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REVIEW AND PERSPECTIVES

Update on polyglucosan storage diseases

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Abstract

An abnormal structural form of glycogen (with less branching points or amylopectin-like polysaccharide) called polyglucosan (PG) may accumulate in various tissues such as striated and smooth muscles, brain, nerve, liver and skin, and cause a group of nine different genetic disorders manifesting with a variety of clinical phenotypes that affect mainly the nervous system (Lafora disease, adult PG body disease), the heart (glycogen storage disease type XV, hypertrophic cardiomyopathy type 6, PG body myopathy type 1) and the skeletal muscle (glycogen storage disease type IV, glycogen storage disease type VII, PG body myopathy type 2), depending on the organs which are mostly affected by the PG aggregates. The pathological feature of PG storage in tissues is a hallmark of these disorders. Whole-genome sequencing has allowed to obtain a diagnosis in a large number of patients with a previously unrecognized disorder. We describe the clinical, pathological and molecular features of these genetic disorders, for many of which the pathological mechanisms underlying the corresponding mutant gene have been investigated and, at least in part, understood.

Keywords Polyglucosan · Polyglucosan storage · Glycogenin-1 · Lafora · Glycogen storage

Glycogen is the storage form of glucose in the cells, and it is essential for energy supply and glucose homeostasis. It is a highly branched polymer of glucose molecules connected by α-1,4-glucosidic linkages, and branched by α-1,6-glucosidic linkages (Fig. 1).

De novo biosynthesis of glycogen requires the initiator protein called "glycogenin" (EC 2.4.1.186), a glycosyltransferase that catalyzes the formation of a glucose polymer using uridine-diphosphate (UDP)-glucose as donor [1, 2]. An un-glycosylated dimer of apo-glycogenin-1 is auto-glycosylated by an initial glucose-1-O-tyrosine linkage formed with the hydroxyl group of tyrosine-195 residue [3, 4], followed by addition of 8–13 glucose molecules by several α-1,4-glucosidic linkages (Fig. 1). This glycogenin-oligosaccharide molecule constitutes the primer for the reactions of elongation [at the non-reducing end by glycogen synthase (EC 2.4.1.11)], and branching [by glycogen branching enzyme (EC 2.4.1.18)]. Each glycogen granule (β-particle) contains approximately 55,000 glucose residues with numerous non-reducing ends for rapid access to glucose. A glycogen molecule is soluble because its glucose chains are short (13 units), each chain is a branch of another, and the whole molecule has a spherical structure [4].

An abnormal quantity or quality of glycogen accumulating in tissues causes a group of disorders called "glycogen storage diseases (GSDs)", which were among the earliest inborn errors of metabolism for which specific enzyme deficiencies were identified. Research on GSDs has identified enzyme deficiencies and their underlying gene mutations for nearly all steps of the pathways of glycogen and glycolysis metabolism ("primary GSDs") (Table 1), but GSDs can also be caused by mutations in proteins which have an indirect impact on glycogen metabolism (e.g. regulatory proteins), leading to "secondary GSDs" [5].

In GSDs, an increased quantity of glycogen is most frequently found, but rare defects of glycogen synthesis may lead to reduced glycogen level. Conversely, an abnormal quality of

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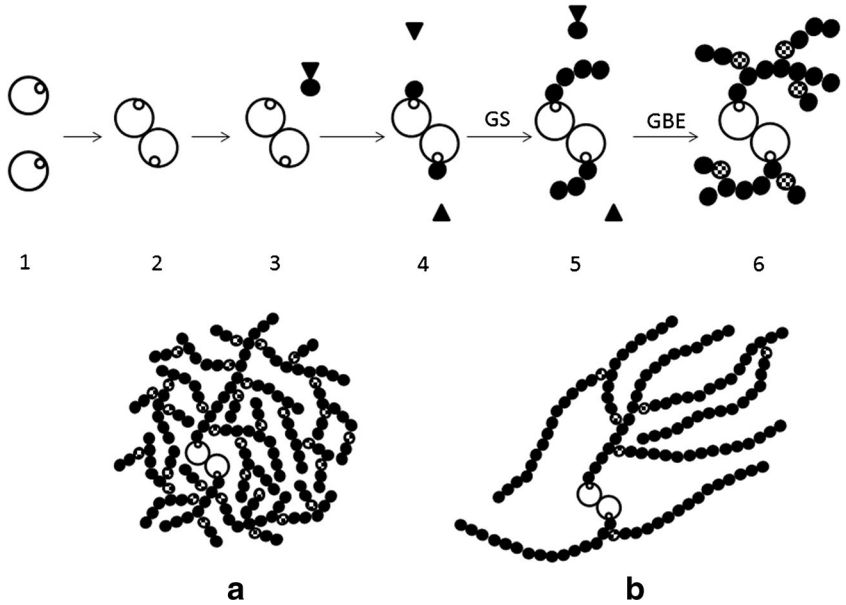

Fig. 1 The upper panel shows the step-by-step synthesis of polysaccharide molecules. Two molecules of apo-glycogenin-1 (1) dimerize (2), and autoglucosylate by UDP-glucose (3) in which a glucose molecule (filled circle) is added by O-glycosylation linkage with the hydroxyl group of tyrosine-195 residue (small empty circle) and release of UDP (filled triangle) (4); the reaction ends with the formation of a glycogenin-oligosaccharide molecule which constitutes the primer or core for the following reactions (4). Glycogen synthase (GS) catalyzes the reaction of glycogen chain elongation (5) with numerous α-1,4-linked glucose units (filled circles). Glycogen branching enzyme (GBE) catalyzes the glycogen branching process (6), in which several α-1,6-linked glucose units (dotted circles) are added. The lower panel shows the schematic representation of glycogen molecule (**a**), and of polyglucosan molecule (**b**). Glycogen is a heavily branched molecule characterized by numerous α-1,6-linked glucose units (dotted circles) and short peripheral chains, conferring a spherical structure with soluble property. Conversely, polyglucosan is characterized by less numerous α-1,6-linked glucose units and long peripheral chains of α-1,4-linked glucose units (filled circles), conferring an abnormal structure with insoluble property.

glycogen indicates that the branching structure of glycogen is anomalous (compromising its function as a readily convertible energy storage), leading to rare forms of GSDs which are characterized by storage of a polysaccharide called "polyglucosan" (PG) (Fig. 1). As compared with glycogen, PG has fewer branched points, more numerous α-1,4-linked units, excessively long peripheral chains, and a structure resembling that of the plant polysaccharide called "amylopectin". PG lacks glycogen's spherical structure, and, similar to amylopectin or starch, it is insoluble, precipitates and accumulates in deposits called "PG bodies", which can be easily identified by their typical histopathological and ultrastructural features. Unlike glycogen molecule, PG is resistant to α-amylase or diastase digestion.

We focus on disorders caused by PG storage, omitting GSDs such as the most frequent forms of glycogenosis: GSD2 (Pompe disease) and GSD5 (McArdle disease) (Table 1). PG storage in tissues characterizes a variety of disorders that clinically affect mainly the nervous system (e.g. Lafora disease and adult-onset PG body disease—APBD), the heart (e.g. GSD15 and dominant hypertrophic cardiomyopathy—HCM6) or the skeletal muscle (e.g. PG body myopathy type 1 and type 2—PGBM1, PGBM2, GSD4—Andersen disease, GSD7—Tarui disease).

