In this selective review, we consider a number of unsolved questions regarding the glycogen storage diseases (GSD). Thus, the pathogenesis of Pompe disease (GSD II) is not simply explained by excessive intralysosomal glycogen storage and may relate to a more general dysfunction of autophagy. It is not clear why debrancher deficiency (GSD III) causes fixed myopathy rather than exercise intolerance, unless this is due to the frequent accompanying neuropathy. The infantile neuromuscular presentation of branching enzyme deficiency (GSD IV) is underdiagnosed and is finally getting the attention it deserves. On the other hand, the late-onset variant of GSD IV (adult polyglucosan body disease APBD) is one of several polyglucosan disorders (including Lafora disease) due to different etiologies. We still do not understand the clinical heterogeneity of McArdle disease (GSD V) or the molecular basis of the rare fatal infantile form. Similarly, the multisystemic infantile presentation of phosphofructokinase deficiency (GSD VII) is a conundrum. We observed an interesting association between phosphoglycerate kinase deficiency (GSD IX) and juvenile Parkinsonism, which is probably causal rather than casual. Also unexplained is the frequent and apparently specific association of phosphoglycerate mutase deficiency (GSD X) and tubular aggregates. By paying more attention to problems than to progress, we aimed to look to the future rather than to the past.

Key words: glycogen storage diseases, GSD, polyglucosan disorders

Why writing of glycogen storage diseases (GSD), which are “old hats” among the metabolic disorders affecting skeletal muscle? Why writing of GSD to honor Valerie Askanas and King Engel? Why writing with Ronen Spiegel? The answer to the first question is deeply personal: the very first paper of the senior (chronologically, not academically) author (SDM) described a little girl with Pompe disease (1) and his first project as a postdoctoral fellow with Dr. Lewis P. (Bud) Rowland was to unravel why glycogen accumulation is not limitless in muscle (2). The answer to the second question is an exciting, if ancient, collaboration showing that both morphological and biochemical features of Pompe disease were reproduced in muscle culture (3). The answer to the third question is a much more recent collaboration on a patient who had phosphoglycerate kinase (PGK) deficiency (GSD IX) and a pure myopathy, or so we thought initially (see below) (4).

The truth is that GSD are still very much an open chapter, where new entities are discovered (5, 6), apparently paradoxical myopathies due to lack, rather than excess, of glycogen (aglycogenosis or GSD 0) are being reported (7, 8), old disorders, such as Lafora disease, are now recognized as GSD (9), and therapy based on enzyme replacement is reasonably successful in GSD II (10, 11). This is not meant to be a comprehensive review of the muscle glycogenoses. Rather, we will consider puzzling aspects of some GSD, following the Roman numerical order shown in Figure 1.

GSD II (acid maltase deficiency, Pompe disease)

The first and oldest conundrum about this disorder was its clinical heterogeneity, with a severe generalized infantile form and a later-onset form largely confined to skeletal muscle and presenting in children (juvenile onset) or in adults (late-onset). As acid maltase (acid α-glucosidase, GAA) is a single ubiquitous protein, it is not surprising that initial findings of different residual GAA activities in muscle (12) have been confirmed and related to the severity of GAA mutations, with nonsense mutations prevailing in the infantile form and missense
and splicing (i.e. “leaky”) mutations prevailing in later onset cases (13, 14).

A second riddle regarded the pathogenesis of weakness: was it simply due to the mechanical disarray of the contractile material caused by the glycogen-laden lysosomes or to an energy defect? The former mechanistic hypothesis is questionable both because it does not explain the weakness of later onset GSD II and because equally disruptive overloads of lipid droplets in muscle were compatible with normal strength in a preclinical case of neutral lipid storage disease with myopathy (NLSDM) (15). The latter energetic hypothesis is supported by the notion that GAA activity normally “returns” to the cytoplasm the end product of lysosomal glycogen digestion, glucose. It is also telling that in muscle from patients with the infantile form of GSD II most glycogen is free in the cytoplasm, probably released by “burst lysosomes” (13).

