THESIS

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Genetics of the tying-up syndrome in warm-blooded horses

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Abbreviations

AMP Adenosine monophosphate

AST Aspartate Aminotransferase

ATP Adenosine triphosphate

CBC Complete blood count

CK Creatine Kinase

CPK Creatine Phosphokinase

DHPR Dihydropyridine receptor

DNA Deoxyribonucleic acid

EDM Equine Degenerative Myeloencephalopathy

ER Exertional Rhabdomyolysis

ERS Exertional Rhabdomyolysis Syndrome

GBED Glycogen Branching Enzyme Deficiency

HYPP Hyperkalemic Periodic Paralysis

IMM Immune Medicated Myositis

IMP Inosine monophosphate

MFM Myofibrillar Myopathy

MH Malignant Hyperthermia

MHS Malignant hyperthermia susceptibility

MYHM Myosin Heavy Chain Myopathy

NSC Non-structural carbohydrates

PSSM Polysaccharide Storage Myopathy

PSSM1 Polysaccharide Storage Myopathy type 1

PSSM2 Polysaccharide Storage Myopathy type 2

RER Recurrent Exertional Rhabdomyolysis

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1. Introduction

“Tying-up is a syndrome or description of a horse with muscle damage that has many different causes. It probably is one of the most misunderstood and controversial syndromes in the athletic horse.”[1]

The tying up syndrome, also known as exertional rhabdomyolysis, is a condition that affects horses, particularly warm blooded breeds. It is characterized by the breakdown of muscle fibers and the release of myoglobin. This can lead to muscle cramping, stiffness, pain and kidney diseases in severe cases.

“Tying-up or Exertional Rhabdomyolysis (ER) was previously known as Monday-morning disease.”[2] “The first descriptions symptoms of so called Monday morning disease in continental Europe were given in 1840 for cold blood type horse by the French army veterinarian Berger.”[2] “Monday morning disease was associated with work horses that was given a day of rest after a week of hard work. When the horses were supposed to return to work on the following Monday, they developed stiffness and pain in the hindquarter musculature, and reluctance to move.” [3] “The classical form of the disease, Monday morning disease, occurs in horses at work or in training that are rested for one or more days while receiving full grain rations. These horses may tie-up as soon as they are started back to work.” [4]

In 1926 Hutyra and Marek reported a second clinical picture of tying-up - a post exercise myopathy in racehorses that decades later was titled ‘tying up’ by Meginnis. [2]

“Tying-up is not a single disease, but a collection of clinical signs or symptoms that may have several causes. Horses may have similar symptoms, but the actual cause of muscle cramping may be different in each horse.”[5] It “is an intermittently occurring condition that primarily affects the muscles of horses, resulting in clinical signs ranging from slight stiffness to immobility.”[6] Tying-up is a synonym for various kinds of muscle disorders.

“There has been much confusion over terminology of equine exercise induced rhabdomyolysis. Because of the association with exercise, these problems are now referred to as Equine Exertional Rhabdomyolysis or Equine Rhabdomyolysis Syndrome (ERS)”.[2]

ER or ERS are myopathies that can occur sporadic or chronic.

Figure 1 gives an overview about the classification of muscle disorders.

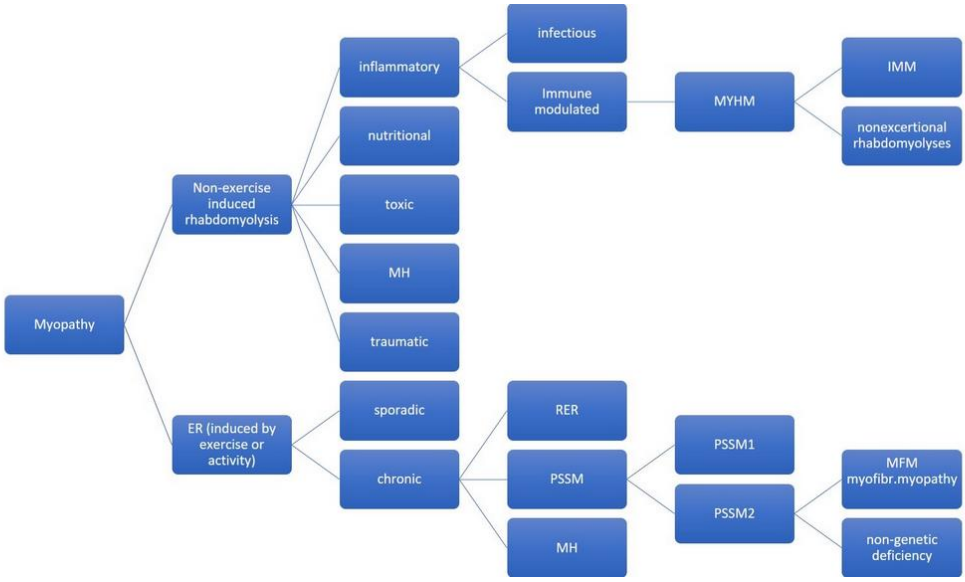

Figure 1: Classification of muscular diseases in horses related to tying-up (own depiction)

Myopathies in horses are a heterogeneous group of muscle disorders that are either induced by activity or exercise or are noticed as non-exercise related rhabdomyolysis including toxic, nutritional or inflammatory myopathies and also traumatic injuries.

"Four forms of chronic tying-up have been identified on the basis of muscle biopsies or genetic testing: type 1 polysaccharide storage myopathy (PSSM), type 2 PSSM, malignant hyperthermia (MH) and recurrent exertional rhabdomyolysis." [7]

Ongoing research led to genetic tests for malignant hyperthermia (MH), polysaccharide storage myopathy type (PSSM 1) and myosin heavy chain myopathy (MYHM). For diseases like PSSM2, myofibrillar myopathy and RER elucidation of accurate phenotype characterisation and the development of genetic tests is under investigation.

Symptoms of tying up raise numerous questions for owners, breeders, veterinarians and others related to a horse. Knowledge about incidence, risk factors, mechanisms to avoid the disease or erase the symptoms, about treatment and possible limitations for breeders are essential.

2. Relevance of genetic research for ER/ERS and aims of this thesis

Aetiology research remains problematic and protracted, particularly in the case of multifactorial diseases with dramatic clinic manifestations and potentially fatal consequences. Tying up is a syndrome that is still being studied intensively.

"High costs, prolonged duration of studies, poor breeders' cooperation, long gestation time,

and uniparous nature limit, in most cases, the investigation of inherited diseases of horses. To study the mode of inheritance in the horse, it would be ideal to identify susceptible individuals, have access to their pedigrees, and screen their relatives." [8]

"Today, horses are used primarily for race, show, and pleasure."[5] "Diseases affecting any muscle group can influence performance and intended function."[9] "Despite the changes in breeds and usage, tying-up still persists as the most common muscle problem in horses."[5]

Horses, considered as supreme athletes, depend on their musculature, which approximately compromises up to 55% of their body mass. [10] Consequentially the focus on specific physical and performance traits increased the frequency of heritable diseases.[10] "The impact of even minor or intermittent perturbations in muscle structure, contraction, and energy metabolism is readily apparent in horses owing to the rigorous expectations of athletic performance."[10]

Genetic muscle disorders are defined as a dysfunction of muscle fibres due to substance storage in sarcoplasm or due to single biochemical abnormality caused by gene mutation. [11]

"Although exertional rhabdomyolysis was previously considered a single disease described as azoturia, tying-up, or cording up, it is now known to comprise several myopathies, which, despite similarities in clinical presentation, differ notably in etiopathogenesis." [12]

"Muscle disorders encompass a wide variety of clinical manifestations that range from exertional or non-exertional rhabdomyolysis, myalgia, myotonia, myasthenia, stiffness, fasciculations, postural and gait abnormalities, exercise intolerance, muscle atrophy and others." [13]

"Myopathies can involve the disruption of metabolism of glycogen and lipids (e.g. channelopathies, myasthenic syndromes), sarcoplasm and cytoskeleton structure (e.g. dystrophies, myofibrillar), and mitochondrial function." [13]

Inflammatory myopathies include infectious and immune modulated (e.g. primary/genetic or acquired) causes. [14]

"Furthermore drugs and toxicants (e.g. plants and chemicals) can also cause muscle dysfunction." [13]

This thesis aims to summarize the current state of knowledge about muscular disorders in horses described as tying up syndrome with specific focus on the heritable diseases RER, PSSM type 1 and 2, MH, MFM and partly MYHM. Genomic research, clinical symptoms, therapy and methods of prevention of these hereditary muscular diseases in warm-blooded

horses will be described. A short guide has been prepared for each of the diseases discussed in this thesis.

3. Diseases causing Tying-up

"Exertional rhabdomyolysis syndrome is recognised in many athletic horse breeds and in recent years specific forms of the syndrome have been identified." [15]

RER, MH and PSSM 1 and 2 as well as MFM belong to the group of chronic exertional myopathies while malignant hyperthermia and MYHM as well as nutritional, toxic or traumatic myopathies occur as non-exertional diseases. To which extent all of the previously described muscle diseases have an underlying genetic basis is still under investigation.

ER describes a painful dissolution of muscle cells with exercise that is induced by different cellular malfunctions.