Genes associated with PG storage diseases (Table 2)

Several genes are known to be associated with PG storage diseases, including:

- *GYG1 (NM_004130)*: encodes for glycogenin-1; causes PGBM2 and GSD15
- *GBE1 (NM_000158)*: encodes for glycogen branching enzyme; causes GSD4 and APBD
- *RBCK1 (NM_031229)*: encodes for ring-B-box C-kinase-1; causes PGBM1 and immunodeficiency with cardiomyopathy
- *PFKM (000289)*: encodes for muscle phospho-fructo-kinase; causes GSD7
- *EPM2A (NM_005670), EPM2B (NM_198586), PRDM8 (NM_020226)*: encode for laforin, malin and E3-ubiquitin ligase; cause Lafora disease
- *PRKAG2 (NM_016203)*: encodes for AMP-protein kinase γ-2; causes dominant hypertrophic cardiomyopathy type 6 (HCM6).

Of these, *GYG1* and *GBE1* genes encode for enzymes involved in glycogen synthesis, *PFKM* encodes for an enzyme

Table 1 Classification of glycogen storage diseases (GSDs)

		GSD type	Enzyme defect	Disease name	Gene symbol	Gene locus	Inheritance
Primary GSDs*	Disorders of glycogen synthesis	GSD0	Glycogen synthase		*GYS1* *GYS2*	19q13.33 12p12.1	AR
		GSD15	Glycogenin-1		*GYG1*	3q24	AR
		PGBM2	Glycogenin-1	PGBM2			
		GSD4	Brancher (amylo-1,4-1,6 transglucosidase)	Andersen	*GBE1*	3p12.2	AR
	Disorders of glycogen breakdown (glycogenolysis)	GSD3	Debrancher (amylo-1,6 glucosidase)	Cori-Forbes	*AGL*	1p21.2	AR
		GSD5	Muscle glycogen phosphorylase	McArdle	*PYGM*	11q13.1	AR
		GSD6	Liver glycogen phosphorylase	Hers	*PYGL*	14q22.1	AR
	Disorders of glycolysis	GSD1	Glucose-6-phosphatase	Von Gierke	*G6PC*	17q21.3	AR
		GSD7	Muscle phospho-fructo kinase	Tarui	*PFKM*	12q13.11	AR
			Phospho-glycerate kinase		*PGK1*	Xq21.1	XR
		GSD10	Phospho-glycerate mutase		*PGAM2*	7p13	AR
		GSD11	Lactate dehydrogenase	Fanconi-Bickel	*LDHA*	11p15.1	AR
		GSD12	Aldolase-a		*ALDOA*	16p11.2	AR
		GSD13	β-enolase		*ENO3*	17p13.2	AR
		GSD14	Phospho-gluco mutase		*PGM1*	1p31.1	AR
	Lysosomal glycogenoses	GSD2	Acid maltase (acid α-1,4-glucosidase)	Pompe	*GAA*	17q25.3	AR
		GSD2B	Lysosomal associated membrane protein 2	Danon	*LAMP2*	Xq24	XD
Secondary GSDs*		GSD9A	Phosphorylase kinase		*PHKA2*	Xp22.13	XR
		GSD9b			*PHKB*	16q12.1	AR
		GSD9C			*PHKG2*	16p11.2	AR
		–	Laforin (glycogen phosphatase)	Lafora	*EPM2A*	6q24	AR
		–	Malin (E3 ubiquitin ligase)	Lafora	*EPM2B*	6p22.3	AR
		–	PR domain containing protein 8	Lafora	*PRDM8*	4q21.21	AR
		PGBM1	RBCK1 (E3 ubiquitin ligase)	PGBM1	*RBCK1*	20p13	AR
		–		Immunodeficiency cardiomyopathy			
		HCM6	AMP-protein kinase-γ2	Hypertrophic cardiomyopathy	*PRKAG2*	7q36.1	AD

*Primary GSDs are caused by enzyme/protein deficiencies for the steps of the pathways of glycogen and glucose metabolism; secondary GSDs are caused by deficiencies in enzyme/proteins which have an indirect impact on glycogen metabolism (e.g. regulatory proteins) [5]

PGBM, polyglucosan body myopathy; *HCM*, hypertrophic cardiomyopathy; *AR*, autosomal recessive; *XR*, X-linked recessive; *XD*, X-linked dominant; *AD*, autosomal dominant

involved in glycolysis, and *RBCK1, EPM2A, EPM2B, PRKAG2* genes encode for regulatory proteins (Table 1).

In the recent years, the use of wide-range genetic screening in identifying the causative genes of PG storage disorders among patients with unclassified forms of myopathy or cardiomyopathy has demonstrated its value as a powerful diagnostic tool.

Pathogenetic mechanism of PG storage

The investigations of the pathogenetic events causing PG accumulation have suggested a role of both ubiquitin-proteasomal system (UPS) and lysosomal-autophagic pathway.

PG inclusions are strongly reacting for ubiquitin, suggesting that the stored material is recognized as abnormal and it becomes ubiquitinated to be degraded by the UPS, and develops amylase resistance. Proteasomal degradation is initiated by labelling the targeted misfolded proteins with poly-ubiquitin chains, with the coordinated action of enzymes including the E3-ubiquitin ligases. Ubiquitin is only known to recognize abnormal proteins but not polysaccharides; however, glycogen molecules include the enzyme protein glycogenin, which is the molecule targeted by ubiquitin [6].

Table 2 Glycogen storage diseases with PG body accumulation

Disease name	Disease MIM #	Organs clinically affected	Tissues with storage of PG bodies	Gene symbol	Protein
Lafora disease (LD)	254780	Brain, nerve	Brain, heart, nerve, skin, muscle, liver, retina	*EPM2A*	Laforin (glycogen phosphatase)
				EPM2B	Malin (ubiquitin ligase)
				PRDM8	PR domain containing protein-8
Adult PG body disease (APBD)	263570	Brain, nerve	Brain, nerve	*GBE1*	Glycogen branching enzyme
Glycogen storage disease type IV (GSD4)	232500	Muscle, liver, heart, brain	Muscle, heart, brain, liver, nerve		
Glycogen storage disease type VII (GSD7)	232800	Muscle	Muscle	*PFKM*	Phospho-fructo kinase
Immunodeficiency, auto-inflammation, cardiomyopathy	615895	Immune system	Heart, muscle, liver	*RBCK1*	Ring-box protein C-kinase (ubiquitin ligase)
Polyglucosan body myopathy type 1 (PGBM1)	615895	Muscle, heart	Muscle, heart		
Polyglucosan body myopathy type 2 (PGBM2)	616199	Muscle	Muscle	*GYG1*	Glycogenin-1
Glycogen storage disease type XV (GSD15)	613507	Heart, muscle	Heart		
Hypertrophic cardiomyopathy type 6 (HCM6)	600858	Heart, muscle	Heart, muscle	*PRKAG2*	PR kinase AMP-activated γ-subunit

A functional link between proteasomal function and PG accumulation has been provided by the demonstration that blocking the proteasomal activity results in the formation of PG storage in cell models [7]. The role of the UPS in muscle from patients with PG body myopathy has also been suggested by overexpression of MuRF-1 [8], a muscle-specific E3-ubiquitin ligase involved in protein degradation through the UPS [9]. The activities of UPS and autophagy occur at low basal levels to perform homeostatic functions including the control of protein quality and quantity, but when either the proteasomal or the lysosomal degradation is impaired, or when the amount of material to be degraded exceeds the normal capacity, ubiquitinated proteins may aggregate and form autophagic vacuoles. In PG body myopathy, the accumulated PG are likely to be insufficiently degraded by the UPS, and may cause both an induction of autophagy and an impaired autophagic flux.

