histopathological abnormalities will likely reflect the fibre type proportion of a particular muscle. It is intriguing that

similar muscle pathology has recently been described in a large number of marine mammals, although unsurprisingly the clinical histories for these species are unknown (Sierra et al. 2012).

How the characteristic polysaccharide inclusions relate to the clinical signs in patients with PSSM is unclear. There is evidence to support a metabolic defect leading to a reduction of energy availability within affected muscle fibres (Annandale et al. 2005). This may explain the observation of clinical signs in young foals with the disease in the absence of extensive change on muscle biopsy (Byrne et al. 2000; De La Corte et al. 2002), Therefore the polysaccharide inclusions may be a co-incidental marker of the disease rather than causative. In addition, it has been proposed that the physical presence of the polysaccharide inclusions and subsarcolemmal vacuoles may disrupt the arrangement and function of the myofibrillar proteins and their attachments, a theory supported by the correlation between the severity of histopathology and muscle enzyme activity (Naylor et al. 2012). It is difficult to reconcile the clinical improvement observed in response to management changes if physical interference was solely responsible for the clinical signs observed, as the polysaccharide inclusions do not change with such husbandry modifications.

Aetiology of PSSM

In contrast to Thoroughbred horses with Recurrent Exertional Rhabdomyolysis (RER) no abnormality of muscle contracture was detected in *in-vitro* studies of muscle from horses with PSSM, suggesting a different pathogenesis of the two diseases (Lentz et al. 1999). The absence of any derangement in the ability of

affected horses to utilize glycogen or produce lactate during exercise led to the suggestion that PSSM results from abnormal glycogen storage rather than a defect affecting glycogen utilization (Valberg et al. 1999). This is also supported by the finding that the greatest increases in CK activity were observed in PSSM horses performing submaximal rather than maximal exercise (Valberg et al. 1999). Studies evaluating the role of insulin sensitivity in this abnormal glycogen storage disease have yielded conflicting results in different breeds of horses (Annandale et al. 2004; Firshman et al. 2008). Quarter horses with PSSM were shown to have increased insulin sensitivity relative to control horses (De La Corte et al. 1999; Annandale et al. 2004) whilst no difference was found between Belgian draught horses and controls (Firshman et al. 2008). These differences may reflect inherent breed differences in other genes regulating insulin sensitivity or differences in muscle fibre type proportions (Firshman et al. 2008). Suggesting that mechanisms other than heightened insulin sensitivity may be resulting in the accumulation of abnormal polysaccharide within skeletal muscle of some horses with PSSM1.

There are eleven skeletal muscle glycogenoses recognized in humans, many of which produce histopathological features similar to PSSM. These diseases are associated with autosomal recessive defects in specific enzymes of glycogen metabolism, lysosomal abnormalities or defects of AMP-dependent protein kinase (DiMauro and Lamperti 2001). The clinical phenotypes observed in some of these diseases are similar to those seen in horses with PSSM1. Initial work into PSSM in the horse logically focused on evaluating the activity of these key enzymes in affected horses, however no abnormalities were identified (Valberg et al. 1998). Similarly no difference in the content of the major insulin sensitive glucose

transporter GLUT4 in muscle from affected horses was found (Annandale et al. 2004), although more recently many more GLUT receptors have been identified in the skeletal muscle of horses, such as GLUT8 and GLUT12 (Lacombe 2014), and their role in PSSM is yet to be evaluated.

Enhanced glycogen synthesis was suggested when horses with PSSM were shown to re-synthesise glycogen more rapidly following exercise depletion than normal horses (De La Corte et al. 1999b). As normal glycogen synthesis is under the control of two enzymes; glycogen synthase and glycogen branching enzyme, an alteration in the activity of one of these enzymes would likely affect the relative branching of the glycogen molecule formed leading to an altered 3-dimensional structure (Annandale et al. 2004) that may impart a resistance to digestion with diastase.

Type 1 PSSM

In 2008, an autosomal dominant, gain of function, mis-sense mutation (R309H) in the glycogen synthase 1 gene (*GYS1*) was identified in association with many but not all cases of PSSM (McCue et al. 2008). Glycogen synthase is an enzyme responsible for the production of glycogen, by joining glucose monomers via alpha 1,4 linkages, under the influence of insulin and glucose-6 phosphate. This point mutation leads to a single amino acid substitution, from arginine to histidine that results in increased glycogen synthase activity (McCue et al. 2008).