Figure 2 gives an overview of the cellular locations of various muscle disorders:

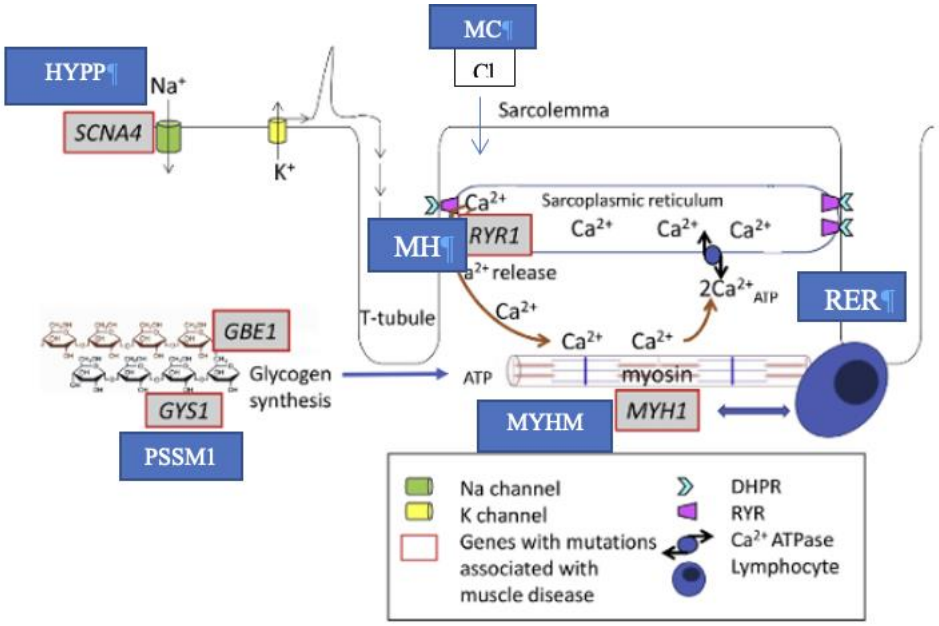

Figure 2: Cellular locations of various muscle disorders [11]-modified

"The cellular locations of the 5 monogenic mutations that cause muscle disease in horses. The mutation causing hyperkalemic periodic paralysis (HYPP) occurs in the SCNA4 gene encoding the sodium channel, the mutation causing glycogen branching enzyme disease (GBED) occurs in GBE1 encoding glycogen branching enzyme, the mutation causing PSSM1 occurs in GYS1 encoding glycogen synthase, the mutation causing malignant hyperthermia occurs in

RYR1, and the mutation causing myosin heavy chain myopathies (MYHM) immune-mediated myositis and nonexertional rhabdomyolysis occurs in MYH1 encoding the type 2X myofiber myosin heavy chain. DHPR, dihydropyridine receptor." [11]

"The publication of the equine genome sequence in 2009 has provided a major advance towards an improved understanding of equine muscle physiology." [16] Furthermore, the complete sequencing of the equine genome is a major advance that will impact the understanding of equine muscle physiology. The rapid technological advances in equine genomics have enabled gene expression profiling to explore muscle responses to exercise and training in equine athletes, to identify muscle related candidate genes useful in early performance evaluation and to detect genomic markers of inherited muscle diseases. [16]

The international equine genome research is mainly concentrating on the bases of these heritable diseases – affecting the skeletal muscle – to develop DNA-based diagnostic tests to reduce or eliminate these genes and provide customized treatments. [10]

Progress in understanding the genetic bases of equine muscle diseases has led to identification of the affected mechanisms of muscle contraction. Knowledge of the exact mechanisms of muscle damage can help to develop new individual therapies as well as management plans for the treatment of affected horses.

3.1. Recurrent Exertional Rhabdomyolysis (RER)

"Recurrent exertional rhabdomyolysis occurs frequently in Thoroughbreds, Standardbreds, and Arabian horses that are in high-stress environments. It is likely due to abnormal regulation of intracellular calcium in skeletal muscles with acute episodes of rhabdomyolysis triggered by stress." [12]

"Recurrent exertional rhabdomyolysis (RER) is a myopathy that is observed in horses during exercise activities such as a training session after a day of rest." [17] "The terminology ´RER` has been proposed to define a chronic rhabdomyolysis observed in exercising Thoroughbreds with aberrant myofibre calcium regulation and caffeine-hypersensitive muscle." [17] "In Standardbreds and French Trotters, RER is one of the most common myopathies related to exercise. In RER cases, some risk factors have been observed, including young age, female, excitable behaviour and a high energy diet, and external risk factors such as cold temperature, humidity and return to work after a rest period." [17]

3.1.1. Definition of RER

"Equine exertional rhabdomyolysis (ER), often referred to as 'tying-up', is a clinical syndrome characterized by painful muscle contractures following exercise as well as skeletal muscle fiber necrosis. Most horses with repeated episodes of ER are believed to have a specific disease, termed recurrent exertional rhabdomyolysis (RER)." [18]

"RER is the most common muscular disorder in Thoroughbred and Standardbred breeds. Five to ten percent of Thoroughbred and Standardbred horses develop exercise-induced rhabdomyolysis at some point during a racing season." [10]

"Approximately 3% of exercising horses are reported to have had an episode of ER in the last 12 months. The prevalence of ER is higher among racehorses and National Hunt Horses (6%); in polo horses, it is as high as 13%. The development of rhabdomyolysis is influenced by factors such as exercise routines, sex, age, and temperament of the horse as well as diet and presence of lameness." [19]

"There is strong evidence that RER susceptibility has an underlying genetic basis […]".[18] An analysis of pedigrees containing the ancestors of RER-affected and clinically normal Thoroughbreds supported dominant inheritance with variable expression originating from a founder stallion.[18] "The pathogenesis of RER is still not fully understood. Genetic studies, including preliminary breeding trials, suggest that RER is an inherited condition in Thoroughbreds, with an autosomal dominant mode of inheritance with variable expression." [20]

"The expression of RER is impacted by a genetic predisposition coupled with environmental factors that complicate the identification of susceptibility loci in both breeds. A lack of a defined genetic predisposition could be due to a polygenic mode of inheritance with small effect size for RER susceptibility, allele specific expression, transcriptional or translational modifications, alternate splicing, or selective isoform expression induced in a race training environment." [21]

"Numerous aetiologies have been proposed including hypothyroidism, electrolyte depletion, lactic acidosis, glycogen storage disorders and altered muscle contractility." [22]

"Increased sensitivity of contractile activity in RER muscle cells could potentially be due to an altered sensitivity of the contractile apparatus to activation by CA^{2+}, increased activity of the sarcoplasmic reticulum Ca^{2+} release channel, or decreased activity of the sarcoplasmic reticu-

lum Ca^{2+}-ATPase."[10] Alterations in these processes have been investigated.

3.1.2. Clinical signs of RER

"A diagnosis of RER is made on the basis of history and recurring signs of muscle stiffness in association with high serum levels of creatine kinase (CK) and aspartate aminotransferase (AST) subsequent to muscle cell necrosis." [18] A genetic test to diagnose RER has not been developed.

"RER is characterized by the intermittent development of muscle pain, stiffness, sweating, and reluctance to move during or shortly following brief periods of exercise. RER is distinct from sporadic forms of exertional rhabdomyolysis that arise in unfit horses that are overexerted or depleted of electrolytes. Horses that develop RER are usually very fit, and episodes are triggered by excitement during exercise, especially in horses fed a high nonstructural carbohydrate diet that have been rested for a few days prior to exercise."[10] "Clinical signs such as lumbar and back stiffness, reluctance to move and cramping, sweating, tachypnoe and tachycardia are observed." [17] "High creatine phosphokinase (CPK) activity is detected within 24 h after the exercise, and arginine succinyl transaminase (AST) activity increases at least 48 h after the rhabdomyolysis and remains high in cases of recurrent rhabdomyolysis." [17]

"The nonspecific finding of increased numbers of central nuclei in muscle biopsy samples from horses with active clinical disease is the only common histopathological feature of RER." [10]

"Various risk factors have been identified. It is especially seen in young, female Thoroughbred racehorses with a nervous temperament. The condition occurs more frequently following a period of rest while being maintained on a high carbohydrate diet. Also restraining the horse from reaching top speed, appears to be an important trigger." [20]

3.1.3. Prevention of RER

Avoiding episodes of RER and elimination of stress factors are recommended, combined with optimal feeding management, no hold back in training and no days off. A quiet surrounding, stable and training to decrease stressful influences should be part at managing horses susceptible to RER.

"Episodes of exertional rhabdomyolysis in susceptible horses can be decreased by providing regular daily exercise and by avoiding high nonstructural carbohydrate diets, which are known

to increase excitability. In practice, dantrolene is also used to reduce episodes of rhabdomyolysis. When given 60–90 min before exercise, dantrolene significantly reduces muscle damage with exercise in RER horses (as indicated by serum creatine kinase activity). Dantrolene reduces the release of calcium into the myoplasm via the calcium release channel and, in so doing, increases the caffeine contracture threshold for muscle bundles from RER and control horses in vitro. "[10]

3.2. Malignant Hyperthermia (MH)

Malignant Hyperthermia is one of two potential disorders impacting muscle excitation contraction coupling in horses: malignant hyperthermia (MH) and recurrent exertional rhabdomyolysis (RER). [12]

"Malignant hyperthermia is caused by an autosomal dominant mutation in the skeletal muscle ryanodine receptor gene (*RYR1*). The mutation is responsible for both anesthesia-related and non–anesthesia-related causes of rhabdomyolysis in Quarter Horses. A diagnosis can be made by genetic testing of blood or hair roots." [12]

"Mutations in the RYR1 gene encoding the calcium release channel of the skeletal muscle sarcoplasmic were one of the earliest discoveries in humans and swine of mutations that compromise muscle contractility. This channel responds to surface membrane depolarization to release calcium from storage within the sarcoplasmic reticulum into the myoplasm to initiate contraction of the myofilaments. A dominant R2454G RYR1 mutation was discovered in Quarter Horses that developed fatal episodes of MH triggered by halothane anesthesia. This region of the protein apparently plays a regulatory role in the control of channel gating and is one of the hot spots for MH-causing mutations in other species. Muscle with RYR1 mutations often responds to triggering agents by releasing excessive amounts of calcium into the myoplasm. A rise in myoplasmic calcium results in a sustained contraction, accompanied by increased glycogen metabolism, that generates heat, lactic acidosis, increased O^2 consumption, and increased CO^2 production. The prevalence of the R2454G mutation in a random sample of 225 Quarter Horses was 1.3%. The incidence of unusual incidents of hyperthermia in Quarter Horses under halothane anesthesia attributable to the RYR1 mutation has not been determined. Other breeds of horses not reported to have the known RYR1 mutation can also develop MH under general anesthesia. Whether there may be other yet-unidentified equine mutations that

produce muscle rigidity and hyperthermia triggered by anesthesia or stress is not yet known." [10]

