

211th ENMC International Workshop: Development of diagnostic criteria and management strategies for McArdle Disease and related rare glyco(geno)lytic disorders to improve standards of care 17-19th April 2015 Naarden, Netherlands

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Introduction

Twenty participants, including one patient representative, from 9 countries (UK, Spain, France, Sweden, Finland, Denmark, Italy, Germany and USA) attended the workshop, the aims of which were to agree best practice strategies for diagnosis and management of McArdle disease (GSDV) and related rare glycolytic disorders of skeletal muscle.

Clinical phenotype of GSDV: Data from the Spanish registry

Alejandro Lucia presented data from the Spanish registry of 239 patients (137 males, 102 females, aged 9-93 years) indicating a prevalence of 1/167,000 [1]. The most prevalent *PYGM* mutations were p.R50X, p.W798R and p.G205S, which accounted, alone or in combination, for about 61% of all *PYGM* genotypes, with p.R50X being the most prevalent. There was no genotype/phenotype correlation, virtually all individuals reported exercise intolerance and 50% reported recurrent myoglobinuria. Episodes of acute renal failure were rare, occurring in 4%.

In a small number of very active people symptoms occurred only during sporting activities, while the majority showed intolerance to almost all types of physical exercise, 58% reported onset of symptoms in the first decade of life. Fixed muscle weakness was seen in 25%, affecting mostly the upper body, 86% were aware of the 'second wind' phenomenon and virtually all had raised baseline serum CK.

Thus, the phenotype in GSDV is remarkably consistent, variability amongst patients most likely relates to epigenetic modulatory factors such as the ACE genotype, fitness level, possibly gender, dietary habits, glycogen storage in skeletal muscle and residual levels of muscle glycogen phosphorylase (MGP).

Unusual GSDV phenotypes

John Vissing described atypical presentations of GSDV including two patients with marked wasting and weakness of the paraspinal muscles leading to a severe axial

myopathy. Muscle biopsy in these patients showed large vacuoles [2]. On the other hand, a rare group of patients who carry a splice site mutation express small amounts of residual enzyme and present with a much milder phenotype with improved exercise performance. Compared with typical patients, they lack the classical description of a 'second wind' which appears blunted on formal exercise testing [3]. Pascal Laforêt and Andrea Martinuzzi presented patients with an 'FSHD-like' phenotype with scapular winging and severe upper limb weakness. Early published descriptions of a fatal infantile phenotype were not molecularly confirmed and may be examples of 'double trouble'. When formally assessed, exercise capacity in GSDV carriers showed no difference from healthy controls [4].

Pathological and molecular changes in GSDV

MGP catalyzes the first reaction in the catabolism of muscle glycogen and is lacking in GSDV. Muscle biopsies show accumulation of sub-sarcolemmal glycogen with absence of MGP [5, 6]. Previously, diagnosis was based on the forearm exercise test (showing no rise in lactate) [7], and muscle biopsy [8]. However, the most accurate diagnostic tool is genetic testing for homozygous or compound heterozygous pathogenic mutations and deletions in *PYGM* [9, 10]. Currently, 147 disease causing mutations have been reported, 91% are located in exonic regions and 9% in intronic regions. Missense mutations account for 50%, deletions 18%, nonsense mutations 13% and mutations that affect RNA splicing 11% [11]. Exon 1 is the most dense in terms of mutations (N=17), including p.R50X which is found in 68-72% of all Northern European and Northern American mutated alleles [12,13,14], followed by exon 17 (N=14) which includes p.F710del, the most common mutation found in Japan [15].

Screening for p.R50X is recommended as the first-line investigation in Caucasian Northern European and Northern American patients. Targeted PCR-RFLP to

p.R50X mutation with restriction enzyme NlaIII, or PYGM-Exon 1 sequencing are two straightforward diagnostic approaches to screen this “common” mutation [20, 21]. Other frequent mutations are: p.G205S (3-10% of mutated alleles), p.W798R in Spanish patients (10-17%) [10, 16, 17, 18, 19]. In some patients, muscle mRNA or cDNA may be required to identify mutations that cannot be detected with DNA analysis [20].