A more compelling scenario for the pathogenesis of GSD II, as well as other lysosomal storage disorders (16), involves a disruption of the vital autophagic process, with accumulation of autophagosomes resulting from defective autophagosome-lysosome fusion (16, 17). In fact, Nishino and colleagues went as far as stating that “Pompe disease can no longer be viewed simply as a glycogen storage disease,” but rather as a problem in handling excessive numbers of autophagosomes (13).

A unique feature of GSD II is the availability of a generally effective – and now widely utilized – enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA). There is already a vast literature on the subject (10), and a few problems have emerged, such as the immune reaction to rhGAA in infants with null mutations and no GAA protein (i.e. no cross-reactive immunological material, CRIM) (18). Assumption of rhGAA into lysosomes is probably hindered by the more general autophagic dysfunction mentioned above and several strate- gies have been proposed to improve uptake, including conjugation of rhGAA with a synthetic oligosaccharide harboring mannose-6-phosphate (19), combining ERT with chaperones (20), and inhibition of glycogen synthesis (21, 22).

One final clinical note: besides skeletal muscle, smooth muscle must also be affected in late-onset GSD II because there have been a few reports of cerebral arterio- pathies, often affecting the basilar artery (23, 24).

**GSD III (debrancher enzyme deficiency; Cori-Forbes disease)**

The debrancher is a “double duty” enzyme, with two catalytic functions, oligo-1,4-1,4-glucantransferase and amylol-1,6-glucosidase. Once phosphorylase has shortened the peripheral chains of glycogen to about four glucosyl units (this partially digested glycogen is called phosphorylase-limit dextrin, PLD), the debrancher enzyme removes the residual stumps in two steps. First, a maltotriosyl unit is transferred from a donor to an acceptor chain (transferase activity), leaving behind a single glucosyl unit, which is then hydrolyzed by the amylo-1,6-glucosidase, at which point the branch is off. A single-copy gene, AGL, encodes the debrancher enzyme.

A comprehensive summary of clinical features and therapeutic options has been published recently (25).

Considering the predominantly myopathic presentation of GSD III, a clinician would likely question why
the defect of an enzyme that acts hand-in-hand with myo-
phosphorylase should cause weakness rather than cramps
and myoglobinuria, the clinical hallmarks of McArdle
disease. One reason for this discrepancy may be that in
McArdle disease glycogen cannot be metabolized at all,
whereas in GSD III the peripheral chains of normal gly-
cogen can be utilized. However, this explanation postu-
lates that the intact glycogenosynthetic pathway allows
some turnover between normal glycogen and PLD, which
is not unreasonable.

Another explanation for the fixed and mostly dis-
tal weakness of patients with GSD III (26) is the si-
multaneous involvement of muscle and nerve, as
documented both electrophysiologically and by nerve
biopsy (27, 28).

GSD IV (branching enzyme
deficiency, Andersen disease)

The glycogen branching enzyme (GBE) is a single
polypeptide encoded by one gene (GBE1). GBE deficien-
cy results in the deposit of an amylopectin-like polysac-
charide that has fewer branching points and longer outer
chains than normal glycogen and is known as polyglu-
cosan. Polyglucosan is periodate/Schiff (PAS)-positive
and only partially digested by diastase, which makes it
easily recognizable in various tissues and offers an im-
portant clue to the correct diagnosis.

It is gratifying to see that in the just published 22nd
edition of Rudolph’s Pediatrics, the neuromuscular pres-
tentation of GSD IV is given as much space as the hepatic
form (29), which dominated previous textbook descrip-
tions.

In fact, the neuromuscular presentation has been un-
derdiagnosed, judging from the flurry of recent papers.
As recognized in a seminal paper of 2004 (30), there are
two main infantile presentations. The first is a perinatal
disorder known as “fetal akinesia deformation sequence”
or FADS, characterized by multiple congenital contrac-
tures (arthrogryposis multiplex congenita), hydrops fe-
talis, pulmonary hypoplasia, craniofacial abnormalities,
intrauterine retarded growth (IURG), abnormal amniotic
fluid volume, and perinatal death. The second, labeled
“congenital,” should probably be called “fetal infantile,”
as it presents at or soon after birth with hypotonia, muscle
wasting, neuronal involvement, inconsistent cardiomy-
opathy, and early death.