"Malignant hyperthermia (MH) has been described in Quarter Horses, Thoroughbreds, Appaloosa, Arabs and ponies undergoing general anaesthesia." [23]

"Malignant hyperthermia and hyperkalaemic periodic paralysis are diseases associated with genetic mutations that can be triggered during general anaesthesia and may be fatal if not recognised and treated immediately. Morbidities are reported less frequently, presumably because often they do not cause permanent harm and may resolve within a short period of time. Complications in equine anaesthesia are numerous and include injuries at induction and recovery, damage to the airway associated with orotracheal or nasotracheal intubation, post-anaesthetic myopathy or neuropathy, regurgitation and aspiration of stomach contents, ocular injuries and complications associated with intravascular cannulation." [23]

"MH is rare, with less than 1% of 24,000 genetic tests for MH in quarter horses and paint horses being M/N. Halter and pleasure horse lines have the highest prevalence." [11]

3.2.1. Definition of MH

"Malignant hyperthermia (MH) is a life-threatening pharmacogenetic disorder of skeletal muscle elicited by exposure to volatile anesthetics, depolarizing muscle relaxants, or stress." [8]

"It has been established that RYR1 mutation is responsible for malignant hyperthermia in the horse."[24]

It is not known whether this mutation is present in all horses that develop malignant hyperthermia or whether there may be other yet unidentified mutations that cause signs of hyperthermia and metabolic acidosis during anesthesia. [25]

3.2.2. Clinical signs of MH

"Clinical signs related to inhalation anesthesia include tachycardia, tachypnoea, hyperthermia, and muscle rigidity accompanied by severe lactic acidosis, increased serum CK activity, and electrolyte derangements. Exertional rhabdomyolysis in Quarter Horses with malignant hyperthermia can result in sudden death or clinical signs of excessive sweating, tachycardia, tachypnoea, hyperthermia, and muscle rigidity." [12]

"During anesthesia, clinical signs of hyperthermia (41°C or 105F), hypercapnea (partial pres-

sure of carbon dioxide, 274 mm Hg), and acidosis (pH of 6.7) have been reported in horses with MH." [11]

"Clinical signs of MH are inconsistently triggered by exposure to halogenated anesthetics, succinylcholine, or in some cases stress or excitement. Muscle contracture and extreme rigidity occur during an episode, followed quickly by hyperthermia >40°C, hypercapnia, lactic acidosis, and severe muscle cell damage (rhabdomyolysis)." [10]

"Halothane maintenance in combination with succinylcholine triggered MH. Most horses were treated symptomatically and recovered from anesthesia with a few exceptions." [23]

Post-anesthetic myopathy may be encountered after recovery. [23]

"Following exercise, horses intermittently develop signs of exertional rhabdomyolysis with the unusual feature of increased body temperature during episodes. Some MH-affected horses have died suddenly after an episode of exertional rhabdomyolysis. Horses can possess both the *RYR1* mutation and the *GYS1* mutation for PSSM1. Double *RYR1, GYS1* mutation–positive horses have more severe episodes of exertional rhabdomyolysis, higher serum CK activity after exercise, and a poorer response to the diet and exercise regimes recommended for PSSM1." [11]

3.2.3. Prevention of MH

For Quarter horses and related breeds DNA testing before anesthesia is recommended to avoid MH induced crisis situations.

The prognosis for horses with MH under anaesthesia is poor. During episodes of MH treatment with alcohol, mechanical ventilation and chilled intravenous fluids containing sodium bicarbonate are recommended.

"Horses with malignant hyperthermia may benefit from premedication with dantrolene (4 mg/kg, PO) 60–90 minutes before exercise, particularly under hot conditions." [12]

3.3. Polysaccharide Storage Myopathy (PSSM)

"Polysaccharide storage myopathy (PSSM) is a collective name for a group of muscular diseases that occur in horses of various breeds."[26] Two types of PSSM are described until today; PSSM type 1 and type 2.

"The term type 2 polysaccharide storage myopathy (PSSM2) was derived to classify horses

that lacked the glycogen synthase 1 (*GYS1*) mutation which causes type 1 PSSM (PSSM1) and yet had abnormal aggregates of amylase-sensitive or amylase-resistant polysaccharide in muscle fibres. Polysaccharide storage myopathy 1 has been reported in various breeds." [27]
PSSM was not identified as a skeletal muscle glycogenolysis until Valberg in 1992 described an exertion related glycogen accumulation and muscle damage. [28]
"Valberg et al. showed that horses with recurrent exertional myopathies had excessive glycogen storage and unphysiological, complex polysaccharides primarily in their fast-twitch Type II muscle fibres, commonly known as "white muscle." This condition, Polysaccharide Storage Myopathy (PSSM), has a genetic predisposition, likely following a recessive mode of inheritance, in American Quarter Horses (AQH) and related breeds like Appaloosas, Paints, and others. However, tying-up syndrome has also been documented in Warmblood horses, Morgans, Arabians, and Welsh Ponies. The syndrome is particularly prevalent in heavy draft breeds, such as Percherons and Belgians. It is worth noting that, in contrast to trotting and galloping racehorses, excessive polysaccharide and glycogen storage does not seem to underpin tying-up syndrome."[29]

3.3.1. PSSM 1

PSSM 1 "is caused by a dominantly inherited mutation in the glycogen synthase 1 (*GYS1*) gene. A diagnosis can be made by genetic testing of blood or hair samples." [12] "Type 1 PSSM is a worldwide cause of neuromuscular disease in horses." [30]

3.3.1.1. Definition of PSSM 1

PSSM1 is a muscle disorder characterized by an anomalous build-up of sugar molecules in muscle cells due to a genetic mutation in the glycogen synthase 1 gene (GYS1). This ailment is prevalent in multiple breeds, such as Quarter Horses, Paint Horses, and several draught horse breeds, and is hereditary through an autosomal dominant mechanism. This implies that it is inherited through one mutation on one gene. In PSSM 1, heterozygotes may exhibit symptoms. [26]
"In Warmblood horses, the polysaccharide storage myopathy (PSSM) prevalence is higher than that of RER, according to a retrospective study of the cases presented at the University of Minnesota Neuromuscular Diagnostic Laboratory." [17]

Distinct variants of PSSM, namely PSSM1 and PSSM2 exist. PSSM1 is a defined syndrome with a documented genetic basis. “Each horse has two copies of each gene in its DNA, including the glycogen synthase 1 gene. Horses with two 'healthy' copies of this gene (homozygous healthy) are negative and hence do not possess PSSM1. Horses with one mutated copy of the gene (heterozygous) test positive for PSSM1 and are at risk of developing mild symptoms. In 50% of cases, they pass on the gene to their offspring. Horses with two mutated copies of the gene (homozygous false) for PSSM1 also test positive and are likely to experience severe symptoms. They always pass on the gene to their offspring.” [26]

3.3.1.2. Clinical signs of PSSM 1

In affected horses, even gentle activity frequently elicits symptoms resembling tying-up. [29] Horses affected by PSSM may exhibit various clinical symptoms such as muscle stiffness, excessive sweating, reluctance to move, decreased performance, muscle atrophy, muscle tremors, ER attacks, and dark urine. These signs are occasionally known as 'equine exertional rhabdomyolysis' (ER) or 'tying up', although not all types of ER are attributable to PSSM. [26] A muscle biopsy is a reliable method of identifying abnormal sugar molecule accumulation (PSSM) in horses. When such accumulation is present in muscle fibres, the diagnosis with 100% certainty is that the horse suffers from some form of PSSM. However, the muscle biopsy does not differentiate between PSSM1, a genetic defect that can cause muscle problems to varying degrees, and PSSM2, a group of muscle disorders with the same clinical and microscopic abnormalities but with an as-yet-unknown genetic background. Moreover, a horse may have a predisposition to PSSM without displaying an abnormal accumulation of sugar molecules. This is because abnormal sugar molecules typically take some time to accumulate in the muscle cells and become detectable in a muscle biopsy for horses suffering from PSSM. This may be particularly true for young horses that have not yet undergone training. As a result, a horse with a normal muscle biopsy may still develop PSSM later on. However, if horses exhibit clinical symptoms related to PSSM, these will always be apparent in a muscle biopsy. The investigation of a muscle biopsy is particularly advantageous in horses displaying clinical muscle symptoms. If there is an abnormal accumulation of glucose observed in the muscle biopsy, then there is a dependable indication of PSSM. Nevertheless, it remains unclear which form of PSSM that would manifest. [26]

“Quarter Horse–related breeds and other crossbred or light breeds of horses with type 1

PSSM often develop episodes of rhabdomyolysis at a young age with little exercise. Rest for a few days before exercise is a common triggering factor. Episodes are characterized by a tucked-up abdomen, a camped-out stance, muscle fasciculations, sweating, gait asymmetry, hind limb stiffness, and reluctance to move. Some horses paw or roll, with signs resembling colic. Serum CK and AST activities are increased during an episode (usually >1,000 U/L) and, unlike in other forms of rhabdomyolysis, subclinical episodes characterized by persistently abnormal CK activity are common. Serum CK activity remains elevated for longer periods of time than with sporadic or other forms of chronic rhabdomyolysis. Chronic clinical signs in draft horses may include loss of muscle mass, progressive weakness, and recumbency with normal serum CK and AST activities. When draft horses develop rhabdomyolysis, CK and AST activities may be markedly increased, and horses can become myoglobinuric, weak, and reluctant to rise." [12]

"Glycogen is a highly branched glucose polymer consisting of a-1,4-glycosidic linkages produced by glycogen synthase and numerous a-1,6 linkages formed by glycogen branching enzyme. Whereas in humans most glycogen storage disorders are caused by defects in enzymes of glycogen metabolism, both mutations now known to cause glycogen storage disorders in horses are due to defects in glycogen synthesis: type 1 polysaccharide storage myopathy (PSSM1) and glycogen branching enzyme deficiency (GBED). There are other suspected glycogen storage disorders in horses that do not possess either the PSSM1 or GBED mutations but also have an apparently abnormal deposition of glycogen in histological sections of skeletal muscle. These cases are at present termed type 2 PSSM (PSSM2)." [10]

Glycogen branching enzyme deficiency is a rare genetic disorder characterized by a lack of an enzyme responsible for normal branching of glycogen.