Transcript studies have unveiled the pathogenic effect of some mutations such as intronic mutations, large deletions and silent mutations [21, 22]. Mutations in the donor or acceptor splicing site of *PYGM* have been described in a small number [23]. Interestingly, some silent mutations that do not alter the amino acid sequence, e.g. c.645G>A (p.K215=) or c.1827 G>A (p.K609=) have been demonstrated to affect splicing [24, 25].

Besides the invasive nature of the muscle biopsy procedure, the usefulness of analyzing cDNA synthesized from tissue-specific transcripts is limited by a cellular homeostatic mechanism known as ‘nonsense mediated mRNA decay’ (NMD), which eliminates aberrant transcripts that contain nonsense and frameshift mutations [26, 27, 28, 29, 30]. A study of 28 GSDV patients found that NMD was important 92% [31], this high prevalence of patients showing NMD of *PYGM* transcripts can be largely explained by the high frequency of the p.R50X nonsense mutation.

There is some residual transcription of tissue-specific genes in virtually all body cells, a phenomenon originally described as ‘illegitimate’ [15] or ‘ectopic transcription’ [30]. *PYGM* transcripts can be obtained from peripheral blood mononuclear cells from GSDV patients allowing detection of *PYGM* mutations enabling insight into the actual functional consequences and pathogenicity of NMD-affected mutations without the need for a muscle biopsy. This is exemplified by the silent mutation c.645G>A, whose effect at the transcription level was previously unknown [25]. Further, the upcoming use

of targeted gene-panels [31] or whole exome/genome sequencing in the neuromuscular clinic framework, will presumably result in identification of several novel *PYGM* mutations [32, 33] whose pathogenicity would have to be corroborated using for example mRNA-PYGM in blood cells.

Exercise as treatment for GSDV

Alejandro Lucia presented evidence for physical training in GSDV. In a study by Haller et al, training effects in 8 patients (4 male) [36] using a cycle-ergometer (4 times/week) for 14 weeks were assessed. Exercise intensity corresponded to 60-70% of their maximal heart rate and the duration of the sessions ranged from 30-40 minutes. Training induced significant improvement in peak work capacity, VO_2 peak and cardiac output, as well as in key aerobic enzymes. Matez-Munoz reported training effects in 9 people with GSDV [37], who either walked or trained on a cycle-ergometer 5 times/week for 8 months. Intensities and session duration were similar to the Haller study [36]. Several indicators of exercise capacity, notably ventilatory threshold (also known as the ‘anaerobic threshold’) and VO_2 peak, improved with training. No adverse effects were observed.

Santalla et al assessed the effects of a supervised weight lifting program of light-moderate intensity (~65-70% of one-repetition-maximum (1RM); 2 sessions/week) in a 15 year-old male for 6 weeks [38]. A carbohydrate drink was ingested before each session. After training, his bench press and multi-power squat 1RM performance increased by ~27% and ~6%, respectively. Importantly, no episodes of myoglobinuria were reported and he became virtually asymptomatic after the training intervention [38, 39]. Santalla et al. [39] also studied effects of a weight lifting training circuit program of 4-month duration and light-moderate intensity (2 sessions/week) followed by a 2-month period of ‘de-training’ in 7 GSDV adults (2 male, 5 female). Outcome measures were:

muscle mass (assessed by dual-energy X-ray absorptiometry) and muscle peak power, as well as serum-CK and clinical severity. This training induced a significant increase in total lean mass and peak power generated in bench press and half-squat tests. Importantly, there were no severe adverse events and symptoms improved.

Resistance exercise training should not be recommended unless training sessions are individually supervised by experienced fitness specialists with experience of looking after people with GSDV and patients should always consult their physician first. Pre-exercise ingestion of carbohydrate drinks should always be taken prior to resistance exercises [40].

For children with GSDV, it is important to provide parents, caregivers and educators (especially physical education teachers) with appropriate information to ensure that the best possible management strategies are implemented. It is recommended that all patients with GSDV should adopt as active a lifestyle as possible [40].