Detailed neuropathology in a girl who died at 3
months showed PAS-positive polyglucosan inclusions in
neurons of basal ganglia and thalamus, oculomotor and
pontine nuclei, and in periaqueductal neurons (31). In
the medulla, polyglucosan deposits were noted in the hy-
poglossal nucleus, the dorsal motor nucleus of the vagus,
and the nucleus ambiguus. Similar findings were reported
in two more infants (32, 33). The motor neurons of the
spinal cord are also severely affected (32), explaining how
one of the patients we studied was initially diagnosed as
spinal muscular atrophy type I (SMA I) until mutations in
the SMN1 gene were ruled out (34).

At the other end of the clinical spectrum, there is
adult polyglucosan body disease (APBD), a neurological
variant of GSD IV presenting late in life with progressive
upper and lower motor neuron dysfunction (simu-
lating amyotrophic lateral sclerosis), sensory neuropathy,
neurogenic bladder (APBD patients often see a urolo-
gist before they see a neurologist), and – in about 50%
of patients – dementia. The disease predominates among
people of Askenazi Jewish descent (probably due to a
founder effect) and is most commonly due to the Y329S
mutation in GBE1. Not too surprisingly, this is a “mild”
mutation, which probably explains the late onset of
symptoms. Thanks to the energy and compassion of one
patient, Gregory Weiss, a research foundation (APBDRF;
www.apbdrf.org) has been created to develop therapeutic
strategies.

GSD V (myophosphorylase
deficiency, McArdle disease)

The clinical picture and the block in muscle glycogen
breakdown were elegantly described by Brian McArdle
in 1951 (35), the enzyme defects was discovered 8 years
later (36-38), and it took 12 more years before the first
mutations in PYGM were identified (39).

Despite its long history, McArdle disease still presents
several riddles. First, although it was long considered a
clinically homogeneous disease, its expression can vary
from relatively mild exercise intolerance to a crippling
condition with frequent cramps and recurrent episodes of
myoglobinuria. Explanations have ranged from rare cases
of “double trouble” (i.e. the coexistence in the same indi-
viduals of one mutation in PYGM and another in the gene
encoding adenylylate deaminase) to the association with
insertion/deletion polymorphisms in the angiotensin-
converting enzyme (ACE) (40). Probably more important
is the protecting effect of even small amounts of myo-
phosphorylase residual activity, which is determined by
the type of mutations: for example, splice mutations are
associated to milder clinical phenotypes (41).

What remains unexplained is the fatal infantile form
of McArdle disease, which has been reported in a handful
of cases (42-45). In these unfortunate infants muscle mor-
phology, biochemistry, and molecular genetics [showing
the “common” R50X null mutation (39, 45)] are no dif-
ferent from typical McArdle patients, despite the dismal
outcome.
**GSD VII (phosphofructokinase [PFK] deficiency, Tarui disease)**

PFK is a tetrameric enzyme under the control of three autosomal genes: **PFKM** encodes the muscle subunit, **PFKL** encodes the liver subunit, and **PFKP** encodes the platelet subunit. Mature human muscle expresses only the M subunit and contains exclusively the M4 homotetramer, whereas erythrocytes, which express both the M and the L subunit, contain five isozymes, the two M4 and L4 homotetramers and three hybrid forms. In patients with typical PFK deficiency, mutations in **PFKM** cause total lack of activity in muscle but only partial deficiency in red blood cells.

Clinically, PFK deficiency, first described in 1965 in a Japanese family (46), is indistinguishable from McArdle disease, except for the absence of a typical second wind phenomenon and for the occasional presence of gouty arthritis due to “myogenic hyperuricemia” (47).