A dysfunction in the UPS and/or autophagic processes has been implicated in many pathological conditions with PG storage. An increased level of sequestosome-1 (SQSTM1 or p62), a protein that binds aggregates of ubiquitinated misfolded proteins and LC3 protein, and of the lipidated form of LC3-II was demonstrated in the muscle of patients with PG body myopathy type 2 [8], and was found to cause PG accumulation in the animal model of Lafora disease [10]. Lafora disease results from mutations in the genes encoding either for the glycogen phosphatase "laforin" or the E3-ubiquitin ligase "malin". In the absence of laforin, the abnormal glycogen remains hyper-phosphorylated and aggregate in PG bodies. The fact that different E3-ubiquitin ligases (i.e. malin, RBCK-1) are responsible of PG storage diseases suggests that a non-canonical glycogen degradation system may be implicated in such disorders [11]: glycogen-selective autophagy might be effective in removing PG and in relieving their damage [12]. The mechanism generating PG bodies remained elusive [13]. In transgenic mice overexpressing glycogen synthase, PG inclusion might derive from an imbalance between the activities of glycogen synthase and branching enzyme [14]. It is accepted that PG body formation derives from a deregulation of enzymes involved in glycogen synthesis.

Pathological features of polyglucosan bodies

On haematoxylin-eosin stained sections of skeletal muscle, PG bodies are characterized by violet or hyaline vacuoles localized in subsarcolemmal and cytoplasmic position in about 10–50% of fibres (preferentially of type 1), whereas the cytoplasmic areas surrounding the inclusions may be depleted of glycogen (Fig. 2). PG bodies are strongly reacting with PAS stain [15], show absent staining with Lugol's iodine and acid phosphatase (excluding their lysosomal origin), and are resistant to pre-digestion to both diastase/α-amylase and proteinase-k. The vacuoles are outlined by a thin rim of intense oxidative enzyme reaction.

On immunohistochemical analysis, PG bodies are strongly labelled for KM-279 antibody (against PG isolated from heart of Lafora disease), UPS proteins including ubiquitin [6, 8, 16, 17], p62 (a marker of protein aggregates) (Fig. 2), LC3 (a

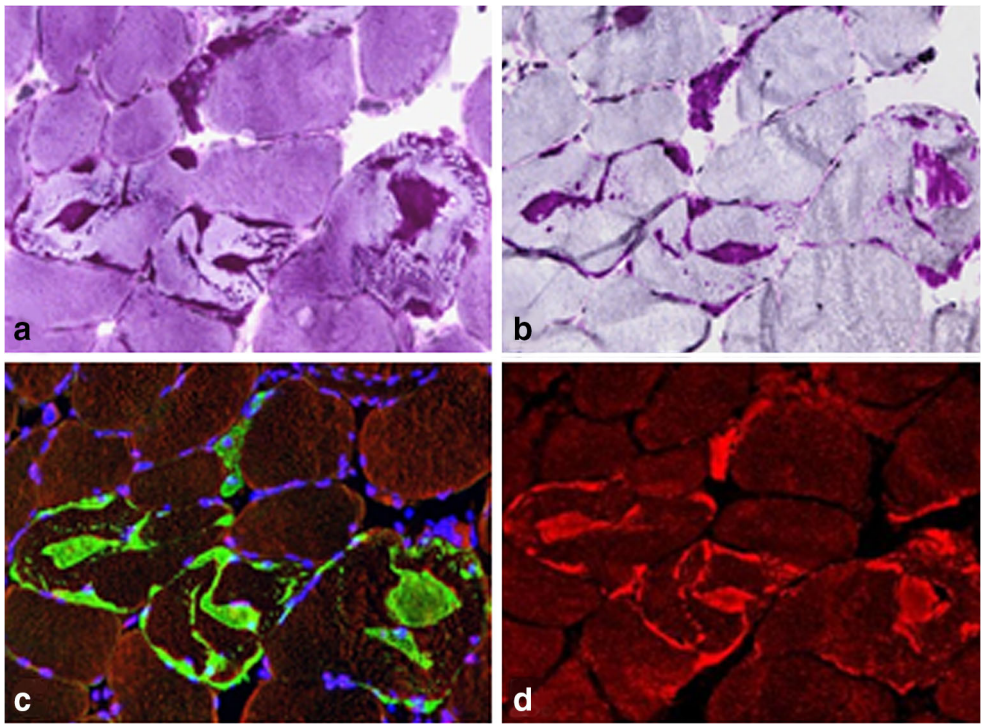

Fig. 2 Serial muscle biopsy sections from a PGBM2 patient showing PG storage. On PAS (**a**) and PAS-diastase (**b**) stained sections, the vacuoles are filled with strongly PAS-positive reacting material localized in subsarcolemmal and intracytoplasmic position; sometimes the areas surrounding the inclusions resulted depleted of glycogen. PG are strongly reacting for p62 (**c**) and ubiquitin (**d**)

marker of autophagosomes), and antibodies against numerous myofibrillar proteins including desmin and myotilin.

On electron microscopy, PG bodies appear as round, non-membrane-bound cytoplasmic structures, formed by amorphous granules and irregular branched filaments. In muscle, these structures often displace myofibrils, leading to Z-disc streaming and Z-line fragmentation, sarcoplasmic reticulum disorganization, accumulation of bizarre mitochondria with para-crystalline inclusions [18], and are often surrounded by β-particles of glycogen (Fig. 3). Conversely, in Lafora disease, PG are finely granular or granulo-filamentous, and are usually membrane-bound.

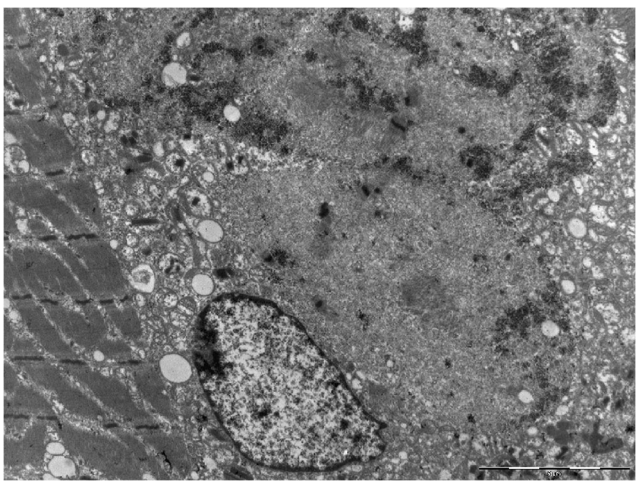

Fig. 3 Ultrastructural features of polyglucosan storage material in the skeletal muscle biopsy from a PGBM2 patient (magnification × 5800). Note myofibrillar large oval areas without delimiting membranes filled with filamentous and granular material

Polyglucosan accumulation in tissues (Table 3)

PG storage can be observed in different tissues, including striated and smooth muscle, brain, nerve, skin, liver and retina, leading to a variety of disorders that clinically impair various organs.

PG accumulation in the brain

PG bodies may accumulate in the brain as a non-specific phenomenon, which can be observed either in different disorders, e.g. mucopolysaccharidosis type VII [19], and, as a para-physiological event, in ageing [20]. PG bodies have been given different names according to their localization:

"*Lafora bodies*", localized in the perikarya of neurons with diffuse distribution, and in skin and muscle in Lafora disease. Lafora bodies are composed of abnormal insoluble glycogen which is hyper-phosphorylated, and contains a small amount of protein [4].

"*Bielschowsky bodies*", localized in the neuronal perikarya restricted to the globus pallidus in double athetosis [21, 22].