Diet and Dietary supplements to Aid Exercise Performance

Richard Godfrey summarized the evidence for diet and dietary supplements to aid exercise performance. In general, patients are encouraged to follow the guidelines for healthy eating issued by the World Health Organization. These suggest a mean daily energy intake of 2500 kcal/day (10 500 kJ/day) for men and 2000 kcal/day (8500 kJ/day) for women. Of course, this will vary considerably with physical activity and so energy intake should vary accordingly. Recommendations suggest that ingested food should comprise 10-15% protein, <35% fat and >50% carbohydrate (CHO).

Skeletal muscle utilizes large amounts of ATP which comes from several sources; ATP that is already attached to the contractile apparatus inside the muscle cells is from reconstitution of ATP by donation of Pi from the intramuscular store of creatine

phosphate (CP) to ADP and direct synthesis by mitochondria. Normally around 500g of CHO is available for glycolysis (400g stored as glycogen in muscle, 100g stored as glycogen in liver and around 3g of glucose in the systemic circulation). Healthy individuals, when working above 50% of the intensity that elicits VO₂ max, derive more than 50% of the required energy for activity from metabolism of lactate. In GSDV, metabolism of muscle glycogen is blocked and no lactate is produced, thus, at least 80% of the CHO substrate available to healthy individuals is simply not available in GSDV and abnormal glycogen storage results in disruption of intramuscular contractile structure.

Dietary interventions explored for GSDV have been summarized in a Cochrane review [41] and include supplementation with creatine, glucose, short and medium chain triglycerides, carbohydrate, high protein and ketogenic diet. High dose creatine supplementation impairs function, increases muscle pain and may even increase the risk of rhabdomyolysis and has no clinical benefit. Glucose ingestion prior to exercise improves exercise tolerance and may protect against exercise induced rhabdomyolysis [42, 43]. Short and medium chain triglycerides may also be of value since, unlike long chain triglycerides, they can simply diffuse across mitochondrial membranes.

Benedikt Schoser discussed ketogenic diet (KD) for GSDV. The effects of KD in muscle tissue include downregulation of oxidative phosphorylation, mitochondria depletion, ineffective mitochondrial respiration and a decrease in glutamate and glutamine levels. In contrast, during starvation, KD can provide up to 70% of the energy requirements for the brain. KD side-effects include metabolic acidosis, nausea, vomiting, weight loss, constipation, or diarrhea. Less common side effects include hyperlipidaemia, selenium and vitamin deficiencies, prolonged QT interval, pancreatitis, renal calculi, and renal tubular acidosis.

In GSDV skeletal muscle is unable to mobilize glycogen during anaerobic exercise due to impaired oxidative phosphorylation and insufficient substrate flux through the tricarboxylic cycle [44]. KD substitutes the lack of glucose with ketone bodies thus bypassing the absence of carbohydrate-based stimulation of insulin secretion and glycogen synthesis, and repletion of acetyl-CoA from ketone bodies [45]. Vorgerd & Zange piloted KD in one patient with GSDV and found improvement in muscle symptoms and performance, although no changes in muscle energy metabolism were identified [46]. They subsequently undertook an open labeled study of KD in five patients, although 13 patients were screened, the most common reason for non-recruitment was that KD was too restrictive. Primary study endpoint was the total maximal tolerated distance of a 30-minute exercise cycling test after week 12 on KD. Secondary outcomes were CK and KB measurements from baseline compared to week 12. One patient dropped out after 6 weeks. By increasing the fat content of the diet to 80% with 15% protein (1 g/kg/d), a stable ketosis > 3 mmol/l 3-OH-butyrate was established. Four patients (1 female, 3 males, median age 46+11 years) remained ketotic during the study period of 12 weeks. All four extended KD to a mean period of 5.8 months with a range of 4 to 9 months, in these participants exercise tolerance and maximal cycling distance improved by 15% (range 9-25%) and baseline CK dropped by 30% (range 18-58%) at week 12. KD was well tolerated, and no severe or moderate side effects were reported. The major limitations of the study were the short duration, small study group size and the use of a non-validated primary outcome measure without a matched control group. Further studies of KD are necessary to determine its effect in GSDV [46].