Two peculiar aspects of GSD VII are worth discussing: the presence of polyglucosan in muscle and the severe infantile presentation.

The presence — in addition to normal-looking glycogen — also of abnormal glycogen with the histochemical (diastase-resistance) and ultrastructural (fine granules and filaments instead of β-particles) features of polyglucosan was first noted in the muscle biopsy of two patients (48) and confirmed in a woman who had developed late-onset fixed weakness (49). We reasoned that this surprising finding could best be explained by the excessive accumulation of glucose-6-phosphate (G6P) upstream of the glycolytic block (49). As G6P is a functional activator of glycogen synthetase (GS), the finely balanced activity ratio of GS and GBE would be tilted in favor of GS and result in a polysaccharide with abnormally long and poorly branched chains, i.e. polyglucosan.

This pathogenic concept was confirmed by two experiments, one in the laboratory, the other an experiment of nature. First, when Nina Raben upregulated the expression of GS in the muscle of GAA-deficient mice, she unexpectedly obtained polyglucosan accumulation (50). Second, after a long search for the molecular basis of polyglucosan myopathy in horses, Stephanie Valberg and co-workers identified a gain-of-function mutation in GS, again altering the GS/GBE activity ratio in favor of GS (5).

The second riddle concerns the fatal infantile variant of GSD VII, reported in a dozen patients between 1987 and 2008. All infants were severely hypotonic at birth and a few developed joint contractures either in utero (51-53) or postnatally (54, 55). Decreased fetal movements were noted in two pregnancies (52, 53) and polyhydramnios in one (53). In all but two cases (53, 55), death occurred in infancy or early childhood due to pulmonary failure.

Most children showed evidence of multisystem involvement, including seizures, cortical blindness, developmental delay, dysmorphic features, and corneal ulcers. The encephalopathy was documented by neuroradiology or neuropathology, which showed dilated ventricles and cortical or cerebellar atrophy (51, 54-57).

Because of the early onset, multisystem involvement, and lack of any molecular evidence of mutations in the **PFKM** gene, the infantile variant of phosphofructokinase deficiency appears to be a separate entity from GSD VII, and its genetic basis (or bases) remain to be clarified, despite evidence that a transgenic **PFKM-null** mouse mimics the infantile more than the typical muscular form of the human disease (58).

**GSD VIII (Phosphorylase b kinase [PHK] deficiency)**

PHK is a multimeric enzyme composed of four different subunits, α, β, γ, and δ and the enzyme composition is (αβγδ)₄. The γ subunit is catalytic and is regulated by the degree of phosphorylation of the α and β subunits. Calcium sensitivity is conferred by the δ subunit, which is tightly bound to calmodulin.

PHK deficiency has been associated with five main syndromes distinguished by inheritance and by tissue involvement: (i) a benign X-linked recessive hepatopathy of infancy or childhood (59); (ii) an autosomal recessive liver and muscle disease (60); (iii) a pure myopathy predominant in men (61); (iv) an autosomal recessive severe liver disease with cirrhosis (62); and (v) a fetal infantile cardiopathy, reported in a handful of patients (63-68).

The pure myopathy has thus far been described in detail only in men and is due to mutations in the X-linked gene (**PHKA1**) encoding the muscle-specific α subunit (69-73). Not surprisingly, patients with PHK deficiency have a clinical picture resembling McArdle disease, except much milder, a sort of “McArdle light.” For example, patients usually have normal venous lactate rise after forearm ischemic exercise, no evidence of second wind, and modest accumulation of glycogen in the muscle biopsy. Formal cycle ergometry studies confirmed the mild impairment of glycogenolysis: there was no change in lactate during dynamic, submaximal exercise and IV glucose administration improved exercise tolerance, but less than in McArdle patients (72).