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

the earlier described azoturia." [31]

"Unexercised horses with PSSM1 usually appear normal, but clinical signs of a short stride, firm musculature, stiffness, pain, sweating, and reluctance to move forward develop with light exercise." [10] Indicators for the disease can become observable when horses are put on forced exercise at two or three years of age. [10]

"Skeletal muscle glycogen concentrations in horses with PSSM1 are typically two to four times higher than the levels in normal horses. Abnormal amylase-resistant polysaccharide accumulates in PSSM1 skeletal muscle by 16 months of age in as few as one or two fibers to as many as 30% of the type 2 skeletal muscle fibers. The abnormal polysaccharide consists of both b glycogen particles and filamentous material and has a less branched structure than normal glycogen. Large aggregates of abnormal polysaccharide are often associated with autophagic rimmed vacuoles and are tagged for degradation by ubiquitin. Although abnormal polysaccharide is occasionally found in cardiac muscle fibers of severely affected horses, cardiac dysfunction is not a feature of PSSM1." [10]

As described by Baird et al. 2010 the prevalence's and percentages of horses tested positive were as follows: PSSM 1 occurs in warmbloods but only in less than 10% of the PSSM cases. [32]

3.3.1.3. Prevention of PSSM 1

PSSM is incurable. [26] Diagnosis is established via muscle biopsy for horses older than 2 years (m. semimembranosus) or via genetic testing of whole blood or hair roots. The only form of prevention that can be considered so far for the occurrence of the disease is a holistic exclusion of breeding animals that show a genetic disposition.

3.3.2. PSSM 2

"An early study identified polysaccharide storage myopathy (PSSM), affecting lumbar and gluteal muscles, as a primary cause of back soreness, poor jumping and poor dressage performance in Warmblood horses. Subsequently, the molecular basis for PSSM was identified and, based on this genetic test, the condition split into type 1 PSSM (PSSM1) caused by a glycogen synthase 1 mutation and type 2 PSSM (PSSM2) of unknown cause." [33]

"Both PSSM1 and PSSM2 have been identified in Warmbloods; however, the majority of cases of PSSM in Warmbloods fall into the category of PSSM2." [33]

“A definitive cause for PSSM2 has yet to be identified and clinical signs vary among breeds, suggesting there may be several aetiologies grouped under the histopathological descriptor PSSM2. Ultrastructural and immunohistochemical evaluation of skeletal muscle recently identified ectopic accumulation of the cytoskeletal protein desmin, myofibrillar disarray and Z disc disruption, and novel proteomic and transcriptomic profiles in a subset of Arabian and Warmblood PSSM2 horses.” [27]

Absence of the mutation in combination with clinical signs of tying up plus an increase of intracellular glycogen assigns the horse to PSSM2-suspicious. [27] PSSM2 is the most common form of PSSM in Warmblood (WB) and Arabian (AR) horses at >80% and 100% of PSSM cases, respectively, whereas PSSM1 predominates in the continental European-derived draught breeds, Quarter Horses and related stock breeds. [27]

“The onset of abnormal exercise responses and behaviors in PSSM2 WB was insidious with an average age of onset of 6 years, an age when WB horses are expected to be advancing in their training. The three most common complaints of respondents owning PSSM2 WB were reluctance to collect under saddle, decline in performance, and reluctance to go forward reported by 58%, 67%, 76% of respondents, respectively.” [34]

“PSSM 2 refers to other muscular disorders that entail the abnormal build-up of sugars in muscle cells but do not have the genetic anomaly responsible for PSSM 1. Thus, PSSM 2 is an umbrella term encompassing a collection of muscle diseases characterized by the accumulation of sugar molecules in muscle cells.” [26]

3.3.2.1. Definition of PSSM 2

PSSM2 comprises a range of muscular disorders that entail the anomalous build-up of sugar molecules in muscle cells, thereby giving rise to symptoms resembling those of PSSM1. Nevertheless, affected horses lack the genetic mutation linked with PSSM1 and, thus, cannot be identified via the genetic test for this condition. The different disorders encompassed by the term PSSM2 are prevalent in several breeds such as various American breeds, KWPN horses, Swedish Warmbloods, Hanoverians, Friesians, Selle Francais, Westphalians, Canadian Warmbloods, Irish, Icelanders, Quarter Horses, among others. It remains unclear if genetic mutation(s) establish the conditions that constitute PSSM2, and if so, which mutation(s) is/are involved. However, several genetic variants have been identified that may be more widespread in horses with PSSM2. However, these genetic variants are found in horses without clinical

issues who do not show sugar accumulation in their muscles. On the other hand, there are also horses who display clear clinical signs of PSSM2 (including the build-up of sugar molecules in their muscle cells) who do not possess the aforementioned genetic variants. In these patients, there needs to be an alternative explanation for the horses to develop PSSM2. No conclusive relationship has been established between specific genetic mutations and the onset of PSSM2. It is probable that multiple factors contribute to the emergence of the disorders encompassed by the PSSM2 label. [26]

3.3.2.2. Clinical signs of PSSM 2

About 80% of PSSM diseases in warmbloods belong to PSSM 2; breeds showing the disease are Dutch and Swedish warmbloods, Hanoverians Friesians, Selle Francais, Westphalians, Canadian Warmbloods, Irish Sport Horses, Gerdlanders, Itusien and many others. [35]
Abnormal exercise responses reported by owners, began at approximately 6 years of age and included a decline in performance, a reluctance to collect and reluctance to go forward in over 50% of horses. [34]
Median muscle glycogen concentrations did not differ between PSSM2 WB and WB with no evident myopathy. [34] Arabians and Quarter horses with PSSM2 show tying up through clinical signs and have increased CK and AST serum levels in most cases. Chronic episodes of muscle stiffness, soreness, and muscle atrophy with normal to modest increases in serum CK activity are common in horses with type 2 PSSM. Many cases of type 2 PSSM in Arabian and Warmblood horses have been reclassified as myofibrillar myopathy. [12]

3.3.2.3. Prevention of PSSM 2

For horses with PSSM 2 rest is contra productive. A daily training accompanied by a balanced diet is recommended.
The same diet and exercise regime prescribed for PSSM1 is recommended for PSSM2; however, the benefit of these recommendations for PSSM2 is undocumented. [34]
With the recommended diet and exercise regime, 80% of PSSM2 WB owners reported an overall improvement with significant decreases in the proportion of horses showing a decline in performance and rhabdomyolysis. [34]
In conclusion, diet and exercise recommendations ideal for PSSM1 improve but do not eliminate the decline in performance and reluctance to go forward under saddle characteristic of

PSSM2. [34]

3.4. Myofibrillar Myopathy (MFM)

"Myofibrillar myopathy is a newly recognized disorder in Arabian and Warmblood horses. In Arabian horses, it occurs most commonly in endurance horses and is characterized by muscle pain, stiffness, and elevated serum CK and AST activities." [12]

"This occurs at the end of endurance rides or about 5 miles into rides that are preceded by 1–2 weeks of rest." [12]

"In horses, myofibrillar myopathy is a late-onset disease of unknown origin characterized by poor performance, atrophy, myofibrillar disarray, and desmin aggregation in skeletal muscle." [36]

Desmin maintains myofibrils orderly in a healthy muscle; in myofibrillar myopathy the myofibrils break and the desmin is found in clumps in the cells.

"In a recent study molecular and ultrastructural signatures of myofibrillar myopathy in Warmblood horses were evaluated through gluteal muscle tandem-mass-tag quantitative proteomics (5 affected, 4 control), mRNA-sequencing (8 affected, 8 control), amalgamated gene ontology analyses, and immunofluorescent and electron microscopy." [36] "93/1533 proteins and 47/27,690 genes that were significantly differentially expressed were identified. The top significantly differentially expressed protein CSRP3 and three other differentially expressed proteins, including PDLIM3, SYNPO2 and SYNPOL2, are integrally involved in Z-disc signaling, gene transcription and subsequently sarcomere integrity." [36]

3.4.1. Definition of MFM

"Myofibrillar myopathy (MFM) is a clinically and genetically heterogeneous group of hereditary muscle diseases characterized by ectopic protein aggregates and a distinct pattern of myofibrillar disorganization."[37] "Myofibrils contain myofilaments aligned within contractile units called sarcomeres that are bordered by Z discs." [38]

"The term myofibrillar myopathies (MFMs) was proposed in 1996 as a noncommittal designation for a group of chronic neuromuscular diseases associated with common morphological features. These consist of a distinct pathological pattern of myofibrillar disorganization that begins at the Z-disk, followed by accumulation of myofibrillar degradation products and ec-

topic expression of multiple proteins."[39] "Z discs provide structural support and mechanosignaling. The tension during muscle contractions leads to Z discs being sends to the nucleus that activates genes which initiate training adaptions." [38]

"Based on the similarity of the histopathological findings in Arabian and Warmblood horses to a described myopathy in humans, the term myofibrillar myopathy (MFM) was applied to those horses in which abnormal desmin aggregates were identified." [27]

"The possibility exists that type 2 PSSM is an early indicator of MFM; however, this has not yet been substantiated. MFM has thus far not been described in Quarter Horses or similar stock breeds (e.g. Appaloosa or Paint Horses)." [27]

"Because of the marked phenotypic and pathomorphological variability, establishing the diagnosis of MFM can be a challenging task." [40]

3.4.2. Clinical signs of MFM

In Arabians the most common sign of MFM is intermittent tying up. The severity of muscle stiffness can be milder than seen in classic typing up. Slight stiffness, dark urine and an increase of ALT - not as high as in classical tying up - can occur.