GSDV: A patient's perspective

Andrew Wakelin described his experience of living with GSDV, symptoms were present from the age of 4 which led to several incorrect diagnoses of ‘growing pains’, ‘knock-knees’ and ‘rheumatism’; at 16 years of age he was told “we won’t investigate further”. He was finally diagnosed with GSDV at the age of 30 but received no advice on management until he was 50 years old. Late diagnosis may lead to exercise avoidance leading to deconditioning and fatigue, or, muscle damage due to inappropriate isometric activities leading later to muscle atrophy and weakness. Many patients are diagnosed late and do not have access to specialist care.

People with GSDV have constant ruminations: ‘is this going to be a problem?’ ‘Can I do it another way?’ ‘Can I split the task down?’ ‘How are my muscles feeling?’ ‘Do I need to slow down or pause?’ ‘My legs are getting so heavy’. ‘I can hardly move’. ‘My heart is pounding’. ‘The pain is too much’. After overdoing it: ‘How badly am I cramped?’ ‘How many muscles are affected?’ ‘What is my likely CK level?’ ‘How dark is my urine?’ ‘Do I need medical care?’ In terms of social impact, people with GSDV deal with other thoughts: ‘Can I go out with my friends?’ ‘Will I get left behind?’ ‘How can I avoid situations?’ ‘I look normal – my friends don’t understand’. From a patient perspective referral to a specialist centre where there is multi-disciplinary team care, including a psychologist is essential to prevent inappropriate and possibly harmful advice such as ‘avoid exercise’.

Guidance for patients should include when to seek medical care and when is it safe, and how to “home treat” myoglobinuria and when to seek emergency care. Emergency departments should focus on the management of rhabdomyolysis and treatment of pain, monitoring for acute renal failure and compartment syndrome are important. The CK level will not normalize and thus the serum CK should not be an indication for discharge.

Outcome measures in McArdle disease for clinical trials

John Vissing gave an overview of clinical outcome measures for GSDV. Most validated neuromuscular outcome measures do not apply to this group of patients who need dynamic exercise testing. Performing a max test using cycle ergometry for 15 minutes has been validated in GSDV and used for clinical trials where heart rate, Borg rating of perceived exertion (RPE) and power are the key outcome measures. This test demonstrates a second wind in both GSDV and GSDXIV (PGM deficiency) but not in GSDVII (PFK deficiency) [47].

The 12-minute walk test measures heart rate and Borg rating of perceived pain (RPP), demonstrates a second wind and the primary outcome measure is the maximum distance walked [48]. Accelerometers can be used for studies of physical training to determine compliance [37]. The handgrip test, otherwise known as the forearm exercise test, shows a flat lactate response curve with exaggerated rise in ammonia [49]. Recording the frequency of myoglobinuria and measuring the serum CK are not reliable outcome measures. Muscle biopsy for enzyme and glycogen content may be useful, depending on the type of study. Measuring fat and carbohydrate turnover are good tools for metabolic studies although the procedure is complicated, in GSDV fat metabolism is higher than healthy controls [50]. NMR imaging has been used in GSDV [46]

Phosphofructokinase deficiency, Tarui's disease, GSDVII

Andrea Martinuzzi gave a summary of the clinical and pathological features of GSDVII: In 1965 Tarui and co-workers reported a woman with exercise intolerance from childhood resulting in cramps, pain, nausea and sometimes vomiting with exercise. She had mild jaundice with an otherwise unremarkable neurologic examination. She didn't experience any deterioration over time, nor any problem during pregnancy and delivery. The biochemical investigation of muscle biopsy revealed a

block in the phosphorylation of fructose 6 phosphate to fructose 1, 6 biphosphate, the committed step of glycolysis, a reaction catalyzed by phosphofructokinase [51]. The enzymatic defect was later shown to be caused by a homozygous IVS15+1G/T deletion of 75 bp in exon 15 of the M-PFK gene [52]. The enzyme is homo/heterotetramer of 3 isoforms coded by three highly homologous, but different, genes on three different chromosomes: the muscle type (PFK-M, chromosome 12q) the liver type (PFK-L, chromosome 21q) and the platelet type (PFK-P, chromosome 10p). The three isoforms differ in their kinetic properties. Skeletal muscle expresses only PFK-M, which is also the prevalent isoform in heart (90%), brain (55%) and red blood cells (43%). PFK-M is transcribed in 3 mRNA (A-C) that differs from their promoter and the retention/deletion of intron 2. PFK-M A and B are found in muscle while the type C is found in non-muscle tissues [52].