"*Corpora amylacea*", localized in the astrocytic process and representing a para-physiological change observed in

Table 3 Pathogenetic significance of PG storage in various tissues

Tissue	Pathogenicity	Disease or physiological condition
Skeletal muscle	Yes	PGBM1, PGBM2, GSD4, GSD7, HCM6, RBCK1-immunodeficiency, LD
Cardiac muscle	Yes	PGBM1, GSD4, GSD15, HCM6, RBCK1-immunodeficiency, LD
Smooth muscle	Yes	RBCK1-immunodeficiency, GSD4
Brain	No	Ageing
	Yes	LD, APBD, double athetosis, GSD4, mucopolysaccharidosis-7
Peripheral and intramuscular nerves	No	Ageing
	Yes	APBD, CIDP, GSD4, LD, diabetic neuropathy, peripheral neuropathy, motor neuron disease, familial spastic paraparesis, denervation atrophy
Skin (apocrine glands)	Yes	LD, APBD, GSD4
Liver	Yes	LD, GSD4, RBCK1-immunodeficiency
Retina	Yes	LD
Am\niocytes, fibroblasts	Yes	GSD4

old healthy subjects. Corpora amylacea are glycoproteinaceous PAS-positive structures that were first described by Purkinje in 1837 in the brain of elderly patients, where they usually increase in number with age. The presence of ubiquitin in these structures supports the idea that they are waste containers involved in protective or cleaning mechanisms of the central nervous system (CNS), in which potentially deleterious cellular products are sequestered to be later eliminated via phagocytosis or other mechanisms involving the innate immune system [23]: during formation of corpora amylacea, some epitopes emerge and these are recognized by innate IgM antibodies [23].

PG bodies accumulation in the nerves

PG bodies have been observed in both myelinated and unmyelinated peripheral nerves of patients with late-onset chronic inflammatory demyelinating sensory-motor polyneuropathy (CIDP) [21, 24], adult PG bodies disease (APBD) [25], Lafora disease, diabetic neuropathy [26], peripheral neuropathy [27, 28], motor neuron disease, familial spastic paralysis, as well as in elderly people without neurologic diseases [29, 30].

PG bodies have also been observed within intramuscular nerve branches in elderly people without neurologic diseases [30, 31], and in 3% of muscle biopsies from patients with different diseases, including lower and upper motor neuron disease, spasticity, Lafora disease, neuropathies, mild cognitive impairment and sphincter dysfunction [32]. These findings suggest this PG storage is a non-specific change correlated with ageing associated with muscle denervation atrophy [30], but PG accumulation might have neurotoxic potential causing neuronal-selective apoptosis [12].

PG bodies accumulation in the skeletal and cardiac muscles

PG accumulation in skeletal and cardiac muscle has never been observed as an unspecific lesion [17], as it represents the morphological hallmark of an organ-related disease.

PG accumulation limited to skeletal muscle characterizes several clinically different disorders, such as GSD type VII (GSD7) due to deficiency of phospho-fructokinase (PFK), PG body myopathy type 2 (PGBM2) due to deficiency of glycogenin-1, and other polysaccharide storage myopathies with undetermined enzyme defect or genetic aetiology [17, 33–40].

PG accumulation observed mainly in skeletal and occasionally also in cardiac muscle or liver, or mainly in cardiac and occasionally also in skeletal muscle, has been observed in GSD type IV (GSD4 or Andersen disease) due to deficiency of glycogen brancher enzyme (GBE), PGBM1 due to mutations in *RBCK1* gene, hypertrophic cardiomyopathy type VI (HCM6) due to mutations in *PRKAG2* gene, and immunodeficiency with cardiomyopathy due to mutations in *RBCK1* gene.

PG accumulation limited to the heart (not liver or muscle) and partially resistant to diastase digestion has been observed in patients with GSD type XV (GSD15) due to deficiency of glycogenin-1, which is associated with severe cardiomyopathy often requiring cardiac transplantation [41, 42].

The development of cardiomyopathy and myopathy as a consequence of PG storage is likely to be due to myofiber death and secondary fibrosis. A cytotoxic effect of stored material in addition with depletion of normal glycogen could be important for the physiology in striated muscle [42].

Disorders with polyglucosan storage

Lafora disease

Lafora disease (LD) or "Myoclonus epilepsy with Lafora bodies" is a neurodegenerative disorder with onset in childhood or adolescence, characterized by epileptic seizures that become intractable, combined with myoclonus, dysarthria, ataxia and dementia, leading to death within 10 years after onset (usually before 25 years of age). The pathological hallmark of the disease is the presence of the inclusions described by Lafora in 1911, which are mainly localized in the perikarya of neurons in the brain cortex, basal ganglia, thalamus, cerebellum, spinal cord, and also in cortical neuritis and dendrites. Lafora bodies (LB) gradually replace the cytoplasm of dendrites, likely leading to onset and intractability of the disease, since dendrites are the chief determinant of a neuron's excitability state. LB can be also observed in other tissues beside the brain (Fig. 4), including skeletal muscle, peripheral nerves, liver, heart, apocrine glands in the skin [43], and retina (causing no symptoms in the skin and eye), indicating this is a generalized disorder [4, 21].

LD is an autosomal recessive disease characterized by genetic heterogeneity, since it may be caused by mutations in different genes encoding for proteins involved in glycogen synthesis.

The 331-aminoacid protein called "laforin" is encoded by the Epilepsy Progressive Myoclonus type 2A (*EPM2A*) gene, a glycogen phosphatase that hydrolyses phospho-tyrosine and phospho-serine/threonine substrates. Laforin is polyubiquitinated by a 395-aminoacid protein called "malin", an E3 ubiquitin ligase which is encoded by the Epilepsy Progressive Myoclonus type 2B (*EPM2B*) gene. About 50% of LD patients have mutations in the *EPM2A* gene, 40% have mutations in the *EPM2B* gene, and the remaining have mutations in *PRDM8* gene, which was identified in early-onset forms of LD, and encodes a PR domain containing protein 8, probably interacting with malin and laforin.

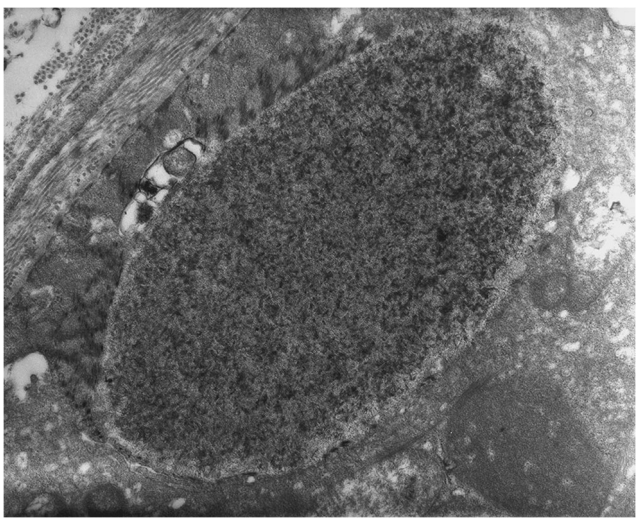

Fig. 4 Ultrastructural feature of a Lafora body in the skin biopsy from a patient affected with Lafora disease (magnification × 10,000). Note round or oval polyglucosan bodies in sweat gland myoepithelial cells characterized by filamentous and non-membrane-bound cytoplasmic material

In LD, excessive glycogen phosphorylation or insufficient glycogen synthase degradation leads to excessive glycogen elongation and accumulation of PG bodies. The major factor causing PG aggregation is a defective removal of the erroneously inserted phosphate in glucose residues of glycogen, leading to inability to degrade glycogen. Since malin is essential for removal of laforin after de-phosphorylation, its absence causes accumulation of laforin in glycogen, making it prone to aggregate. It also causes secondary cytosolic absence of laforin, adding further to the deleterious effects on glycogen and malin deficiency.