"The most common signs of myofibrillar myopathy in Warmbloods are mild shifting lameness, exercise intolerance, and loss of muscle mass that is not accompanied by a concomitant rise in serum CK activity." [12]

Especially in warmbloods satisfactory performance as young horses but decline at the age of 8 years are typical. Most frequent in warmbloods are poor performance without elevation of CK or ALT. Poor performance, unwillingness to move forward and hindlimb lameness can be observed.

"Recently, a subset of Arabian horses originally diagnosed with PSSM2 was found to have a newly identified muscle disorder termed myofibrillar myopathy (MFM). This myopathy was identified in athletic Arabian or Arabian cross horses that competed in 100 mile (161 km) endurance rides and intermittently developed signs of muscle pain and stiffness after exercise." [33]

3.4.3. Prevention of MFM

"(The) evaluation of a Warmblood family revealed that desmin positive aggregates were present in the founding dam, three offspring and a second generation offspring. Thus, the poten-

tial exists for a heritable form of MFM in Warmblood horses. Development of a DNA based test for MFM in Warmblood and in Arabian horses is appealing because the sensitivity of desmin positive aggregates in muscle biopsies could well vary depending upon the age of the horse, size of the muscle biopsy, muscle sampled and degree of biopsy preservation prior to performing IHC." [33]

"MFM is rare to nonexistent in QH based on muscle histopathology." [27]

"Since 2016, a commercial company has been offering genetic tests with the claim that these tests are diagnostic of PSSM2 and/or MFM in horses. There are no data published by the company to show that a positive result with the commercial tests corresponds to a histopathological diagnosis, the means used to discover these diseases. The term "P variant" is used by the company and in this paper to define the alternate (non-reference) allele at each of the loci used to putatively diagnose PSSM2 or MFM." [27]

The finding of Valberg clearly states that MFM and PSSM2 in warmbloods and Arabians cannot be diagnosed based on the use of existing commercial P-variant tests. [27]

3.5. Myosin Heavy Chain Myopathy (MYHM)

"Myosinopathies are a heterogeneous group of congenital myopathies clinically ranging from late onset mild muscle dysfunction to early lethal symptomatic manifestations." [41]

3.5.1. Definition of MYHM

"Myosinopathies are defined as a group of muscle disorders characterized by mutations in genes encoding myosin heavy chains. Their exact molecular and cellular mechanisms remain unclear. " [41]

"The head domain of myosin heavy chains is essential for molecular force production and motion. Hence, subtle amino acid substitutions in this particular region are likely to lead to severe molecular and cellular contractile impairments followed by negative muscle consequences." [41]

"MYHM is caused by a missense mutation in the myosin heavy chain 1 gene (*MYH1*) that results in a glutamic acid for glycine substitution in the myosin type 2X heavy chain. This myosin heavy chain is present in the fastest contracting muscle fibers, type 2X (previous designation type 2B). The common designation for genotypes is: heterozygous affected, My/N;

homozygous affected, My/My; and normal unaffected, N/N. MYHM has an autosomal co-dominant mode of inheritance with variable penetrance. My/My homozygotes usually have more severe clinical signs and a recurrent presentation compared with heterozygotes." [11]

3.5.2. Clinical signs of MYHM

"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." [42]

"Clinically, horses homozygous or heterozygous for $MYH1^{E321G}$ experience rapid onset of malaise, stiffness, skeletal muscle atrophy and weakness; histopathologically, the affected horses display inflammatory infiltrates within type IIx myofibres, particularly CD^{4+}-, CD^{8+}-, and CD^{20+}-positive lymphocytes." [41] "Another $MYH1^{E321G}$- related phenotype has also been discovered in young Quarter Horses in which heterozygous and homozygous animals suddenly develop a profound non- exertional rhabdomyolysis notably characterized by muscle stiffness, pain and inability to rise. Additionally, these symptoms are often accompanied with muscle atrophy in homozygous horses." [41]

"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." [42]

"These horses initially were presented with notable muscle stiffness and markedly increased serum creatine kinase and aspartate transaminase activities with or without muscle atrophy. Lymphocytic infiltrates in muscle fibers were present in <18% of horses with $MYH1^{E321G}$ nonexertional rhabdomyolysis. " [42]

"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 (the) study did not have clinical signs of MYHM." [42]

"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. 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)." [42]

“In conclusion, My/My QH were relatively rare in (the) 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.” [42]

3.5.3. Prevention of MYHM

“Further investigation for MYHM is needed. 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.” [42]

4. Genetics of the diseases in Tying-up

“Diagnostic tests to determine the cause of chronic tying-up include a CBC, serum biochemical analysis, measurement of serum vitamin E and selenium concentrations, dietary analysis, exercise testing, genetic testing, and muscle biopsy. An exercise challenge test is useful to detect subclinical cases; serum CK activity is measured before and 4 hours after light exercise. In addition, quantifying the extent of exertional rhabdomyolysis during mild exercise is helpful in deciding how rapidly to reinstate training.” [12]

“Whilst certain neuromuscular diseases of horses require muscle biopsy for accurate classification, a handful can be diagnosed by genetic testing. (…) Examples of horse muscle diseases with causative genetic mutations include hyperkalaemic periodic paralysis (HYPP), myotonia congenita, malignant hyperthermia (MH) and type 1 polysaccharide storage myopathy (PSSM1). Whilst mutations can arise spontaneously, often they are passed down from parents to offspring: as such, some mutations and diseases occur more commonly in some breeds, or lines of horses than in others.” [43] Due to the complexity of the respective diseases as well as the possibilities of dealing with them, graphics have been created for the purpose of overview, which can be consulted in the diagnosis of these diseases. These provide indications for differentiating the diseases mentioned and can be used as a guide for the further procedure in dealing with the corresponding diagnoses.

4.1. Genetics of RER

“Both environmental factors and a genetic predisposition impact the expression of RER with young, nervous, female horses in race training on high starch diets affected at highest frequencies. Although heritability of RER is estimated at 0.40, linkage analyses and genome-wide association studies so far have been ineffective at identifying a consensus chromosomal locus or gene associated with RER in Thoroughbred horses.” [27, 44]

“A diagnosis of recurrent exertional rhabdomyolysis is based on history, clinical signs, increases in serum CK and AST activities, and muscle biopsy. Muscle biopsy is useful for the evaluation of horses during periods when they have clinical signs of muscle disease and during which a diagnosis can lead to beneficial changes in management. Muscle biopsy is not recommended for use in prepurchase examinations or breeding decisions.” [12]

“Some aspects of RER aetiology have been identified, such as the genetic component that has been demonstrated to be autosomally inherited in Thoroughbred bloodlines of two affected sires. An in vitro contracture test of the isolated RER muscle samples showed abnormal contracture under stimulation with potassium, halothane and caffeine, which demonstrated some calcium regulation dysfunction. Consequently, several candidate genes involved in calcium regulation such as RYR1, ATP2A1 and CACNA1S were investigated and excluded as being causative for the disease.” [17]

“Further, a multiyear controlled breeding trial demonstrated segregation of RER when individuals were phenotype by the in vitro contracture test. Another study estimated the heritability of typing-up in Thoroughbreds to be approximately 0.42. Clinical evaluation of RER horses from across the United States also indicate that gender, age, temperament, diet and exercise routines all play roles in the sporadic expression of the clinical phenotype.” [10]

“After filtering, the transcriptome analysis revealed 191 significant genes in RER vs. control muscles.”[17]

“A total of 54 genes were significantly down-regulated below a normalized ratio of 0.71 and 13 genes were up-regulated.” [17]

Figure 3 gives an overview about up- and down regulated genes in research of RER aethiology.

“The aerobic pathway of ATP synthesis was down-regulated, while glycolysis was activated by hexokinase 2 up-regulation. Signs of hypoxia and oxidative stress were also detected.

Down-regulated genes are in grey (red) boxes with their normalized ratio and P-value. Up-regulated genes are in black (green) boxes with their normalized ratio and P-value." [17]

"Predominantly affecting young mares, this condition is assumed to be inherited in an autosomal-dominant manner with variable expression. While traditionally considered multifactorial diseases, there are indications that EDM, PSSM, and even "Tying up" are primarily influenced by a major gene, similar to HYPP." [45]