To date the *GYS1* mutation has been identified in a large number of breeds across Europe and North America (McCue et al. 2008b; McCue et al. 2009; Stanley et al.

2009b; McCue et al. 2010; Johlig et al. 2011; Schwarz et al. 2011; Herszberg, et al. 2009; Baird et al. 2010) (table 1). A particularly high prevalence has been identified in Quarter horses, Percheron and Belgian draft horses (McCue et al. 2008b), whilst to the authors knowledge it remains to be identified in a pure bred Thoroughbred horse. Given that genotyping affected horses has only been commercially available for the last five years, it is highly likely that as more cases are genotyped we can expect to find the mutation in a greater range of breeds. The particularly high prevalence in hardy draught breeds led some authors to suggest that the disease phenotype may have imparted an evolutionary advantage in these breeds, which is partly supported by a recent hereditary study (McCoy et al. 2014).

Clinical presentation

Whilst there is a distinct correlation between the *GYS1* genotype and the severity of histopathology (Naylor et al. 2012) there remains considerable variation between the clinical signs associated with PSSM1, from exertional rhabdomyolysis to vague signs of poor performance, suggesting that other genetic and environmental factors may act to modify the disease phenotype (Valberg et al. 2011). McCue and co-workers have shown that the ryanodine receptor (*RYR1)* mutation associated with malignant hyperthermia leads to a more severe phenotype in Quarter horses with PSSM1 (McCue et al. 2009b), whilst environmental factors such as diet and exercise are known to attenuate clinical signs (Firshman et al. 2003; Ribeiro et al. 2004; Borgia et al. 2010). It is plausible that many of the previously suggested acquired causes of rhabdomyolysis, such as hormonal imbalances or anti-oxidant deficiencies may exert an effect by modifying the phenotype of an already genetically susceptible individual.

Whilst many horses with PSSM1 are asymptomatic (Johlig et al. 2011; Naylor et al. 2012), exertional rhabdomyolysis is the most commonly and perhaps easily recognized clinical syndrome. Exertional rhabdomyolysis was reported in over 90% of affected cases in one study of horses where biopsies were obtained to investigate poor performance (McCue et al. 2009). In one family of Warmblood horses 59% of those with the *GYS1* mutation also had a history of exertional rhabdomyolysis (Johlig et al. 2011), and those with the mutation were 7.1 times more likely to show signs of exertional rhabdomyolysis than those without. However more subtle clinical signs may easily be over-looked and these include poor performance, muscle fasciculations, muscle atrophy, gait abnormalities, generalized or pelvic limb stiffness, undiagnosed lameness, paresis or back pain (Quiroz-Rothe et al. 2002; McCue et al. 2009). Interestingly PSSM affected horses are often described as having a calm demeanor (Valberg et al. 2011).

Shivers

Early reports suggested a possible link between PSSM and the incidence of Shivers, a neuromuscular condition characterized by a reluctance to lift the pelvic limbs, and to back-up, often associated with fasciculations of the musculature of the pelvic limb and tail (Firshman et al. 2005). This was supported by the high prevalence of weakness in horses with PSSM and a report of two Belgian horses with weakness and Shivers that were diagnosed with severe PSSM on histopathology of skeletal muscle at post-mortem examination (Valentine et al. 1999). However no association between the two conditions was identified in two larger studies of 103 Belgian draught horses (Firshman et al. 2005) or 132

205 Warmblood horses (Hunt et al. 2008). It appears that both PSSM and Shivers are
206 neuromuscular disorders that commonly occur in similar breeds of horses,
207 occasionally concurrently, and that there is no causative relationship.
208
209 **Cardiac disturbances**
210 Human glycogenoses are often associated with specific cardiac phenotypes that
211 contribute to exercise intolerance. In particular enhanced atrio-ventricular
212 conduction leading to arrhythmogenesis and cardiac failure are seen (Arad et al.
213 2005; Soliman, et al. 2008). Given that polysaccharide inclusions have been
214 reported in the myocardium of affected horses at post-mortem examination
215 (Valentine et al. 1997; Valentine et al. 2001; Larcher et al. 2008) and that sudden
216 death has been described in horses with PSSM, further investigation of the cardiac
217 phenotype of horses with PSSM1 was performed (Naylor et al. 2012b). No
218 significant structural changes or arrhythmias were detected in affected horses
219 when compared with matched controls (Naylor et al. 2012b).
220
221 **Diagnosis**
222 Resting muscle enzyme activity may be used to screen for subclinical muscle
223 damage in possible PSSM1 cases, however increases in basal creatine kinase (CK)
224 and aspartate transferase (AST) activity may not be observed in affected horses,
225 particularly in those that are heterozygous for the *GYS1* mutation (Naylor et al.
226 2012). Measuring skeletal muscle enzyme activity following 20 minutes of
227 submaximal exercise (e.g. trot and canter work) may increase the sensitivity of
228 this assessment, particularly for horses with signs of exercise intolerance (Valberg
229 et al. 1999). A significant difference was observed between horses with PSSM1