Over 100 patients with GSDVII have been described from various ethnic backgrounds and 21 different mutations have been reported thus far. There is no clear-cut genotype/phenotype correlation. The disease is most prevalent in the Ashkenazy population, where an exon 5 splicing defect is found in 68% [52]. There are four clinical presentations of GSDVII: “classical” exemplified by the first Tarui case description; “late onset” presenting with progressive muscle weakness in the lower limbs during or after the 5th decade; “infantile” presenting with neonatal hypotonia, arthrogryposis, seizures, developmental delay and early death, and, the “hemolytic form” presenting with non-spherocytic hemolytic anemia.

Hemolysis, accompanied by reticulocytosis, which also characterizes the classic form, is due to a 50% reduction in PFK activity in red blood cells, where the only source of energy is glycolysis and where a drastic reduction of 2,3 diphosphoglycerate modifies the hemoglobin affinity for O₂. The hemolysis is typically non-exertional

because ammonia, produced in excess during exercise, activates any residual PFK thus partially correcting the defect. An increase in ammonia during exercise is due to deamination of AMP by myoadenylate deaminase (MADA). AMP is removed to allow ATP synthesis from ADP, a reaction otherwise blocked by excess AMP. The increase in ammonia, inosine and hypoxanthine, the other two catabolites of AMP, leads to hyperuricemia which is frequently found in GSDVII and GSDV patients and often leads to gout [53].

The fatal infantile form [54], described thus far in 13 cases [55], presents with variable but diffuse involvement of the brain (seizures, developmental delay, cerebellar hypoplasia,) profound hypotonia, arthrogryposis, respiratory failure, and cardiomyopathy [55]. PFK activity is absent in both liver and muscle, but normal in red blood cells. Since no mutation in PFK-M or L has been detected, it is hypothesized that the defect may be due to defects in a regulatory gene of all PFK isoforms.

In the classic form of GSDVII the muscle biopsy shows sub-sarcolemmal glycogen accumulation (exceeding 1.5 g/100). In some patients diastase-resistant granulo-filamentous deposits (polyglucosan bodies) are also visible [56]. Hypertrophic cardiomyopathy has been described in few individuals and could be underestimated clinically [57].

The diagnosis of GSDVII is based upon the clinical history, raised CK, hemolytic anemia and blunted or absent lactate production during forearm exercise. Absence of the second wind phenomenon is due to the inability to utilize blood borne glucose to bypass the metabolic block and this differentiates GSDVII from GSDV. Indeed, during exercise testing administering a glucose infusion worsens performance with lower maximal workload and decreased O₂ uptake with higher peak heart rate [58, 59]. ³¹P-NMR evaluation reveals 30% ATP muscle content at rest, continuous decline

of ATP with rise in phospho-mono-esters with no acidification during exercise and slow phosphor-creatine/phosphate (PCr/P) recovery and persisting low Mg⁺⁺ post exercise [60]. Other elements differentiating GSDVII from GSDV are the frequency of exercise induced vomiting and the relative rarity of myoglobinuria in GSDVII. Unsolved mysteries include the etiology of the fatal infantile form and the modulators of phenotype in the other clinical presentations. In this respect, wider international collaboration with systematic registration of all cases could provide the needed critical mass to determine the natural histories of the disorder.

Paivi Piirilä described results of one male patient with GSDVII, another with GSDV and four healthy controls who underwent cycle testing with venous sampling of lactate and ammonia. A late increase of lactate, about three times the basal level, was seen 10-30 minutes after exercise in the subject with GSDVII but the lactate response in the GSDV patient remained flat. When the GSDVII patient was started on a KD the muscular symptoms alleviated and the oxygen uptake increased [61].