Laforin-malin complex downregulates glycogen synthase through malin-dependent ubiquitination and proteasomal degradation, and ensures a blockade of neuronal glycogen synthesis even under intense glycogenic conditions (since neurons usually keep the machinery for glycogen synthesis inactive) [44]. In the absence of laforin-malin complex, unfolded protein accumulates.

It was proposed that, by suppressing excessive glycogen phosphorylation, laforin prevents PG accumulation [45]. Laforin may be acting as an ancillary protein bringing proteasome or autophagy substrates to malin for ubiquitination or upon ubiquitination, directing them to degradation. The compromised autophagosome formation and overexpression of laforin, which increases the LC3-II levels, suggest that laforin activates autophagy [10]; a combination of impaired autophagy and glycogen synthesis dysregulation would result in the formation of LB [45]. Indeed, LB stained positive for laforin and ubiquitin, consistent with an early defect in autophagy, which could enhance the accumulation of glycogen and ubiquitinated proteins. The laforin-malin complex promotes the clearance of cytosolic proteins via the UPS: in LD, there are increased levels of ubiquitinated proteins and p62, suggesting the presence of defective autophagic process [13]. The block of proteasomal activity results in the formation of PG bodies that are resistant to diastase digestion [7].

The accumulation of LB was demonstrated to be due to a regulatory mechanism operating through the UPS degradation of glycogen synthase and the adaptor protein targeting to glycogen [44], and finally, from a combination of impaired autophagy and glycogen synthesis dysregulation [45].

AMP-activated protein kinase deficiency (*PRKAG2* gene)

Familial hypertrophic cardiomyopathy with Wolff-Parkinson-White (WPW) syndrome (or HCM type 6, MIM#600858) is

an autosomal dominant disorder due to mutations in the *PRKAG2* gene. The defective 569-aminoacid protein is the non-catalytic and regulatory γ-2 subunit of the heterotrimeric AMP kinase (AMPK) enzyme complex, which is composed of a catalytic α-subunit, and a non-catalytic β-subunit. The regulatory γ-subunit functions as a serine/threonine protein kinase: the enzyme activation is regulated by a decrease in ATP levels and an increase in AMP levels.

Since PRKAG2-related cardiomyopathy is not due to sarcomere protein mutations and is not associated with myocyte and myofibrillar disarray (but rather to myocytes vacuole formation), the disease should be considered as a HCM phenocopy, sharing with HCM some clinical features but differing for biological and pathological basis [46, 47]. Indeed, the disease is essentially a cardiac-specific non-lysosomal glycogenosis, which is clinically characterized by hypertrophic cardiomyopathy, atrio-ventricular conduction block and ventricular pre-excitation (WPW), predisposing to supraventricular arrhythmias and high risk of sudden death in midlife [48, 49]. The clinical spectrum of the disease includes:

- a severe congenital and rapidly fatal form with cardiomegaly, cardiac and respiratory failure [50, 51]
- the classical phenotype with onset in late adolescence, in which arrhythmias and conduction abnormalities usually dominate the clinical picture, sometimes requiring cardiac transplantation [46, 52]
- milder forms of HCM with onset in the third decade of life, sometimes associated with skeletal muscle involvement, myalgia, muscle weakness, exercise intolerance and PG in muscle [53].

Histopathological features of the disease are large, subsarcolemmal vacuolar deposition of PAS-positive aggregates in the heart [50], and occasionally also in skeletal muscle [53]. These inclusions may be either negative after α-amylase digestion [50, 53], or amylase-resistant [46], and this latter pattern is characteristic of PG. Among 5 unrelated patients, 3 had the severe fatal phenotype and the same p.R531Q mutation in the *PRKAG2* gene [50], whereas in other 6 families, there were 3 different missense mutations (the p.R302G recurring in 3 of them), but similar pathological features [46].

On electron microscopy, cardiomyocytes appeared enlarged, but myofibrillar disarray (a characteristic feature of HCM) was not detected [46], and interstitial fibrosis was minimal.

It is conceivable that the activation of AMP kinase consequent to *PRKAG2* gene mutations increases glucose uptake and hexokinase activity, modifying the balance of the different enzyme activities that participate in glycogen synthesis and degradation, thus affecting the structure of the branched glycogen polymer, which accumulates [50, 52]. The increase in the phosphorylation and in the activity of AMPK explains the dominant, gain-of-function action of *PRKAG2* gene mutations, and the p.R531Q mutation is associated with the most severe clinical phenotype [50].

Possible explanations that might account for the abnormal atrio-ventricular accessory pathways consequent to glycogen accumulation are either metabolic deposits that activate quiescent accessory pathways, or embryonic atrio-ventricular connections that normally regress during heart development and persist in *PRGKA2* mutant patients [46].

Glycogen storage disease type VII or Tarui disease

Phospho-fructo-kinase (PFK) deficiency was first reported by Tarui in 1965 in three siblings from a Japanese family affected with exercise intolerance and muscle weakness after exercise. Glycogen storage disease type VII (GSD7) is typically characterized by muscle symptoms (exercise intolerance, cramps, recurrent myoglobinuria) associated with compensated haemolytic anaemia, with onset in childhood. Other clinical phenotypes include exercise intolerance or permanent weakness starting in juvenile or adult age [18, 54, 55].

PFK is an ATP-D-fructose-6-phosphate-1-phospho-transferase (EC 2.7.1.11) which catalyzes the conversion of fructose-6-phosphate to fructose-1,6-diphosphate. PFK is a tetrameric enzyme under the control of three genes: *PFKM*, which encodes the muscle subunit, *PFKL*, which encodes the liver subunit, and *PFKP*, which encodes the platelet subunit. Mature muscle expresses only the M-subunit and contains exclusively the M-mono-tetramer (M4), whereas erythrocytes contain five different enzymes (M4, L4, M1L3, M2L2, M3L1). In patients with typical PFK deficiency, mutations in *PFKM* gene cause total lack of activity in muscle, but only partial enzyme deficiency in erythrocytes, where the residual 50% activity is due to the L4 isoenzyme. GSD7 is common in Ashkenazi Jewish descent because of two recurring mutations in the population: the c.237+1G>A (a splicing defect resulting in skipping of exon 5), which is present in 68% of mutant alleles, and the c.2003delC (resulting in a frame-shift and premature stop codon), which is present in 26% of mutant alleles [56].

Muscle pathology may show either glycogen storage in subsarcolemmal blebs, or inclusions of PG bodies in about 10% of fibres, all of type 1 [18], that stain positive to Lugol's iodine reaction. Some fibres may contain single vacuoles in the centre of fibre, others contain concentric masses at the periphery, partly or completely surrounding a core of sarcoplasm. All muscle fibres show a lack of histoenzymatic reaction for PFK. On electron microscopy, PG bodies appear to be composed of finely granular and filamentous material that is not membrane-bound. A plausible explanation for PG accumulation in PFK deficiency is a skewed activity ratio of glycogen synthase and GBE, probably due to the accumulation of glucose-6-phosphate (a physiological activator of glycogen synthase) secondary to the metabolic block [57].