and controls in CK activity at 4 hours post-exercise but not AST activity 4 or 24 hour post-exercise in one study of Belgian draught horses (Naylor et al. 2012), whereas significantly higher post-exercise AST activities were observed in horses with PSSM1 relative to controls in an earlier study of Haflinger horses (Schwarz et al. 2011). These studies suggest that there maybe breed differences in muscle enzyme responses to exercise in horses with PSSM1 or may simply reflect small sample sizes. Furthermore the changes in muscle enzyme activity following exercise may be relatively small (increases of less than 50% above resting levels) compared to those typically seen in other diseases such as RER and importantly there is considerable overlap between the response of unaffected control horses and those heterozygous for the *GYS1* mutation. Therefore raised muscle enzyme activity should increase the index of suspicion of a myopathy and prompt further investigations such as muscle biopsy or GYS1 genotyping. However, PSSM cannot be excluded in the absence of large changes of muscle enzyme activity following exercise.

A diagnosis of PSSM has traditionally been made on histopathology of muscle biopsy samples, however this technique is unable to clearly differentiate between PSSM1 and PSSM2. The identification of the *GYS1* mutation has allowed the development of a restriction fragment length polymorphism (RFLP) assay to diagnose type 1 PSSM (McCue et al. 2008). This is performed on DNA extracted from EDTA whole blood samples or hair roots (approximately 30 required- easily collected from the mane or tail). This assay is a less invasive method for testing for PSSM1 than the traditional muscle biopsy, and is particularly useful in breeds known to have a high prevalence of the *GYS1* mutation. The blood test may also be

255 useful in younger individuals where changes on histopathology are fewer, as it is
256 known that the severity of the polysaccharide accumulations increases as an
257 animal ages (De La Corte et al. 2002). Furthermore genotyping affected animals
258 may provide useful prognostic information for making decisions with regards to
259 training and breeding, as it has been shown that the severity of the skeletal muscle
260 pathology correlates with the number of copies of the mutant allele with
261 homozygotes having more severe histopathology than those heterozygous for the
262 *GYS1* mutation (Naylor et al. 2012).
263
264 It is often useful to consider the breed of the animal when deciding which
265 diagnostic test(s) to perform. In breeds with a particularly high prevalence of
266 PSSM1 such as Draught and Quarter horse related breeds it may be preferable to
267 genotype the horse for the *GYS1* mutation initially. A particularly high prevalence
268 is found in continental European breeds, such as the Percheron, whilst the
269 prevalence in UK derived breeds such as the Clydesdale or Shire is much lower
270 (McCue et al. 2010). Conversely in breeds with a lower prevalence of the *GYS1*
271 mutation, such as Cobs and Welsh ponies or indeed those where the mutation has
272 yet to be described such as Thoroughbred horses, that would more likely have
273 PSSM2 or another myopathy, a skeletal muscle biopsy remains the most
274 appropriate diagnostic test currently available. Skeletal muscle biopsy samples
275 should be harvested from the *gluteal* or the *semimembranosis* muscles, and are
276 easily obtained from the standing sedated animal (Ledwith and McGowan 2004).
277 Biopsy samples are best preserved when placed in an empty sterile pot and
278 transported immediately on ice packs to the laboratory (Stanley et al. 2009). It is
279 recommended to liaise with the diagnostic laboratory prior to collecting the

biopsy and avoid posting samples at the end of the week, to avoid unnecessary delays in transport.