Renata Scalco presented unpublished data from a cohort of six GSDVII patients attending a clinic in the UK; all patients reported more severe exercise intolerance than GSDV, 3/6 had experienced vomiting with strenuous exercise. Episodes of myoglobinuria were reported in 3/6 (none severe enough to warrant hospital admission), improved exercise tolerance with fasting was reported by 2/6. Baseline serum CK levels varied from normal (95iu/l) to 10x normal (1796 iu/l) three patients had cardiac abnormalities seen on echocardiography which included: minor LV trabeculation (1/6), left ventricular hypertrophy (1/6) and mitral valve disease requiring replacement (1/6). Three patients showed evidence of mild haemolysis, one of whom had been investigated for iron overload thought to be secondary to chronic haemolysis.

Muscle disorders of glycogen metabolism associated with GYS1, GYG1 and RBCK1 deficiency

Anders Oldfors presented data on two novel glycogen storage diseases due to defects of the E3 ubiquitin ligase RANBP-type and C3HC4-type Zinc Finger-Containing 1 (RBCK1) and the autoglucosylating protein glycogenin-1 (GYG1) that acts as a primer for glycogen synthesis. Glycogen synthase is essential for glycogen synthesis by elongating the oligosaccharide primer formed by GYG1 by the addition of glucose molecules through α 1,4 glucosidic linkages.

Deficiency of muscle glycogen synthase (GYS1) causes muscle glycogen depletion in both skeletal and cardiac muscle. Onset is in childhood presenting with exercise intolerance and high risk of sudden cardiac arrest [62, 63].

Deficiency of GYG1 and RBCK1 share characteristic features in skeletal muscle [63, 64]. There is accumulation of abnormal glycogen partly with features of polyglucosan in fibers that show depletion of normal glycogen. The storage material is ubiquitinated and labeled with sequestosome-1 / p62 in both conditions. It also reacts with antibodies to desmin. Despite similar light and electron microscopic features, the storage material is in general less amylase resistant in GYG1 deficiency than in RBCK1 deficiency.

Cardiac involvement is usually not present in GYG1 deficiency but may be severe when it does occur in rare cases [65]. Cardiomyopathy is a consistent finding in RBCK1 deficiency and may require cardiac transplantation. As in skeletal muscle the cardiac storage material is less amylase resistant in GYG1 deficiency than in RBCK1 deficiency. Unlike GYG1 deficiency some patients with RBCK1 deficiency develop an auto-inflammatory disease in childhood reflecting the fact that RBCK1 deficiency affects many different tissues [66].

The pathogenesis of GYG1 deficiency involves reduced amount of the glycogen priming protein and/or loss of its ability to autoglucosylate. However, total lack of functional GYG1 is compatible with formation of apparently normal glycogen in addition to the abnormal storage. The substitute for GYG1 in glycogen priming in cases with GYG1 deficiency remains enigmatic as well as the pathogenesis of the abnormal glycogen. The pathogenesis of glycogen storage disease with polyglucosan in RBCK1 deficiency remains to be elucidated.

Renata Scalco described a patient with a limb girdle dystrophy phenotype with abnormal glycogen storage on muscle biopsy and homozygous mutation in GYG1. She presented with slowly progressive asymmetric muscle weakness affecting axial, proximal and distal limb muscles from the 4th decade. There was no evidence of respiratory or cardiac involvement and life-expectancy was not reduced.

Other rare muscle glycolytic disorders

Ron Haller described physiological studies in seven patients with phosphoglycerate kinase 1 deficiency (PGK1, GSDX), a sex linked recessive disorder caused by mutations in PGK1 at Xq21.1. Five subjects had a pure muscle phenotype with exertional cramps and myoglobinuria, two patients had central nervous system involvement and haemolytic anaemia ('muscle-plus' phenotype). All patients showed a blunted lactate response with exaggerated ammonia response on forearm testing, ³¹P-MRS showed delayed recovery following maximum exercise, peak VO₂ was around 30 kWatt or less. For all the parameters, exercise capacity was greater in the patients with the 'pure muscle' phenotype compared with those with the 'muscle-plus' phenotype.

