

Glycogen Branching Enzyme Deficiency in an 11-week-old German Quarter Horse filly

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Summary

A six-week-old Quarter Horse filly was presented with flexural deformity of the front limbs, stiff gait and difficulties to stand up. Blood serum chemistry showed elevated triglycerides, AST, LDH and CK (aspartate aminotransferase, lactate dehydrogenase, creatine kinase). Despite intensive treatment the foal became recumbent and was finally euthanized at the age of eleven weeks. Postmortem examination and histopathology revealed periodic acid Schiff's (PAS)-positive cytoplasmic inclusions in skeletal and cardiac myocytes, liver cells and nervous tissue, indicating accumulation of amylopectin. By commercial PCR testing the foal was found homozygous for a mutation of the glycogen branching enzyme 1 (GBE1); the dam was diagnosed heterozygous.

Keywords: glycogen branching enzyme / glycogen / Quarter Horse / foal / amylopectin / genetic defect / myopathy

Glycogen Branching Enzyme-Defizienz bei einem 11 Wochen alten deutschen Quarter Horse-Fohlen

Ein sechs Wochen altes Quarter Horse-Stufffohlen wurde mit einer vorbiegenden Stellung im Karpus beidseits, steifem Gang und Schwierigkeiten beim Aufstehen vorgestellt. Es wurden erhöhte Triglyzeride, AST-, LDH- und CK-Werte (Aspartat-Aminotransferase, Laktat-Dehydrogenase, Creatinkinase) im Blut des Fohlens festgestellt. Trotz intensiver Behandlung kam das Fohlen zum Festliegen und wurde schließlich im Alter von elf Wochen euthanasiert. Die postmortale histopathologische Untersuchung zeigte Periodsäure-Schiff (PAS)-positive cytoplasmatische Einschlüsse in Herz- und Skelettmuskelzellen, in Leberzellen sowie im Nervengewebe, die auf eine Akkumulation von Amylopectin hinweisen und somit eine genetische Muskelerkrankung vermuten lassen. Daraufhin wurde ein kommerzieller PCR-Test auf Glycogen Branching Enzyme-Defizienz durchgeführt: das Fohlen war homozygot und die Mutterstute heterozygot für eine Mutation des Glycogen Branching Enzymes 1 (GBE1).

Schlüsselwörter: Glycogen Branching Enzyme / Glykogen / Quarter Horse / Fohlen / Amylopektin / Gendefekt / Myopathie

Introduction

Glycogen branching enzyme deficiency (GBED) is a fatal glycogen storage disease in fetuses and neonatal foals of Quarter Horses and Paint Horse breeds, which is caused by an autosomal recessive inherited nonsense mutation in codon 34 of the GBE1 gene (Valberg et al. 2001, Ward et al. 2004). Affected foals are homozygous for the mutant GBE1 allele, their dams and sires are heterozygous. In the United States, 8.3% of Quarter Horses and 7.1% of Paints and 0% of Thoroughbreds are heterozygous carriers (Wagner et al. 2006). Approximately 2.5% of fetal and neonatal deaths in Quarter Horses are attributed to a homozygous mutation of the GBE1 gene, the majority of them being abortions (Wagner et al. 2006). Most of the carriers of the GBE1 mutation in the USA are related to the sire "King P234", but it is not possible to use pedigree analysis to diagnose GBED, because the majority of Quarter Horses are descendants of this stallion and its father "Zantanon", who may also have carried the mutation (Valberg and Mickelson 2006). This is the first report about GBED in horses in Germany.

Glycogen is the vital storage form of carbohydrates in most tissues and is composed of straight-chain α -1,4 glucose linkages with α -1,6 branch points. While glycogen synthase catalyses the formation of the α -1,4 glycosyl linkages from UDP-glucose, the glycogen branching enzyme produces α -1,6 linkages resulting in a branched glycogen molecule (Kreutzig 2002). Glucose delivered out of glycogen is essen-

tial for muscle contraction of the cardiac and skeletal myocytes and for maintaining blood glucose homeostasis.