The molecular basis underlying the fatal infantile cardiomyopathy has been a puzzle for many years because there is no heart-specific PHK isozyme. The riddle was solved when Burwinkel et al. definitely excluded mutations in any of the PHK genes (74) but detected a single dominant mutation in the gene (**PRKAG2**) encoding the γ2 subunit of the AMP-activated protein kinase.
(AMPK). Later, we identified a second mutation in another infant (75). AMPK is an aβγ heterotrimer functioning as a “cellular fuel gauge,” which is switched on by increases in the AMP:ATP ratio, an indicator of cellular energy deficit (76).

What remains a mystery is why mutations in AMPK should inhibit PHK and cause a “pseudo-PHK deficiency.” We suspect that a similar mechanism may operate in the fatal infantile PGK deficiency that we discussed above.

**GSD IX (phosphoglycerate kinase [PGK] deficiency)**

PGK is a single polypeptide encoded by a gene (PGKI) on Xq13 and present in all tissues except spermatogenic cells. Although this enzyme is virtually ubiquitous, clinical presentations depend on the isolated or combined involvement of three tissues: erythrocytes (hemolytic anemia), skeletal muscle (exercise intolerance, cramps, myoglobinuria), and the central nervous system ([CNS] seizures, mental retardation, stroke).

In a recent review (4), we found that the most common association, seen in 11 of 33 patients (34%) was hemolytic anemia and CNS involvement. Isolated myopathy was a close second (9 of 33 patients, 27%). Isolated blood dyscrasia was reported in 6 patients (18%), the association of anemia and CNS dysfunction in 4 patients (12%), the association of anemia and myopathy only in one patient (3%), and the involvement of all three tissues in 2 patients (6%).

However, the plot thickened when we found yet another patient with the association of myopathy and a peculiar CNS dysfunction, namely, severe juvenile Parkinsonism (77). What we found strange and a little disconcerting was that this young man harbored the same previously unreported mutation (p.T378P) that we had identified in our latest patient with pure myopathy (4). However, we recovered some confidence in genotype:phenotype correlation when Dr. Spiegel’s patient also developed severe Parkinsonian symptoms and signs. Although this is an n of 2 series, our findings raise two interesting questions. First, is there, in fact, a causal relationship between the T378P mutation and Parkinsonism? Second, PGK deficiency was suspected in both patients because they presented initially with exercise intolerance, cramps, and myoglobinuria: Parkinsonism was a surprising clinical development. One cannot help wondering whether in some patients with PGK deficiency juvenile Parkinson disease may precede and overshadow the myopathy, thus escaping diagnosis. Certainly, this association has to be kept in mind.

**GSD X (phosphoglycerate mutase [PGAM] deficiency)**

PGAM is a dimeric enzyme composed of a muscle-specific (M) subunit and a brain-specific (B) subunit. Normal adult human muscle contains predominantly the MM homodimer, which accounts for about 95% of the total activity.

Fourteen patients with PGAM deficiency in muscle have been reported, of whom nine were African American (78, 79). Although the first reported patient could not be studied at the molecular level (80), all other African American patients harbored the W78X mutation, at least in heterozygosity, suggesting a founder effect.

The most striking peculiarity of GSD X is its common association with tubular aggregates (TAs), which were seen in the muscle biopsies of 5 patients (36%) whereas they have never been reported in other glycogenoses. TAs are ordered stacks of tubules originating from the sarcoplasmic reticulum. Although they are a nonspecific pathological change seen in diverse conditions, including exposure to drugs, toxins, and hypoxia, their association with PGAM deficiency does not appear to be casual although the specific trigger remains unknown.

**Conclusions**

As stated at the outset, we did not intend to review all the muscle glycogenoses, but only to consider some conundrums still presented by “old” GSD. We have not considered Lafora disease because muscle involvement is overshadowed by the devastating encephalopathy. Likewise, we have not discussed some recently described glycogenoses, such as aldolase deficiency (81), β-enolase deficiency (82), and the two forms of glycogenosis type 0 (aglycogenosis?) (7, 8) because they have been described in single patients.

Thus, although we discussed more the problems than the progress promised in the title, we hope our considerations are an adequate homage to Valerie Askanas and W. King Engel.

**References**