Antonio Toscano presented two patients with Beta Enolase 3 deficiency (GSDXIII), one was Italian from Italy and the other of Turkish origin living and diagnosed in the UK. Both presented with exercise intolerance and myoglobinuria. Beta

Enolase 3 is an essential enzyme of terminal glycolysis which catalyses the interconversion of 2-phosphoglyceric acid (PGA) and phosphoenolpyruvate. Enolases from most species are dimeric. Mammals have three genes for beta enolase3, coding for the α , β , γ subunits; the subunits associate to form both homo- and heterodimers. The α subunit is expressed in many tissues, primarily in neurons and muscle. Both patients had normal baseline CK and a blunted lactate response on a forearm exercise test. EMG was normal in one patient and mildly myopathic in the other. Muscle biopsy showed normal histology, but a severe reduction of beta enolase was identified on enzymology. In both compound heterozygote mutations were identified in *ENO3* [66].

Debranching enzyme deficiency (GSDIII)

Pascal Laforêt gave an overview of GSDIII (debranching enzyme deficiency, Cori Forbes disease) an autosomal recessive disorder caused by mutations in *AGL*. The condition presents with liver disease by two months of age with hypoglycaemic seizures and hepatomegaly. Type 111b affects 15% patients and is a pure liver disease, in types 111c and 111d, in teenagers and adults fasting becomes less of a problem although the liver remains enlarged and the main problem becomes progressive proximal and distal muscle weakness together with cardiomyopathy. In these patients, serum CK is raised, usually around 4000 iu/l, and diagnosis is based on measuring debranching enzyme activity in blood and confirmed by genetic testing.

Management consists of high protein diet and overnight feeds. EMG shows mixed neurogenic and myopathic features. Muscle biopsy shows a vacuolar myopathy with increased cytoplasmic glycogen, although this is not necessary for diagnosis. GSDIII is a dynamic metabolic condition and forearm exercise testing shows impaired rise in lactate and raised ammonia, VO_2 max is reduced and during a constant workload cycling test blood glucose decreases while oral glucose supplementation improves

exercise performance with increased oxidation of palmitate and free fatty acid oxidation.

Animal models

Richard Piercy gave an overview of animal models. A number of the recognised glycogen storage disorders of humans have also been described in veterinary (domesticated) species (table 1). Indeed, recessive disorders are especially common in these domesticated mammals because of in-breeding. Further, some diseases are well characterised within certain breeds due to a founder effect and (unless they are maintained as research models of human disease) the disease can be bred-away from. Consequently, the recessive disorders tend to be recognised in ‘outbreaks’ when popular carrier animals are used for breeding within a limited gene pool, followed by a reduction in prevalence, as the disorder is recognised (and avoided) by breeders.

A novel, but highly prevalent glycogen storage disorder, known as polysaccharide storage myopathy type 1 (PSSM1) - perhaps best classified as GSD-0c is associated with a missense mutation in the equine *GYS1* gene (table 1) [67]. The causative R309H mutation is common in more than 30 breeds of horse [78], implicating an ancestral founder, with a possible positive selection in some breeds due possibly to enhanced performance or improved resilience to food shortage. It seems that with modern horse management (in particular high carbohydrate diets and a relative lack of exercise) the disorder manifests either as intermittent episodes of exertional rhabdomyolysis (with sometimes massive elevations in plasma creatine kinase activity), or as paresis or other forms of gait disturbance [78]; it can also be subclinical. Recent evidence confirms that the mutation causes constitutive activation of the mutant enzyme but the mechanism by which this leads to intermittent exercise-associated rhabdomyolysis remains unclear. Histopathologically, muscle from PSSM1-affected

horses has diastase-resistant amylopectate inclusions, increased glycogen deposition and sub-sarcolemmal vacuolation with a sometimes marked regenerative picture characterised by internalised nuclei – changes that resemble several human polyglucosan myopathies with other causes. Pathological changes in affected horses increase with age and can result in endomyseal fibrosis and grossly, muscle atrophy. Typically the disorder is managed by provision of regular exercise and low carbohydrate diets [79]; triheptanoin causes a detrimental response [80].

Serena Pagliarani gave an overview of the AGL knock-out mouse, a model for Glycogen Storage Disease type III which has a deletion of the glycogen binding domain at the 3' end of the gene. These mice have massive glycogen storage in liver, skeletal and cardiac muscle. The liver is darker than normal and enlarged, transaminases increase with age. The mice develop a progressive myopathy with kyphosis and CK is raised.