Foals suffering from GBED have an extremely low or even an absence of measurable tissue GBE activity and are unable to synthesize and store correctly branched glycogen. Glycogenolysis normally occurs via removal of terminal glucose residues from the glycogen molecule. The amylopectin-like structures stored in tissues of the affected foals are large granules with a deficiency of these terminal glucose residues. Therefore the affected foals are unable to properly use the stored polysaccharides for glycogenolysis and glucose homeostasis. The heterozygous ancestors show a reduction of GBE activity by about 50% compared to healthy controls, but are usually clinically unsuspecting (Wagner et al. 2006). The clinical signs in horses vary from stillbirth, flexural limb deformities, seizures, sudden respiratory or cardiac failure and finally persistent recumbency (Render et al. 1999, Sponseller et al. 2003, Valberg et al. 2001, Valberg and Mickelson 2006). Usually the foals die from hypoglycemia or heart failure. Common hematological findings include a low white blood-cell count (often about 4000 cells/mL) and serum biochemistry often reveals elevated CK, AST and GGT (creatin kinase, aspartate aminotransferase, gamma-glutamyltransferase) and in some cases intermittent hypoglycemia. Muscle or liver biopsies can support a presumptive diagnosis, since myocytes and hepatocytes usually contain characteristic periodic acid-Schiff (PAS) positive globular or crystalline intracellular inclusions indicating accumulation of amylopectin (Render et al. 1999) in amounts pro-

portional to the foals' age at death (Sponseller et al. 2003, Valberg et al. 2001). There is no treatment for GBED affected foals, but early recognition can save expense for the owners and support exclusion of dam and sire from breeding.

In Norwegian Forest Cats, the deletion of exon 12 of the feline GBE1 gene results in a fatal form of Glycogen Storage Disease (GSD) type IV in which the striated muscles and the nervous system are predominantly affected while the liver is relatively spared (Fyfe et al. 1997). The human GSD type IV results in amylopectin accumulation within the cells of liver, muscle and nervous tissue (Andersen 1956, Brown and Brown 1966, Bruno et al. 1993). The clinical signs also vary widely, ranging from neonatal death to mild muscle weakness in adulthood (Bornemann et al. 1996, Chen 2001, DiMauro and Lamperti 2001). Different mutations of the GBE1 gene are suspected to be the reason for the different clinical manifestations in humans (Bao et al. 1996). In horses up to now only one mutation (GBE1) has been described (Ward et al. 2004).

Case details

A six-week-old Quarter Horse filly was presented to the Equine Clinic because of flexural deformity of the front limbs, stiff gait and difficulties to stand up. The dam had one healthy foal with a different stallion one year before and no history of abortion, stillbirth or ill foals in the past. The foal had required treatment and supportive care right after birth because of weakness, hypothermia and umbilical bleeding. Two and four days after birth, the foal had been treated with oxytetracycline because of the flexural deformity in the fetlock and carpus. The condition improved but the front limbs remained slightly flexed in the carpus. As the mare did not have enough milk the foal was additionally fed with milk replacers every three hours.

At the time of presentation the foal was 6 weeks old, bright and alert and the clinical examination revealed no abnormalities besides the flexural front limb deformity and a stiff gait. X-rays of both front legs were without special findings. The foal was returned back to the owner with treatment with meloxicam (0.6 mg/kg/day p.o.) and supplements (Haemolytan 400, Equistro p.o.).

At the age of eight weeks the foal became suddenly recumbent. A complete blood count (CBC) showed slight monocytosis (0.5 G/L; reference range 0.04-0.4 G/L), indicating slight inflammation. Serum biochemistry revealed elevated triglycerides (2.6 mmol/L, ref. <0.97 mmol/L) and blood glucose (7.5 mmol/L, ref. 3.05-4.99 mmol/L), mildly elevated glutamate dehydrogenase (GLDH) (15 U/L, ref. < 8 U/L), and high AST (992.2 U/L, ref. <250 U/L), LDH (3576.5 U/L, ref. <400 U/L) and CK (1145.7 U/L, ref. <130 U/L). These changes in triglycerides, liver and muscle enzymes indicated an elevated utilization of fatty acids and a slight damage of muscle cells. Selenium and vitamin E were within the reference ranges. Commercial blood PCR testing for Polysaccharid Storage Myopathy Type 1 (Laboklin GmbH, Bad Kissingen) was negative, the foal was homozygous for the intact gene. A muscle biopsy was not performed. From this time on the foal was assisted in rising every three hours. CBC and serum biochemistry were controlled ten days later, but were unchanged compared to the first measurement.