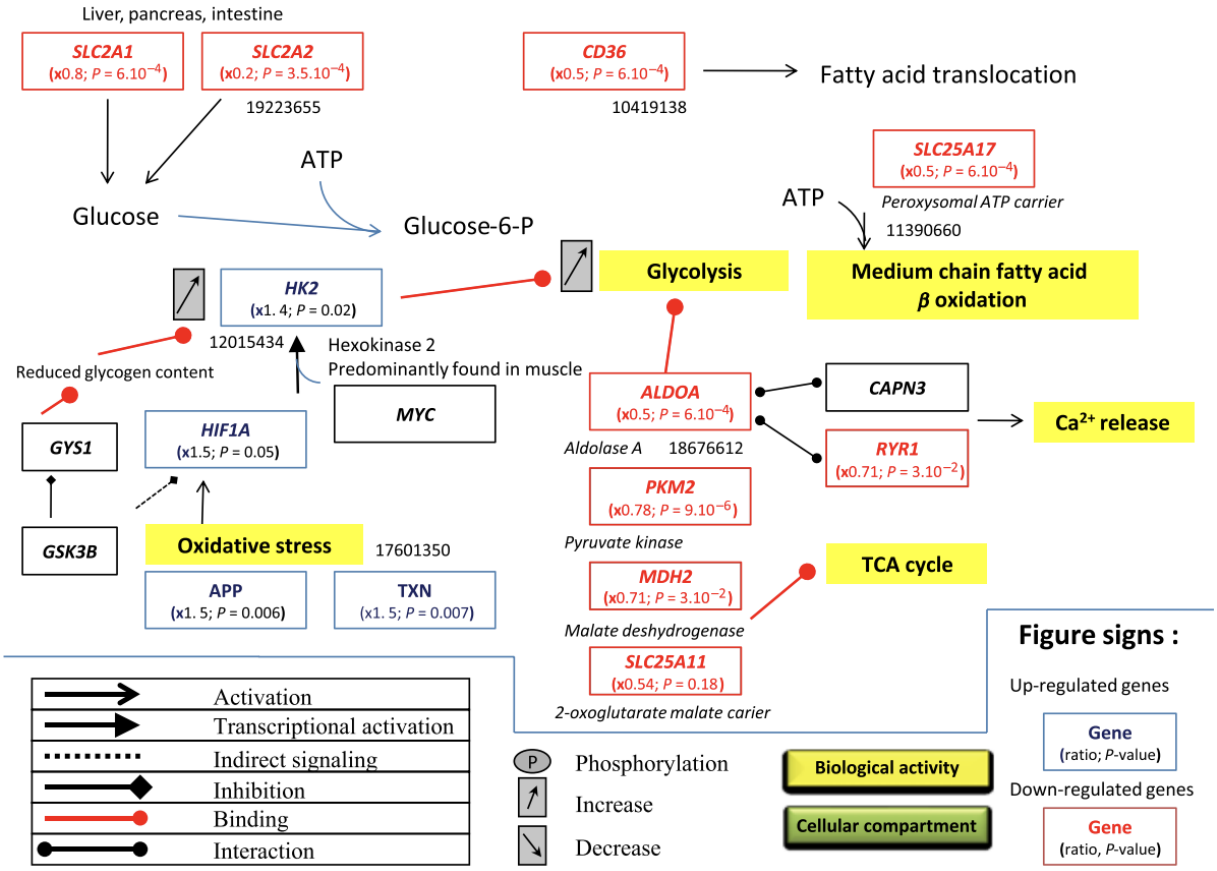

Figure 3: Alterations of pathways of energy synthesis in recurrent exertional rhabdomyolysis muscles [17]

"Multiple lines of evidence suggest that RER susceptibility has an underlying genetic basis. A Markov chain Monte Carlo method was used to analyze 62 RER and 34 control Thoroughbred horses from an extended pedigree for the conditional probability of foundation genotypes. All affected horses shared a common ancestor, and this ancestor and five other stallions had a conditional probability of 1.00 for being affected."[46]

"A study in Japan estimated the heritability of RER in a cohort of 6,538 Thoroughbreds (501 deemed affected) using Bayesian analysis with Gibbs sampling based on the threshold model

for a binary trait. An analysis using three or four generations resulted in a heritability estimate of approximately 0.42."[10]

"Future studies of Standardbred horses could also determine if RER susceptibility loci are shared with Thoroughbreds and if there is any indication of association of RER loci with performance. " [10]

Overview of RER (current state of research 2023)	
- high risk factors: female/ young horses, stress induced - occurs shortly after exercise - pain lumbar/ sacral/ gluteal - sweating - discoloured urine in extreme cases - sporadic + chronic	
Defect genetics	Diagnosis
- enhanced muscle storage of calcium from sarcoplasmic reticulum - stress- related abnormal CA regulation	- history, clinical signs - ADT / CK (release within hours) - exercise test possible - repeated episodes - well trained horses - stress triggered - biopsy: no visible abnormalities or centrally located nuclei in biopsies - typical histology - genetic test for Px encoding CACNA303 suggested risk factor – unvalidated
Therapy	Management
- pain release - fluid Vitamin E / Selen support if necessary - management plan	- quiet stable - train first - no hold back in training - avoid days off - optimal feeding management - dantrolene 1hour before training – orally 2-4 mg/kg - diet: Substitution of fat for NSC, low NSC high fat diet

Figure 4: Overview RER

4.2. Genetics of MH

"In horses, malignant hyperthermia (MH) is caused by a mutation in exon 46 of the ryanodine receptor (*RYR1*), which causes an arginine to glycine (R2454 G) amino acid substitution in the skeletal muscle calcium release channel (ryanodine receptor). The disorder is autosomal dom-

inant. Homozygosity seems to be lethal because no homozygous adults have been reported out of 24,000 horses tested (personal communication, R. Bellone, Veterinary Genetics Laboratory, University of California, Davis). The common designation for genotypes is: heterozygous affected, M/ N; normal unaffected, N/N." [11]

"Mutations in the ryanodine receptor type 1 *(RyR1)* gene cause dysfunction of the calcium release channel of the sarcoplasmic reticulum in skeletal muscle, resulting in the excessive release of calcium into the myoplasm and a hypermetabolic state characterized by intense heat, hypercapnia, lactic acidosis, and, in many cases, death. A genetic basis for the disease has been confirmed in humans, pigs, and dogs. Malignant hyperthermia in humans is also caused by mutations in the gene encoding the dihydropyridine receptor (DHPR)." [8]

"It is associated with a mutation in the ryanodine receptor gene (RyR1) causing increased myoplasmic calcium." [23]

"A missense mutation in the *RYR1* gene decreases the activation and increases the deactivation threshold of the ryanodine receptor (calcium release channel)." [8] "When triggered, the R2454G ryanodine receptor remains open, causing a drastic efflux of calcium from the sarcoplasmic reticulum and inducing a persistent muscle contracture. The process of reuptake of myoplasmic calcium into the sarcoplasmic reticulum consumes large amounts of oxygen and ATP and generates carbon dioxide and excessive heat. Myofibers are damaged by the depletion of ATP and possibly the high temperatures." [11]

"A missense mutation in RyR1 is associated with MH in the horse, providing a screening test for susceptible individuals. Ryanodine-binding analysis suggests that long-lasting changes in RyR1 conformation persists in vitro after the triggering event." [47]

"An additional heterozygous polymorphism (C7360G) in exon 46 generated a R2454G change in $_{MH}$RyR1 horses." [8]

"The point mutation found in the $_{MH}$RyR1 horses is at the same codon reported in two of five mutations for exon 46 in humans. In MHS humans, the mutation results in arginine replacement by cysteine or histidine in different families, whereas, in our $_{MH}$RyR1 horses, arginine was replaced by a glycine". [8]

"The mutation found in the $_{MH}$RyR1 horses is located within amino-acids 1635 to 2636, a region thought to be responsible for orthograde and retrograde signaling in humans." [8]

"The fact that two $_{MH}$RyR1 horses were heterozygous, manifested the disease, and died when

challenged, suggests that the mutated allele is dominant. However, further studies are needed to determine the mode of inheritance in the horse." [8]

Overview MH (current state of research 2023)	
- rare	
Defect genetics	Diagnosis
- autosomal dominant – only one copy with causative mutation → affection of horse	- rigidity of muscles - elevated body temperature - excessive sweating - irregular heart rhythm
Therapy	Management
- poor prognosis - dantrolene 4mg/kg	- no breeding - Premedication with dandrolene - Prevention of MH episodes under anaesthesia via oral dandrolene - 4mg/kg 30 – 60 min. before anaesthesia - No recommendations concerning diets

Figure 5: Overview MH

4.3. Genetics of PSSM

PSSM is characterised by high glycogen accumulation in skeletal muscle and muscle damage with exertion. [44]

"Clinical findings consistent with PSSM were first reported in the early 1900s in working draft horses that developed exertional rhabdomyolysis when returning to work after several days of rest. However, PSSM was not recognized as a skeletal muscle glycogenosis until 1992. Today, as many as 36% of draft horses and 10% of Quarter Horses have PSSM." [48]

"PSSM horses have normal glycogenolytic and glycolytic enzyme activities and are able to utilize glycogen and produce lactate with anaerobic exercise. Thus, PSSM appears to be a unique animal model of abnormal muscle glycogen metabolism." [48]

"The form of PSSM caused by a *GYS1* mutation is termed type 1 (PSSM1), whereas the form or forms of PSSM not caused by the *GYS1* mutation and whose origins are yet unknown are termed type 2 (PSSM2)." [49]

4.3.1. Genetics of PSSM 1

"In 2008, a team of scientists in the US identified a genetic mutation in the equine GYS1 gene, and published the results of their scientific validation process proving that the mutation was associated with PSSM." [48]

"A diagnosis of type 1 PSSM is based on identification of the *GYS1* mutation. If muscle biopsies are examined, muscle fibers with subsarcolemmal vacuoles, dark periodic acid-Schiff (PAS) staining for glycogen, and, most notably, amylase-resistant abnormal complex polysaccharide are usually present." [12]

"It is caused by a dominantly inherited mutation in the glycogen synthase 1 (*GYS1*) gene. A diagnosis can be made by genetic testing of blood or hair samples." [12]

"An accurate genetic test is accessible to identify PSSM1 with an almost 100% accuracy. This test can be performed on horses, whether or not they display clinical symptoms, as their genetic predisposition holds the key. Not all young horses exhibit PSSM1 symptoms, but a positive genetic test outcome for PSSM1 suggests developing clinical manifestations of the disease over time." [26]

"Horses with type 1 PSSM have constitutively active glycogen synthase that is further stimulated by increased blood insulin concentrations, resulting in high muscle glycogen concentrations. When fed a starch meal, these horses take up a higher proportion of the absorbed glucose in their muscles than do healthy horses." [12]

"Genetic testing for PSSM1 has become very helpful not only for diagnosis, but for breeders making informed and ethical decisions about which horses to breed from. By testing DNA (from a blood sample or hair roots) muscle biopsy can be avoided. Over time, it has turned out that many different breeds of horse carry this same gene mutation across the world, strongly suggesting they share a common ancestor." [43]

"Heterozygosity for the H309 allele was shown to be sufficient for horses to be PSSM1 cases, but 22% of PSSM cases were homozygous wild type and 5% of normal horses were heterozygous. This nonconcordance suggested that the GYS1 mutation is not a fully penetrant dominant allele and that one or more phenocopies are present.