Histological and ultrastructural analysis shows progressive vacuolization of skeletal muscle due to glycogen accumulation leading to the disruption of contractile units. Interestingly, glycogen accumulation also occurs in brain and specific mild PAS-positive glycogen deposits can be detected in the granular layer just adjacent to the Purkinje cells in cerebellum.

Euromac Registry

Ramon Martí gave an overview of Euromac, a project funded by the European Commission (Consumers, Health, Agriculture and Food Executive Agency; CHAFEA), the purpose of the registry is to understand the epidemiology and natural history of these rare glycogen storage disorders and facilitate access to specialized care. Euromac also includes two important work packages dedicated to education and dissemination of knowledge of these diseases among patients and clinicians. Thirteen partners from 7

European countries participate in the project (United Kingdom, Denmark, France, Germany, Spain, Italy and Greece), in addition to 5 collaborating partners from Turkey, France, Spain and the USA. The registry is operational and currently has registered almost 300 patients.

The project has ethical approval and all patients willing to participate in the registry must sign an informed consent form. Individual personal data is encrypted and the identity of the patient is only known by the registering physician. The registry accepts contributions from centers of all countries provided their institutional review board have approved the project and the information sheet and consent form has been translated to the local language. Information recorded in the registry is divided in 9 groups of variables: personal data (encrypted), demographics, diagnostic data, clinical data, concomitant diseases, other genetic factors, patient-reported functional data, previous and ongoing treatments and services provided.

EUROMAC aims improve our understanding of the natural history of these disorders and to provide a platform for future clinical trials.

Summary

Disorders of glycogenolysis cause dynamic symptoms of exercise intolerance with myoglobinuria, muscle weakness, with or without, multi-system features such as: haemolytic anaemia, CNS involvement, cardiac and liver disease. The specific phenotype is dependent upon the enzyme deficiency and genetic mutation. Apart from beta enolase deficiency, the baseline CK is usually elevated. The second wind phenomenon is limited to GSDV. In most instances diagnosis can be made by DNA analysis and a muscle biopsy is not required. Muscle biopsy is not necessary for diagnosis, which should always be confirmed with genetic studies. Evidence suggests that patients with GSDV benefit from aerobic training and that exercise is safe in the

other glycolytic disorders. More interestingly, early studies suggest that carefully managed resistance training can alleviate symptoms and is not harmful. Thus regular exercise is recommended for all of these patients. The evidence from clinical trials for diet is less convincing, although a great many GSDV patients are managing themselves with a ketogenic diet and report benefit. Fasting and ketogenic diets have also been reported anecdotally by patients with GSDVII to give benefit. Because of the paucity of published data developing clinical management guidelines was not possible.

The Euromac registry will shed further light on the natural history of these rare disorders and, more importantly, will assist in the recruitment of patients into larger scale international clinical trials. Future research should focus on randomized controlled clinical trials to evaluate physical training (aerobic and resistance), high fat/ ketogenic diet, sodium valproate and Triheptanoin. The non-ischaemic forearm exercise test, cycle ergometry or the 12-minute walk test can be useful outcome measures for such studies.

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Table1: Veterinary models of glycogen storage diseases (GSD). Note that the highly prevalent glycogen synthase 1 (GYS1) mutation found in multiple horse breeds, is a novel glycogen storage disorder, because the missense mutation (R309H) causes a gain of function, unlike GSD-0b (associated with deficiency of glycogen synthase 1).

Glycogen storage disorder	Gene	Species	Breed	Reference
GSD-0c	<i>GYS1</i>	Horse	Multiple breeds	67
GSD-II	<i>GAA</i>	Dog	Lapphund	68
GSD-IIIa	<i>AGL</i>	Dog	Curly Coat Retriever	69
GSD-IV	<i>GBE1</i>	Cat	Norwegian	70
		Horse	Forest Quarter Horse	71
GSDV	<i>PYGM</i>	Sheep	Merino	72
		Cow	Charolais	73
GSD-VII	<i>PFK-M</i>	Dog	English Springer Spaniel	74
			American Cocker Spaniel	75
			Whippet	76
			Wachtelhund	77