At the age of ten weeks the foal was found recumbent again and transferred to the Equine Clinic. Clinical examination was unremarkable besides an increased heart rate of 104 beats/min. The filly showed stultifying chewing and tense musculature at the pectoral girdle, back and the Mm. semi-membranosus and semitendinosus. After assistance in rising the foal was able to walk, but showed a gait stiffer than at the first presentation and slightly uncoordinated. Clinical neurological examination could not define a specific alteration. Ultrasonography of thoracic and abdominal cavity revealed no abnormalities. Radiography of the cervical region was physiologic. CBC and blood glucose were within normal ranges. Symptomatic treatment was started with cefquinome (1mg/kg twice daily i.m.), omeprazole (4mg/kg/day p.o.), vedaprofene (1mg/kg twice daily p.o.), gabapentin (10mg/kg twice daily p.o.) and prednisolone (1mg/kg/day p.o.) for eight days. The foal did not show any improvement, but became reluctant to move and lethargic. It was euthanized on day eight of treatment because of poor prognosis.

Postmortem examination and diagnostics

Postmortem examination was performed in the Department of Pathology of the University of Veterinary Medicine, Hannover. No macroscopic changes besides multiple gastric ulcerations were seen. Samples were collected from various organs, fixed in formalin and embedded in paraffin. Hematoxylin and eosin (HE) stain was performed, and selected sections were additionally stained with PAS (with and without preceding diastase digestion), alcianblue, Ziehl-Neelsen and luxol fast blue stain.

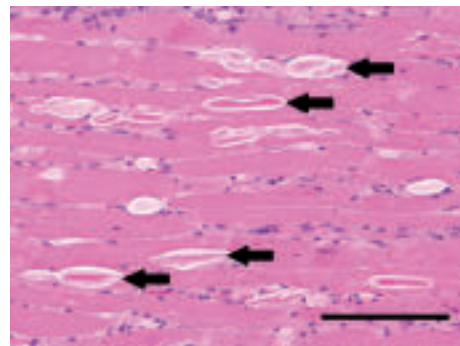


Fig. 1 Histology of skeletal muscle (M. gastrocnemius). Elliptic basophilic inclusions with a bright eosinophilic core in the sarcoplasm of intact myocytes. Hematoxylin eosin. Bar, 100 μ m.

Histological examination revealed prominent cytoplasmic inclusions in myocytes of striated muscles throughout the body, including tongue and diaphragm. They consisted of distinctly bordered, homogenous, pale basophilic material which was arranged as one elliptic body or in a granular fashion. Larger inclusions frequently exhibited a bright pink core. Repeatedly, affected muscle fibers showed degeneration or necrosis of varying degree, which in numerous localizations was accompanied by histiocytic and neutrophilic resorptive inflammation. Identical cytoplasmic inclusions were also detected in cardiomyocytes, purkinje fibers of the cardiac conduction system, and in neurons of cerebrum, cerebellum, brain stem and all segments of the spinal cord; however, they were not associated with degenerative changes in these tissues.

Histochemically, the inclusions were strongly positive for PAS and diastase resistant. Alcianblue staining was also positive, whereas they were negative in Ziehl-Neelsen and luxol fast blue stain. This staining pattern is characteristic for so called amylopectin, which represents an abnormal, poorly branched form of glycogen. Additional histopathologic findings comprised mild periportal lympho-histiocytic hepatitis with single cell necrosis, ulcerative gastritis, and unilateral subacute suppurative keratitis.