Disorders due to mutations in the *RBCK1* gene

The *RBCK1* gene encodes for a 510-aminoacid protein called "Ring-B-box-coiled-coil protein interacting with protein C-Kinase-1" (or Heme Oxidized IRP2 ubiquitin Ligase-1, HOIL1), which is an E3 ubiquitin ligase that, together with HOIP and sharpin, forms the ubiquitin assembly complex LUBAC (linear ubiquitination chain assembly complex), an E3 ligase complex which adds head-to-tail ubiquitin chains to substrate proteins. RBCK-1 has a pivotal role in determining the specificity of the UPS by recognizing target substrates, and it has been implicated in NF-kB signalling pathway through the ubiquitination of inhibitor of NF-kB kinase γ-subunit [58]. NF-kB signalling has an important role in the regulation of the immune system [59].

Recessive mutations in the *RBCK1* gene are associated with two different clinical phenotypes:

- an early-onset fatal immunodeficiency characterized by a chronic auto-inflammation, invasive bacterial infections, severe dilated cardiomyopathy and myopathy, with PG storage in cardiac, skeletal, smooth muscle, and liver [58].
- a childhood or juvenile-onset form of lower limb proximal myopathy associated with dilated cardiomyopathy and cardiac failure often requiring cardiac transplantation [16, 60], called PG body myopathy type 1 (PGBM1), with PG storage in skeletal and cardiac muscles [16, 61].

Early-onset fatal immunodeficiency, auto-inflammation and cardiomyopathy

Few families with this disorder have been so far described. In two French sisters, who presented an auto-inflammatory syndrome, recurrent septicaemia, failure to thrive, hepato-splenomegaly, myopathy and severe dilated cardiomyopathy with PG storage in muscle, two compound heterozygous null mutations were identified: a large intragenic deletion (c.ex1_ex4del) and a nonsense mutation (p.Q185X) [58]. One Italian patient presented a similar phenotype and a homozygous truncating null mutation (c.121_122delCT) [58].

Polyglucosan body myopathy type 1

In muscle biopsy from polyglucosan body myopathy type 1 (PGBM1) patients, PG bodies were observed in about 50% of fibres, they were incompletely digested by α-amylase, resistant to proteinase-k treatment, and positive for ubiquitin and p62 [16], indicating they had been marked for proteasomal degradation. In two patients with liver clinical involvement, PG bodies were found in liver [16]. In PGBM1, PG bodies appear as dots, the inclusions are widespread in the cytoplasm, only occasionally localized in subsarcolemmal region or as centrally round structures [62], they have a strong resistance to α-amylase digestion, and are particularly reactive for p62. On electron microscopy, the lobulated grape structure is always present, and each acinus is mainly separated by mitochondria [62].

To date, about 15 families have been reported with PGBM1. In three patients from two families with childhood onset myopathy and cardiomyopathy without immunodeficiency, three different null mutations in *RBCK1* gene have been reported [61]: two mutations in exon 5 and exon 6, and one c.456+1G>C splicing mutation. In 10 patients from eight families with myopathy and cardiomyopathy with onset in adolescence, the phenotype was associated with null mutations, whereas patients with missense mutations had an apparently milder phenotype, with mild myopathy and either late-onset cardiomyopathy or no apparent cardiac involvement [16]. None of these patients had episodes of sepsis or chronic auto-inflammation, but some had various immunological disturbances (recurrent laryngitis, psoriasis, granulomatous disease).

Genotype-phenotype correlations in *RBCK1* gene mutations

The nature and the localization of the mutation within the *RBCK1* gene might be responsible for the different phenotypes, with null mutations in the N-terminal domain causing the severe immunological dysfunction [58], and mutations in the middle or C-terminal part leading to a milder myopathy/cardiomyopathy phenotype [16]. However, two unrelated patients with the same homozygous truncating mutation (c.896_899del, p.E299Vfs46) in the middle part of the gene suffered from a childhood onset myopathy with cardiac involvement, and also displayed signs of auto-inflammation and immunodeficiency [60].

Disorders due to mutations in the *GBE1* gene

GBE1 gene has 16 exons encoding the 702-aminoacid glycogen branching enzyme (GBE). GBE is an amylo-1,4>1,6 trans-glycosidase that catalyzes the last step in glycogen biosynthesis, by transferring α-1,4-linked glycosyl chains in α-1,6-glucosidic links, thereby adding branches to the growing glycogen molecule. The highest levels of enzyme are found in the liver and muscle.

GBE deficiency can be silent or variably affecting the liver, heart, skeletal muscle and brain, leading to various clinical phenotypes, including disorders which mainly affect either the liver and skeletal muscle (i.e. GSD4), or the central nervous system (i.e. adult-onset PG body disease—APBD, manifesting as a chronic neurodegenerative disease).

Glycogen storage disease type IV or Andersen disease

Glycogen storage disease type IV (GSD4) may present a wide spectrum of clinical phenotypes, involving mainly either the liver or the skeletal muscle. Among the forms of GSD4 affecting mainly the liver there are:

- the classical form originally described by Andersen in 1956, with hepato-splenomegaly and failure to thrive occurring in infancy, progressing to liver cirrhosis and death for liver failure usually before four years of age, unless liver transplantation is performed [63]
- the milder, non-progressive hepatic form, which is less frequent [64].

Among the forms of GSD4 with neurologic or neuromuscular presentation are described:

- the severe perinatal multisystem form called fatal akinesia deformation sequence (FADS) with multiple congenital contractures (arthrogryposis), hydrops fetalis and perinatal death [65, 66]
- the congenital form with severe hypotonia, muscle wasting, neuronal involvement, respiratory insufficiency, death in early infancy, sometimes simulating Werdnig-Hoffmann disease [67–69] with PG bodies in the muscle, heart, liver and nervous system
- a form with arthrogryposis, motor developmental delay, muscle weakness, and rigid spine with PG bodies accumulation in the muscle [70]
- a progressive myopathy and/or dilated cardiomyopathy phenotype with onset in childhood or adulthood [17, 65, 71–74].

Muscle biopsy shows typical PG bodies accumulation [11], which can be also observed in cardiac and smooth muscle, liver, motor neurons, amniocytes, fibroblasts, leukocytes and sweat glands [75].

Adult polyglucosan body disease

Adult-onset polyglucosan body disease (APBD) was first reported by Robitaille [76], manifesting as a leukoencephalopathy with upper and lower motor neuron dysfunction, neurogenic bladder, spastic gait, peripheral neuropathy, sensory loss and dementia, with onset at 40–60 years, or later. Some patients presented an asymmetric motor syndrome mimicking amyotrophic lateral sclerosis [77, 78].

PG bodies can be observed both in central (axons in grey and white matter) and in peripheral nervous system (sural nerve) [25]. Occasionally, PG bodies can be observed in the myoepithelial cells of the axillary apocrine sweat glands, supporting the diagnostic value of a skin biopsy [43]. Skeletal muscle may also show PG bodies, which, on electron microscopy, present short filaments mixed with finely granular material, are not membrane-bound, and are rimmed by normal glycogen particles [78].

Patients with Ashkenazi Jewish background may present common mutations in the *GBE1* gene (c.986A>C, p.Y329S and c.1976A>C), likely due to a founder effect [79, 80].

Genotype-phenotype correlations in *GBE1* deficiency

The severity of varying phenotypes associated to GBE deficiency correlates with the molecular severity of the *GBE1* gene mutations [65]: the most severe and fatal forms are usually associated with null mutations, whereas milder non-lethal neuromuscular forms are associated with residual GBE enzyme activity and at least one mild missense mutation [65]. Mutations in *GBE1* gene in APBD patients are relatively milder (usually of missense type) than in GSD4, explaining the late-onset.