The GYS1 mutation has been identified in a large number of breeds in both North America and Europe. Approximately 6–8% of Quarter Horses and Paint Horses are either heterozygous or homozygous for the GYS1 disease (H309) allele. (…) Many draft breeds originating

in continental Europe have an even higher H309 allele frequency, particularly breeds related to the original Belgian draft, such as the Trekpaard, Comtois, and Breton. Severity of clinical and histological profiles in Belgian and Percheron horses with PSSM1 is influenced by genotype, with homozygosity associated with higher serum muscle enzyme levels (indicative of muscle damage) and increased subsarcolemmal vacuolation and cytoplasmic polysaccharide inclusions. The prevalence of the GYS1 mutation is very low in athletic light breeds, such as Arabians, Thoroughbreds, and Standardbreds." [10]

"PSSM1 horses struggle to achieve maximal speeds and fatigue quickly at their maximal speed but have less muscle damage with this form of exercise than with submaximal exercise." [50, 51]

"A deficit in energy generation in PSSM1 horses during submaximal exercise is supported by metabolic studies. During near-maximal exercise, PSSM1 horses have lower maximal oxygen uptake and higher lactate concentrations than control horses do, indicating decreased flux through oxidative energy metabolism. Further, adenosine monophosphate (AMP) concentrations declined, and inosine monophosphate (IMP) concentrations increased, in muscle fibers of PSSM1 horses relative to control horses performing 20 min of submaximal exercise. When adenosine triphosphate (ATP) levels in muscle cannot be effectively restored by metabolic pathways during exercise, the myokinase reaction increasingly produces ATP and AMP from adenosine diphosphate. AMP is then increasingly degraded to IMP by AMP deaminase. Thus, premature degradation of adenine nucleotides to IMP in PSSM1 horses appears to indicate abnormal regulation of the flux of substrates in aerobic metabolism; such an energy deficit might cause segmental damage to muscle fibers during exercise. (…) The analysis revealed 16 genes upregulated over 1.5-fold and 37 genes downregulated over 1.5-fold. Protein synthesis, apoptosis, cellular movement, growth, and proliferation were the main cellular functions significantly associated with the modulated genes. The authors concluded that PSSM mitochondrial dysfunction, glycogenesis inhibition, and chronic hypoxia may contribute to the PSSM1 disease process." [10]

"Seven hundred fifty horses from diverse breeds diagnosed with PSSM on the basis of abnormal polysaccharide in skeletal muscle were genotyped for the GYS1 mutation. The H allele was found in either heterozygous or homozygous form in 356 horses from 15 different breeds, including Quarter Horses, Paint Horses, Appaloosa horses, five draft horse breeds, three

warmblood breeds, and the Morgan, Mustang, and Rocky Mountain horse breeds, as well as mixed-breed horses and warmblood horses of unspecified breed."[48]

Approximately 80% of Warmbloods, 28% of Quarter Horses, and 20% of draft horses with abnormal polysaccharide in muscle biopsies and clinical signs of exercise intolerance do not possess the GYS1 mutation. (…) It may well be that many different disorders can result in the production of glycogen with an abnormal histological appearance in equine skeletal muscle, and not all horses diagnosed as PSSM2 will have the same cellular or molecular basis for their disease."[10]

Table 1 shows GYS1 genotyping results for various breeds of horses diagnosed with PSSM 1.

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

Table 1: GYS1 genotyping results for various breeds of horses diagnosed with PSSM 1 [10]

<table>
<tr><th colspan="2">Overview PSSM 1 (current state of research 2023)</th></tr>
<tr><td colspan="2">- muscle fibres store 1,5-4 times more glycose
- horses react more sensitive to insulin
- increased frequency in heavily muscled breeds
- autosomal dominant inheritance with 3 genotypes
o N/N= no carrier= no disease/ inheritance
o N/PSSM (only one copy of the gene)
▪ inheritance of the gene with 50%
▪ disease possible
o PSSM/ PSSM: 2 alleles of the mutated gene
▪ 100% inheritance
▪ risk of disease increased</td></tr>
<tr><th>Defect genetics</th><th>Diagnosis</th></tr>
<tr><td>- point mutation of the GYS-1-Gene (in 90 % of the cases)
- glycogen synthesis enzyme in the skeletal muscle cell encoded by the (defective) gene</td><td>- asymptomatic in some cases
- muscle stiffness
- sweating
- reluctance to move
- increased CK-activity
- usually begin after light exercise
- can exhibit symptoms without exercise
- shifting lameness
- tense up their abdomen, development of tremors in flank area</td></tr>
<tr><th>Therapy</th><th>Management</th></tr>
<tr><td>- GYS 1 (Gain of function mutations)
- stop movement
- blanket the horse/ remove sweat (depending on weather)
- provide fluid (add electrolytes)</td><td>- provide opportunity to move (paddock)
- diet: management is necessary, avoid prolonged tests
o remove grain
o feed only hay</td></tr>
</table>

Figure 6: Overview PSSM 1

4.3.2. Genetics of PSSM 2

"There is currently no scientifically validated genetic test for PSSM2." [43]

"A diagnosis of type 2 PSSM is based on the absence of the *GYS1* mutation and the presence of muscle fibers with aggregates of amylase-sensitive PAS-positive staining glycogen and occasionally small amounts of amylase-resistant PAS-positive material." [12]

"Quarter Horse-related breeds with type 2 PSSM also have abnormal glycogen storage, and, although the cause of this myopathy is unknown, they are fed similarly." [12]

"The situation is intricate regarding PSSM2. Currently, a significant amount of research is

being conducted to identify the genetic mutation or mutations responsible for PSSM2. Regrettably, a specific genetic mutation that leads to PSSM2 has not been identified yet. This could be because PSSM2 denotes various clinical syndromes collectively. Several genetic variants have been discovered, which could potentially be associated with PSSM2. However, these variants are also present in healthy horses without a PSSM2 disorder, and hence without any sugar build-up in their muscle cells. Furthermore, certain horses with the sugar build-up in their muscles, as confirmed by a muscle biopsy, do not exhibit these genetic variants. Thus, there is presently no direct link between particular genetic mutations and the syndromes collectively known as PSSM2. The EquiSeq genetic test, offered by the Centre for Animal Genetics, lacks reliability in definitively showing whether a horse has or will develop a muscular disease that falls under PSSM2. At present, the test can only identify a specific genetic variant that may be more frequent in horses with PSSM2. It is important to note that this test alone does not confirm or rule out the presence of a muscle disease with sugar accumulation (PSSM2)." [26]

"It remains unclear whether this is a specific disease at all (with a single cause) or whether the term PSSM2 refers to a whole gamut of muscle disorders in horses – some with possible genetic causes but others caused by environmental or other problems. Currently, the optimal way to identify horses as having PSSM2 is via muscle biopsy." [52]

<table>
<tr><th colspan="2">Overview PSSM 2 (current state of research 2023)</th></tr>
<tr><td colspan="2">- occurs in 10 % of the cases
- increased frequency in light breeds
- defects in muscle structures- lead to myofibrillar myopathies</td></tr>
<tr><th>Defect genetics</th><th>Diagnosis</th></tr>
<tr><td>- genetically not validated – still under investigation</td><td>- clumping of muscle glycogen (PSSM 1 not detected based on genetic testing)
- based on muscle biopsy
- Quarter Horses:
o muscle pain
o stiffness
o reluctancy to move
o muscle atrophy
o high serum CK- activity
o less than 1 year of age- inability to rise or stiff hind-limb gait
- Warmblood horses:
o occasionally high Serum CK
o poor performance without elevations in serum CK
o gait abnormality
o sore muscles
o drop in energy levels
o mean age 8-11 years- median CK and AST activity (323 and 331 U/L)</td></tr>
<tr><th>Therapy</th><th>Management</th></tr>
<tr><td>- reintroduction of exercise needs to be gradual
- pain relief</td><td>- avoid rest
- specific training regime
- regular daily exercise
- diet: moderate starch and sugar content- slightly higher protein content with high quality amino- acids (if necessary fat supplementation)</td></tr>
</table>

Figure 7: Overview PSSM 2

4.4. Genetics of MFM

"A diagnosis of myofibrillar myopathy is made by identifying aggregates of the cytoskeletal protein desmin in muscle biopsy samples." [12] The normal function of desmin is to hold myofibrils in organized alignment in the muscle cell. In horses with MFM, abnormal amounts and shapes of desmin are produced. This leads to the assumption that desmin is a late-stage

marker of MFM and further research to identify more sensitive markers is required.

"The MFM in Arabians seems to damage the cysteine metabolism and results in a deficiency in cysteine-containing antioxidants in the muscle causing chronic oxidation and aggregation of key proteins like desmin. In warmbloods MFM signalling pathways loose their adjustment to demands of exercise resulting in a lack of muscle repair, oxidative stress and mitochondrial dysfunction." [53]

In contrast to Arabians, where MFM in most cases is visible as intermittent tying up, tying up is very rare in warmbloods – they show poor performance without elevations of CK and AST serum levels – often after 8-10 years of satisfactory performance.

Genetic testing for MFM is not recommended at the moment but research is ongoing in order to develop a commercial option to detect MFM. Based on these findings so far it seems to be unlikely that MDM is caused by one specific gene.