A commercial PCR testing for GBE1 mutation (Laboklin GmbH, Bad Kissingen) was performed using frozen muscle samples of the foal and blood of the dam. The foal was homozygous for the mutant gene, the dam heterozygous. Respective information about the sire was not available.

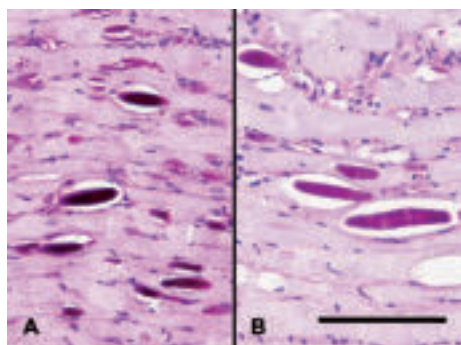


Fig. 2 Histology of skeletal muscle (*M. gastrocnemius*). Cytoplasmic inclusions in the sarcoplasm of intact myocytes. (A) PAS reactivity of inclusions is suggestive of glycogen. PAS reaction. (B) Resistance to diastase digestion is indicative of unbranched glycogen. PAS reaction after diastase digestion. Bar, 100 μm .

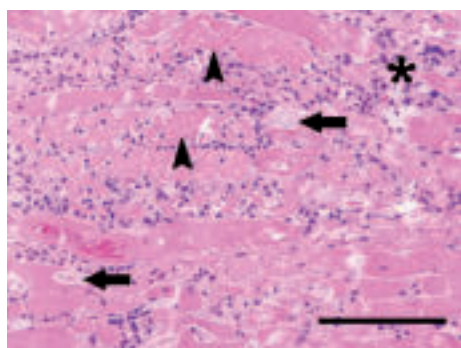


Fig. 3 Histology of skeletal muscle (*M. gastrocnemius*). Marked myocyte necrosis (arrowheads) with resorptive inflammation (asterisk) and remnants of basophilic cytoplasmic storage material (arrows). Hematoxylin eosin. Bar, 100 μm .

Discussion

In the reported case of a Quarter Horse filly, the clinical, biochemical and histopathological findings as well as the final PCR results, are consistent with GBED and parallel the findings in previous equine GBED cases (Render et al. 1999, Valberg et al. 2001) and human GSD type IV cases (DiMau-ro and Lamperti 2001). On postmortem examination, PAS reaction in combination with diastase digestion represents a suitable method to identify the range of tissues and cell types affected by cytoplasmic inclusions. While mere PAS reaction labels all, including physiological glycogen deposits, only

abnormally branched glycogen chains retain their positive staining after enzymatic digestion by diastase. In other studies, amorphous PAS-positive inclusions were identified in a variety of tissues of neonatal foals primarily in cardiac Purkinje fibers and occasional cardiac myocytes. Notable globular PAS-positive inclusions in skeletal muscle were found only in affected foals older than one month (Valberg et al. 2001). In this case PAS-positive inclusions were also found in the nervous tissue, which is a consistent finding in humans (Schroder et al. 1993). Similar nervous tissue alterations in horses were only described by Render et al. (1999) in two Quarter Horse foals, one was stillborn, the other foal was one month old. To a certain degree glycogen is also stored in glial cells in the brain of all vertebrates and also humans. The embryonic mammalian brain usually contains more glycogen than adult

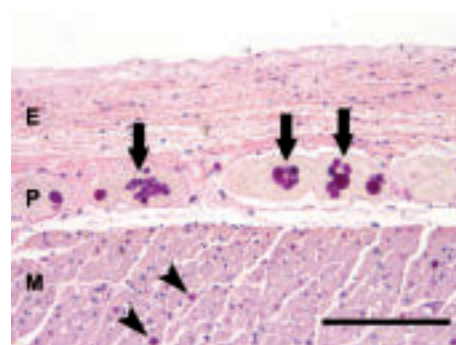


Fig. 4 Histology of myocardium. Globular cytoplasmic inclusions are present in morphologically unaltered Purkinje fibers (arrows) and cardiomyocytes (arrowheads); inclusions display diastase resistance and PAS reactivity. E, endocardium; P, Purkinje fiber layer; M, myocardium. PAS method after diastase digestion. Bar, 100 μm .