Disorders due to mutations in the *GYG1* gene

There are two glycogenin protein isoforms in humans.

Glycogenin-1 is the muscle-specific isoform, but it is also expressed in cardiac muscle, liver and brain [81]. It is encoded by *GYG1* gene (NM_004130) which produces alternatively spliced transcripts including the longest muscle-specific variant 1, which is 2.4 kb large and composed of 8 exons, encoding a 350-aminoacid protein of 37 kDa molecular weight.

Glycogenin-2 is the liver-specific isoform, it is also expressed in cardiac muscle, brain and kidney, but not in skeletal muscle [82, 83]. It is encoded by *GYG2* gene (NM_003918.2), and has a molecular weight of 66 kDa.

In absence of glycogenin-1, proper formation of glycogen in muscle should not be possible because glycogen synthase (required for the elongation of glucose polymer) normally binds to the C-terminus of glycogenin-1 [84], a domain that is lacking as a consequence of truncating gene mutations. However, glycogen with normal granular structure has been detected in patients with *GYG1* gene mutations, implying the existence of an alternative core molecule possessing the ability to auto-glycosylate [85]. Glycogenin-2 is absent in healthy muscle, but it is highly expressed in patients with *GYG1* gene mutations, possibly explaining the rescue of glycogen formation.

Since glycogenin-1 and glycogenin-2 can heterodimerize, it is possible that the functional glycogenin-2 heterodimerizes with the non-functional glycogenin-1, resulting in a non-functional dimer [86]. This may also explain the different phenotypes between primary cardiac versus myopathic forms of glycogenin-1 deficiency.

The spectrum of diseases caused by *GYG1* gene mutations includes:

- a juvenile-onset glycogen-depleted myopathy with severe cardiomyopathy (GSD15) with PG storage in cardiac muscle but not in skeletal muscle
- a late-onset myopathy (PGBM2) without cardiac involvement with PG storage in muscle

Glycogen storage disease type XV

Glycogen storage disease type XV (GSD15) associated with glycogenin-1 deficiency was first identified in a young man with sudden death due to ventricular fibrillation, severe HCM, muscle weakness and exercise intolerance [87]. Few additional patients with severe cardiac phenotype often requiring cardiac transplantation [42] have been described, with onset ranging from 34 to 52 years, and without skeletal muscle involvement.

PG storage, as large vacuoles of PAS-positive material, partially resistant to diastase digestion, has been observed only in cardiomyocytes but not in skeletal muscle, where, conversely, there was a profound glycogen depletion, and an increased proportion of oxidative type 1 muscle fibres with mitochondrial proliferation, indicating a metabolic adaptation of fibre types to the glycogen absence. In cultured myoblasts, there was also lack of glycogen.

The mutant protein is absent or unable to auto-glycosylate, due to non-functional mutations in the *GYG1* gene. In the first patient reported [87], glycogenin-1 immunoblotting showed the exclusive presence of a band corresponding to unglycosylated apo-glycogenin-1, with 1 kDa reduced molecular mass as compared to normal glycosylated protein, suggesting that the p.T83M mutation caused the inactivation of glycogenin-1 and a consequent impaired priming of glycogen synthesis, leading to glycogen depletion in the muscle [88].

Similarly, in 3 unrelated patients homozygous for the p.D102H mutation, glycogenin-1 was still expressed in the heart, but it was unable to auto-glycosylate [42]. The mutation p.D102H appears to be common among British patients, possibly due to a founder effect [42].

Polyglucosan body myopathy type 2

Polyglucosan body myopathy type 2 (PGBM2) is typically characterized by slowly progressive muscle weakness and wasting, with onset occurring usually in the adult life (39 to 65 years of age) [8, 89–92], or, less frequently, in childhood/adolescence [89, 93] or in elderly [94].

According to the pattern of muscle weakness, there is a spectrum of clinical phenotypes, which includes limb-girdle muscular dystrophy, scapulo-peroneal muscle weakness, and late-onset distal myopathy [95]. Indeed, muscle weakness may involve the proximal compartment of the limbs and the axial muscles [93, 95, 96], and/or the distal compartments of the limbs [92], leading to hand/finger atrophy and reduced grip [91, 96], foot dorsiflexion impairment and stepping gait [95].

In many patients, the pelvic girdle is the most affected, whereas in others, there is a more pronounced involvement of the shoulder girdle muscles associated with scapular winging [91]. Muscle involvement may be asymmetrical [91, 95, 97]. Shoulder girdle weakness as initial manifestation of the disease, with asymmetric involvement of wrist extensors and flexors, might be useful to differentiate PGBM2 from PGBM1 [91], in which the upper girdle and arms are relatively spared.

Some patients have also facial muscle weakness, which, in association with highly asymmetric scapulo-humeral and pelvi-femoral involvement, may resemble the phenotype of facio-scapulo-humeral muscular dystrophy [95]. Muscle weakness may be associated with muscle cramps, myalgia [8, 91, 92, 95, 98] or exercise intolerance [91, 92, 95]. Disease progression and pattern of muscle involvement can be quite variable, and heterogeneous also within patients of the same family [90, 96]. Impaired ambulation may sometimes occur, usually at age ranging from 57 to 82 years [90, 95, 96].

Muscle MRI shows atrophy and fibro-fatty replacement of the pelvic girdle and proximal leg muscles (mainly affecting the gluteus, vastus lateralis, adductor magnus, posterior compartment of the thighs) [90–92], the scapular girdle muscles and the upper arms (early involving the deltoid, anterior compartment of the arms and forearms) [90, 91], and paravertebral muscles [91]. There is no evidence of cardiac or respiratory muscle involvement. One patient had a previous diagnosis of axonal motor polyneuropathy and CIDP, and another patient presented an associated autoimmune disease (generalized vitiligo) [8].

PG bodies are the pathological hallmark in muscle biopsy, as they are usually present in 20–50% of fibres [8, 89–92]. On the other hand, some patients may show PG bodies only in scattered muscle fibres [85], or they may not be detected at all. Indeed, PG bodies were absent in one elderly patient [9], in two different biopsies from one patient [92], and in one of two consecutive biopsies from another patient [8]. In studies preceding the molecular era, PG bodies have been reported to vary in number in different muscles from the same patient [99], and to be absent in younger but present in older cases, suggesting that they might form slowly, and that their progressive accumulation may cause the onset of muscle weakness [6]. These results suggest that PGBM2 diagnosis might be missed in absence of PG storage, emphasizing the value of *GYG1* genetic screening.

As compared to PGBM1, in PGBM2, where the C-terminus of glycogenin-1 protein seems to be involved, the inclusions are bigger, round or oval, and subsarcolemmal or cytoplasmic, they appear pinkish matt or uneven, sometimes

including nuclei [62]. In some muscle fibres, PG bodies are surrounded by normal glycogen, but more frequently the cytoplasmic areas surrounding the inclusions show focal depletion of glycogen [8, 85, 94]. Treatment with α-amylase and proteinase-k shows a variable degree of resistance to digestion of stored material [8, 91, 92, 94], but typically only the biggest inclusions show resistance to α-amylase digestion (Fig. 2). PG bodies can be observed more in type 1 than in type 2 fibres [91], possibly reflecting the different metabolisms in these fibres.

PG bodies are strongly immunostained with antibodies against desmin, p62 and ubiquitin (Fig. 2), and scattered LC3-positive reaction may be observed in most fibres [8, 89, 91, 94].