"Myofibrillar myopathy is linked to aberrations in the Z-disk of the sarcomere and alterations in cysteine-based antioxidants such as glutathione and peroxiredoxins." [12]

"The possibility exists that type 2 PSSM is an early indicator of MFM; however, this has not yet been substantiated." [27]

Overview MFM	
- changes in the muscle cells of affected horses - particularly in Arabian and Warmblood horses - breaks in the myofibrils occur and clumps of desmin form near the breaks	
Defect genetics	**Diagnosis**
- desmin in the muscle biopsy - unclear because genetic basis could not be determined until now	- requires special microscopic stain for desmin in a muscle biopsy - Arabians: o episodes of muscle pain o stiffness (can be milder than that seen in classic tying-up) o reluctance to move o increased serum CK and AST levels can occur - Warmblood horses: o decline in performance as reached 8-10 years of age o poor performance without elevations o unwillingness to move forward o reluctance to collect/engage hindquarters o poor quality of canter o slow onset of atrophy
Therapy	**Management**
- pain relief - control serum Vitamin E and supplement if below 3mg/L - co-enzyme co substitution	- core strengthening exercises - consistent exercise with lots of stretching and long, low warm-ups - individual exercise plan has to be determined - diet: control serum Vitamin E and supplement if below 3mg/L, co enzyme co substitution

Figure 8: Overview MFM

4.5. Genetics of MYHM

"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. 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}." [42]

"The term equine myosin heavy chain myopathy (MYHM) is now used to encompass the two types of phenotypes of *MYH1*^{E321G} (equine immune-mediated myositis and profound non-exertional rhabdomyolysis)." [41]

"Many of the heterozygous horses do not develop MYHM." [41]

"Muscle atrophy affected both MYH1^{E321G} and N/N (29/275, 11%) horses." [42]

"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." [42]

<table>
<tr><th colspan="2">Overview MYHM</th></tr>
<tr><td colspan="2">- presents as two overlapping syndromes: immune-mediated myopathy (IMM) and nonexertional rhabdomyolysis
- Quarter Horses, Quarter Horse related breeds and Paints are primary affected breeds</td></tr>
<tr><th>Defect genetics</th><th>Diagnosis</th></tr>
<tr><td>- mutation in the gene MYH1 – can cause horses immune system to attack skeletal muscle cells</td><td>- Immune mediated myositis
o rapid muscle loss within 1-3 days (epaxial and gluteal)
o depression
o loss of appetite
o stiffness
o history of respiratory diseases or recent exposure to horses with respiratory diseases
o chronic increase of CK and AST (up to 10,000 U/L)
o genetic test
o muscle biopsy
- Nonexertional Rhabdomyolysis
o complete blood count – determine current infection
o creatine kinase values over 50,000 U/L
o genetic test
o muscle biopsy</td></tr>
<tr><th>Therapy</th><th>Management</th></tr>
<tr><td>- Immune mediated myositis
o corticosteroids
o 3 days dexamethasone (0.05 mg/kg) → 7-10 days prednisolone (1 mg/kg) → 1-month 100mg/week prednisolone
o in case of leucocytosis, hyperfibrinogenaemia or lymphadenopathy antibiotic therapy
- Nonexertional Rhabdomyolysis
o for S. equi infections – flushing infected guttural pouches and antibiotics
o corticosteroids
o dantrolene 3-4 x day → until reduction of serum CK</td><td>- Immune mediated myositis
o diet:
▪ concentrate with high quality protein balanced vitamins and minerals
▪ Alfalfa hay and amino acids
o extension of time between necessary vaccinations
- Nonexertional Rhabdomyolysis
o extension of time between necessary vaccinations</td></tr>
</table>

Figure 9:Overview MYHM

5. Critical analysis

In conclusion, the cited literature and the elaboration of the various diseases lead to the assumption that the different disorders presented have different origins and an in-depth understanding is necessary for an individual treatment as well as a containment of tying up in breeding.

Genetic predispositions are assumed but no definite results exist which enable a precise demarcation of the different diseases. Genetic tests for Malignant Hyperthermia, Polysaccharide Storage Myopathy Type 1 and Myosin Heavy Chain Myopathy have been developed. Highly problematic is the existence of horses affected by these diseases which show genetic deviations and can therefore not be captured by the already developed genetic tests. This indicates that either the depth of genetic research is not sufficient to precisely define a disease solely relying on the existing genetic tests or that possible mutations of the diseases have not yet been considered and implemented into the tests.

Myofibrillar Myopathy, Recurrent Exertional Rhabdomyolysis and Polysaccharide Storage Myopathy Type 2 are also associated with the tying up syndrome based on the clinical signs, laboratory evaluations and histological findings. For these diseases a genetic basis has not been confirmed in current research.

Despite the lack of genetic research results, the methods available should be used to contain the hereditary spread and to enable the best possible treatment. It would be conceivable to prophylactically test breeding horses for genetic dispositions (within the framework of the existing testing possibilities) in order to prevent the occurrence of the syndrome. Although this does not guarantee that every carrier of the disease can be identified, it could lead to a considerable reduction in the incidence.

The problem with the symptomatic similarity of the different diseases is that differentiated treatment is usually not possible because the core diseases cannot be identified adequately, and generalisation occurs under the term "tying up". In horses with acute symptoms, a holistic approach is essential. Genetic tests, histological findings, blood values as well as the appearance can provide the veterinarian with clues to identify the individual diseases and initiate differentiated treatment methods.

6. Conclusion

The syndrome tying up or exertional rhabdomyolysis is a collection of different diseases characterized by muscle stiffness, pain and a release of muscle enzymes and myoglobin into the blood stream due to the damages of the cellular muscle structures that affects horses, particularly warm-blooded breeds.

Several different diseases like MH, RER, PSSM 1, PSSM2, MFM, MYHM and others are summarized under the term “Tying up syndrome”. These can be identified by blood work, histological findings and in some cases utilizing the genetic basis. To diagnose these diseases, all parameters as well as the clinical display of the horses must be taken into account.

For Malignant Hyperthermia, Polysaccharide Storage Myopathy Type 1 and Myosin Heavy Chain Myopathy a genetic basis has been identified. This leads to the assumption that - for a definite identification of the diseases - further research is required.

Myofibrillar Myopathy, Recurrent Exertional Rhabdomyolysis and Polysaccharide Storage Myopathy Type 2 are diseases of the tying up syndrome for which a genetic basis has not been confirmed through current research.

Genetic tests for RER and MFM have not been developed. Although e.g., a genetic test is described for PSSM2, further research shows a lack of validation of this test. Critical research is essential when new test methods are described in the field of tying up.

The absence of knowledge or proof of a definite genetic basis leads to a focus on evaluations of clinical symptoms, laboratory findings and histological findings as concise features to identify the diseases. These indicators are currently used to adjust treatment decisions.

In contrast to human medicine where gene therapy for hereditary muscular diseases is an active area of research, the path for equine medicine is selective breeding in order to eliminate these diseases. In the future, new gene-therapy methods such as CRISPR/Cas could lead to the development of personalized therapies for genetic diseases such as the tying-up complex.

Genetic evaluations of the diseases are of high importance especially concerning breeding. Horses that are genetically identified as carriers or diseased shall be excluded from the breeding pool to reduce the prevalence of occurrence. Due to the complex appearance of the diseases, a focus solely on genetic findings is not advisable; clinical signs, histological findings and laboratory evaluations always must be taken into consideration before a definite diagnosis can be made.

The syndrome “tying up” does not reveal itself holistically. It remains to be seen whether further research will enable a definitive genetic categorization of the diseases. At the same time, it is important to investigate whether the genetic factors are unalterably transferrable to other breeds or whether differentiated research is needed.

If successful this could enable an exact classification, the presentation of the individual diseases and targeted diagnostic approaches. Furthermore, it could ensure that a differentiation of the various diseases and thus also promising treatments will be possible in the future. Likewise, the occurrence of diseases could be reduced, by excluding diseased horses which would lead to an improvement of the quality of breeding in its entirety. Ongoing research continues to investigate the tying-up syndrome aiming to further unravel its complexity and develop efficient treatment and prevention.

A special focus needs to be put on investigation of the different kinds of mutations visible in different horses.

7. Abstract

Symptoms of ‘tying up’ in horses range from musculoskeletal problems due to stiffening of the muscles, especially the hind limbs and loins to discolouration of the urine (azoturia) and lateral recumbency. According to the current state of research, the term ‘tying up’ covers various diseases that have similar symptoms. Unique definitions of the various diseases covered by the term are challenging due to the similarity of clinical presentation. Inadequate training and electrolyte imbalances are characterized as high-risk factors that may lead to tying up symptoms. The occurrence of the syndrome increases the likelihood of reoccurrence.

Furthermore, clinical studies have proven, that certain families of horses show higher incidences which leads to the assumption of genetic predisposition.

Currently Malignant Hyperthermia, Recurrent Exertional Rhabdomyolysis, Polysaccharide Storage Myopathy Type 1, Polysaccharide Storage Myopathy Type 2, Myofibrillar Myopathy and Myosin Heavy Chain Myopathy are classified under the term “tying up syndrome”.

Based on the knowledge gained, a delimitation on the basis of a genetic assignment is possible for some of the mentioned diseases, for MH, PSSM 1 and MYHM.

In contrast, a definitive genetic allocation for PSSM 2, RER and MFM is not possible at current research status. The existing tests for these diseases are not validated and therefore do not allow a definitive diagnosis. The clinical presentation, the assessment of blood work and histological findings are therefore essential for a diagnosis.

After the genetic code of the equine genome has been identified, errors in the genetic code may in the future be corrected with completely new therapies. The exploration of the Nobel Prize winning CRISPR/Cas technology may lead to selective modifications of mutated DNA and the successful elimination of diseases causing tying up. It therefore is essential that research of these diseases is continued.

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Appendix 4.

Supervisor's certification

I hereby confirm that I am familiar with the content of the thesis entitled

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Written by **Alexander Hausmann**
(Student name)

Which I deem suitable for submission and defence.

Date: Budapest, 2023-11-15

Supervisor's name and signature
Dr. Zöldág László Prof. Emer.

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