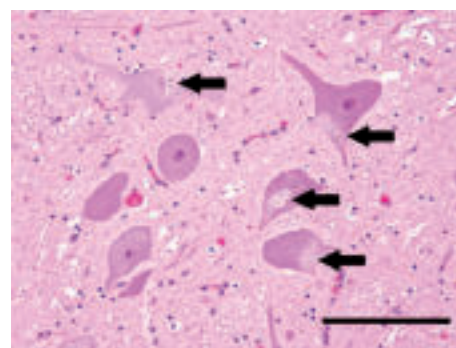


Fig. 5 Histology of central nervous system (medulla oblongata). Storage of granular to globular, eosinophilic to pale basophilic material in the cytoplasm of morphologically intact neurons. Hematoxylin eosin. Bar, 100 μm .

brain tissue (Oksche 1957). The reason why only some GBED affected foals exhibit morphologically detectable nervous system alterations remains unknown, but they do not seem to be age related. In the reported case the gait alteration and slight uncoordinated movements could have been attributed to muscular problems as well as to neuronal problems.

The long survival of the filly in this case might have been due to the closely monitored nutritional intake and care. Most GBED affected foals die or are euthanized by eight weeks of age; but there is a report about one foal that survived till the age of eighteen weeks with close nursing care (Valberg and Mickelson 2006).

Unfortunately a muscle biopsy was not performed in this case. It is likely that at least in the late period of the disease an antemortem diagnosis would have been possible. CK and AST were mildly elevated twice during treatment but were not controlled during the last two weeks of life to keep the costs within the owners' budget. The mild elevation of the muscle enzymes indicates damage to the myocytes due to abnormal polysaccharide accumulation in the cytoplasm of the cells without profound muscle cell necrosis. Muscle cell necrosis presumably aggravated until euthanasia, because multifocal muscle fiber degeneration and necrosis at different graduations were seen during necropsy. The muscles of the hind limbs were severely affected, so a muscle biopsy from this region would have been of diagnostic benefit.

The most common clinical signs of GSD IV in humans are due to progressive liver failure and cirrhosis (Greene et al. 1987). No evidence for liver cirrhosis was found in this foal, but marginal hepatitis with hepatocellular necrosis was seen. Serum biochemistry revealed elevated triglycerides and only mildly elevated AST and GLDH. GGT however was within the reference range. Blood glucose was not monitored closely enough to allow a precise statement. However, the filly showed hyperglycemia at two monitored time points, despite the presumably altered glucose metabolism. This could be due to recent nursing or stress related glucose increase. An altered glucose uptake into the cells has not been investigated or described in foals with GBED. The lack of liver cirrhosis has also been assessed in other affected foals (Valberg et al. 2001) and in Norwegian Forest cats (Fyfe et al. 1992) and could be due to the short life of these animals compared to the chronic course in human patients.

In humans several mutations profoundly affect GBE activity and lead to GSD IV. For example the deletion of an exon results in complete abolished GBE activity and different point mutations lead to reduced but not complete absence of GBE activity (Bao et al. 1996). The exact mechanisms that cause the wide variety of clinical presentations (ranging from neonatal death to liver cirrhosis) are not well understood (Moses and Parvari 2002), but the different levels of GBE activity could be an explanation in human patients.

In contrast to this, only one mutation of the GBE1 gene has been described in horses so far (Ward et al. 2004). This mutation is associated with several clinical disorders like abortion, stillbirth, neonatal death, limb deformities, gait alterations, metabolic disorders and death. The occurrence of a single mutation in horses is likely to be the result of line breeding. This case demonstrates that GBED in Quarter Horses and Paint Horses should be considered as a differential diagnosis in neonatal disorders also in Europe and that routine testing of breeding horses is sensible.

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