Upon ultrastructural level, PG bodies are composed of large lakes of fibrillar material (Fig. 3), are localized in subsarcolemmal or cytoplasmic areas, are non-membrane-bound, and span up to 30–40 sarcomeres. The lobulated grape structure is always present, and each acinus is separated by a thin rim of normally structured glycogen granules [62]. In rare patients, many muscle fibres also contained collections of nemaline rods [17, 94], which appeared as sarcomeric Z-disc disruption close to PG, as a result of perturbed protein turnover.

Mutations in *GYG1* gene have been identified in 33% of patients with PG myopathy and/or cardiomyopathy [89], and in 23% of undiagnosed patients [98], suggesting that this gene is a frequent and underdiagnosed cause of PGBM2 and GSD15, and that *GYG1* gene screening might help diagnosing previously unclassified or unspecific forms of late-onset myopathy or HCM.

The most frequent *GYG1* gene mutation (Table 4), which has been found in patients with different ethnic backgrounds (accounting for about 30–50% of mutant alleles), is the c.143+3G>C, a substitution of one base pair at the donor splice site in intron 2 that results in a frame-shift and insertion of a premature termination codon (p.D3Efs4). This mutation causes complete or nearly-complete aberrant splicing with exon 2 skipping, and profound reduction of functional glycogenin-1 protein after

Table 4 *GYG1* gene mutations

Disease	Reference #	Ethnicity	No. of patients (families)	Age at onset (years)	*GYG1* gene mutations
GSD15	88	Sweden	1	27	c.487delG, p.D163Tfs*5; c.248C>T, p.T83M
	43	New Zealand, UK	2 (1)	34, none	c.304G>C, p.D102H homoz.
		UK	1	46	c.304G>C, p.D102H homoz.
		UK	1	23	c.304G>C, p.D102H homoz.
PGBM2	90	Europe, USA	1	17	c.143+3G>C, p.D3Efs*4 homoz.
			1	Childhood	c.143+3G>C, p.D3Efs*4 homoz.
			1	39	c.304G>C, p.D102H; c.749G>A, p.W250X
			1	65	c.46G>C, p.A16P homoz.
			1	62	c.143+3G>C, p.D3Efs*4; c.7G>C, splicing
		Germany	1	61	c.484delG, p.T163Dfs*5 homoz.
			1	49	c.143+3G>C, p.D3Efs*4; c.970C>T, p.R324X
	92	China	1	39	c.634C>T, p.H212Y homoz.
	91	Italy (Alps)	2 (1)	30, 53	c.143+3G>C, p.D3Efs*4 homoz.
	93	Italy (Sardinia)	5 (4)	40–60	c.143+3G>C, p.D3Efs*4 homoz.
	101	Italy (Northern)	3 (1)	50–62	c.143+3G>C, p.D3Efs*4 homoz.; g.148717967C>G
	99	Italy (Northern)	2 (2)	Adult	c.143+3G>C, p.D3Efs*4 homoz.
			1	Adult	c.970C>T, p.R324X; n.d.
	95	Italy	1	82	c.2T>A, start codon, homoz.
	86	Denmark	1	50	c.166G>C, p.D56H; c.472delT, p.D160Tfs*5
			1	30	c.487delG, p.D163Tfs*5 homoz.
	96	France	1	28	c.646C>T, p.R216X; c.143+3G>C, p.D3Efs*4
			3 (1)	15, 16, none	c.143+3G>C, p.D3Efs*4 homoz.
			3 (1)	47, 72, 81	c.143+3G>C, p.D3Efs*4; c.996_1005del10, p.Y332X
		Denmark	1	50	c.166G>C, p.D56H; c.472delA, p.D159Tfs*5
			1	Teens	c.487delG, p.D163Tfs*5 homoz.
	94	UK	1	Teens	c.487delG, p.D163Tfs*5 homoz.
	97	Germany	2 (1)	64, 66	c.487delG, p.D163Tfs*5; c.403G>A, p.G135R

diastase digestion [89, 90, 92, 98, 100], explaining the late-onset myopathic phenotype which is usually observed in homozygous patients. The levels of normal transcript expression may modulate the deleterious effect of protein deficiency and the age at onset and disease severity [95].

Another frequent mutation identified in unrelated patients is the missense p.D102H [42, 89].

Other mutations recurring in patients form different countries are the c.484delG, p.D163Tfs5 [89, 93, 96], and the nonsense mutation c.970C>T, p.R324X [89, 98], both resulting in a glycogenin-1 protein lacking the C-terminus domain: since this domain is crucial for the elongation of glycogen by glycogen synthase, the resulting protein is unable to auto-glycosylate. It is has been hypothesized that the more this shorter protein is abundant, the more distal the phenotype seems to be [95].

Furthermore, some missense mutations have been associated to a functional enzyme defect that do not affect protein quantity. The missense mutation p.G135R abolished the enzyme function, as determined by an in vitro auto-glycosylation assay [96]. Similarly, the missense mutation p.H212Y was associated with normal levels of glycogenin-1 before diastase digestion and with absent protein after digestion, suggesting preserved protein expression but impaired glycosylation ability [91].

Some patients with null gene mutations showed the absence of glycogenin-1 in the muscle (both before and after amylase digestion) [94, 95], which did not preclude formation of glycogen; since significant amounts of glycogenin-2 were expressed in muscle, glycogenin-2 is the compensating core molecule, explaining the presence of normal glycogen granules [85].

Glycogenin-1 deficiency leads also to an apparent decrease of hexokinase II protein, potentially causing attenuated blood glucose processing, suggesting an energy deficiency that does not originate from loss of glycogen, but rather from an inaccessibility of intrafibrillar glycogen, due to mislocalisation of pooling of glycogen to the interfibrillar space [85].

Genotype-phenotype correlations of glycogenin-1 deficiency

The observation that *GYG1* gene mutations may lead to two distinct phenotypes (i.e. a cardiac phenotype and a limb-girdle myopathy) suggested that the difference may be due to variable residual amounts of glycogenin-1 in the two disorders [89]. The expression of a non-functional glycogenin-1 would lead to the cardiomyopathy phenotype [42, 87], whereas in the myopathic phenotype, there is either a markedly reduced amount or absent protein in the muscle, indicating that high expression of a non-functional mutant protein might be more deleterious to the heart than absent protein [42].

It is worth mentioning that few asymptomatic adult individuals have been identified to be homozygote for a *GYG1* gene mutation which caused either the cardiac phenotype GSD15 [42] or the myopathic phenotype PGBM2 [95] in their affected siblings, underlying the wide clinical heterogeneity of the disease, which can be observed also between individuals belonging to the same family, and suggesting a role for unknown modulating factors in the disease course.

Conclusions

- PGBM2 is probably the most frequent form of limb-girdle myopathy with PG storage.
- *PRKAG2* gene mutations probably cause a frequent form of dominant hypertrophic cardiomyopathy (mutations found in 4/18 probands with cardiac hypertrophy and WPW [46]).
- Skeletal and/or cardiac muscle pathology features are useful to address the subsequent genetic analysis of this group of disorders.
- The use of wide-range genetic screening (such as next generation sequencing [93, 100]) or a panel including all the known genes causative of PG bodies disorders should be used to diagnose patients with unclassified or unspecific myopathy.
- Future progress in understanding the biochemical and genetic defects underlying disorders with glycogen or PG storage will be in the field of secondary GSDs, using whole-exome or whole-genome sequencing strategy [5].

Contribution of authors CG study design, drafting and revision of the manuscript; PV acquisition of data, revision of the manuscript; CR, PV, MR acquisition of data; FM revision of the manuscript; AC study design, drafting and revision of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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