Treatment of PSSM

The aim of managing horses with PSSM is to limit the constant synthesis of glycogen within skeletal muscle by reducing circulating insulin and promoting glycogen metabolism through regular exercise. In addition an alternative energy source such as fat can be provided as long as the horse is not overweight. These recommendations are based on research performed in horses with PSSM1. To date there are no controlled studies in horses with PSSM2 although it is assumed that similar recommendations apply. Regular daily exercise in addition to pasture turnout is advised, in conjunction with a diet low in starch and sugar (<10% digestible energy (DE) as non-structural carbohydrates (NSC)) and relatively high in fat (13-20% DE) (Ribeiro et al. 2004; Borgia et al. 2010). Horses should continue to receive 1-2% of their bodyweight as forage, ideally with a low (<12%) NSC content (Borgia et al. 2011) and in some cases, depending on workload and energy requirements, further caloric supplementation may not be required. Grazing may need to be restricted at certain times of the year when the NSC content of grass is particularly high. There are specifically formulated commercial diets available (such as Dodson and Horrell ERS Pellets or Saracen ReLeve), although adequate fat will only be provided if fed in quantities recommended by the manufacturers. Alternatively a low starch diet may be supplemented with vegetable oil, up to a maximum of 1ml/kg to provide sufficient calories. In some cases diets with a slightly lower fat content may be more palatable yet still be sufficient to control

the condition. A high lipid diet increases the requirement for anti-oxidants, therefore a feed balancer containing vitamin E may be beneficial.

The prognosis is favorable in cases where dietary and exercise recommendations are followed, and these horses are significantly more likely to have an improvement in the severity and frequency of clinical signs relative to those cases where only one (exercise of dietary) recommendation is followed (Firshman et al. 2003). A clinical improvement may be observed within 6 weeks although complete adaptation to these diets likely takes several months (Ribeiro et al. 2004).

Type 2 PSSM

Horses with PSSM2 are not easily distinguishable from those with PSSM1 on the basis of clinical signs and histopathology, although subtle differences in the morphological appearance of polysaccharide inclusions have been suggested (McCue et al. 2009). Fine granular, often diastase negative, inclusions are frequently located close to the sarcolemma in PSSM2 as oppose to the coarse granular diastase positive granules frequently observed in PSSM1 (McCue et al. 2008; McCue et al. 2009). Type 2 PSSM may be a result of one sole enzymatic defect, or perhaps more likely may reflect a group of glycogen storage diseases, with a similar histopathological end-point. Further work is needed to further elucidate the pathophysiologic process(es) involved in these horses.

Conclusion

Polysaccharide storage myopathy is a disease seen in a variety of breeds throughout the UK and Europe as well as North America. The particularly high prevalence of PSSM1 in some Draught breeds likely reflects a prior evolutionary advantage. The recent identification of the *GYS1* mutation could allow for eradication of the disease from these breeds by the implementation of coordinated breeding programmes. This remains controversial, however, in those breeds with a low incidence of clinical signs. The clinical presentation can vary as can muscle enzyme activity in affected horses, therefore muscle biopsy and genotyping for the *GYS1* mutation is required to establish a definitive diagnosis. Whilst no specific treatment is currently available affected horses usually respond well to management changes, in particular a low starch high fat diet in conjunction with regular exercise. With appropriate management the prognosis is favorable.

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502 Table 1: Breeds in which the *GYS1* mutation has been identified in to date.

Breed
Quarter Horse
Paint
Appaloosa
Warmblood
Haflinger
Morgan
Mustang
Rocky Mountain horse
Belgian draught
Percheron
Shire
Suffolk Punch
Hanoverian
Rhinelander
Cob Normand
Connemara x Welsh pony
Connemara x Thoroughbred
Cob
Argentinian polo pony
Polo pony
South German Coldblood
Saxon-Thuringian Coldblood

Exmoor pony

Continental European draught breeds e.g. Ardenner, Belgian trekpaard

Crossbreds

503

530

Figure 1: Characteristic skeletal muscle histopathology in type 1 PSSM compared with muscle from a matched control stained with a) haematoxylin and eosin showing sub-sarcolemmal vacuolation (arrow) and marked variation in fibre size, b) periodic acid Schiff (PAS) showing increased glycogen accumulation (arrow) and c) periodic acid schiff following predigestion with diastase revealing abnormal diastase-resistant polysaccharide (arrow). ×20 magnification.