Late-onset polyucosan body myopathy in five patients with a homozygous mutation in GYG1

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LATE-ONSET POLYUCOSAN BODY MYOPATHY IN FIVE PATIENTS WITH A HOMOZYGOUS MUTATION IN *GYG1*

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Highlights:

Polyglucosan myopathies are due to GBE, PFK deficiencies and to mutations in *RBCK1*, and *GYG-1*.

GYG1-splice site mutation causes polyglucosan myopathy and typical MRI muscle lesions.

We have identified a single intronic *GYG-1* mutation in all five Sardinian patients.

Abstract

Five Sardinian patients presented in their 5th or 6th decade with progressive limb girdle muscle weakness but their muscle biopsies showed vacuolar myopathy. The more or less abundant subsarcolemmal and intermyofibrillar vacuoles showed intense, partially α-amylase resistant, PAS-positive deposits consistent with polyglucosan. The recent description of late-onset polyglucosan myopathy has prompted us to find new genetic defects in the gene (*GYG1*) encoding glycogenin-1, the crucial primer enzyme of glycogen synthesis in muscle.

We found a single homozygous intronic mutation harbored by five patients, who, except for two siblings, appear to be unrelated but all five live in central or south Sardinian villages.

Keywords: polyglucosan myopathy; glycogenin deficiency; glycogenosis; GYG1

1. Introduction

The three main glycogen synthetic enzymes are glycogenin1 (GYG1), glycogen synthase (GYS1), and glycogen branching enzyme (GBE1).

The best known mutations in the gene (*GBE1*) encoding glycogen branching enzyme have been associated with variably severe clinical forms of glycogenosis type IV (GSD IV), all of which cause accumulation of a poorly branched and poorly spherical amylopectin-like glycogen (polyglucosan)[1]. A late-onset severe form of GSD IV, dubbed as adult polyglucosan body disease (APBD), is characterized by central leukodystrophy, peripheral neuropathy, neurogenic bladder, and inconsistently dementia [2, 3].

Recently recognized pathogenic mutations in the gene (*GYS1*) have been associated with profound glycogen depletion in muscle (glycogenosis type 0), resulting in exercise intolerance, weakness, and myalgia with sudden cardiac death [4, 5]. Lack of glycogen substrate was suggested by abnormal ischemic or non-ischemic forearm exercise, a typical test indicating excessive normal muscle glycogen storage due to defects of glycogen breakdown (GSD V, McArdle disease; GSD VII, Tarui disease).

A most unusual glycogenosis type 0 was identified in a 27-year-old man who had exercise intolerance and slight weakness but also suffered from ventricular fibrillation from which he was saved by application of a defibrillator. His muscle biopsy showed severe lack of glycogen due to biallelic mutations in the gene (*GYG1*) encoding the crucial glycogen synthetic enzyme glycogenin present in skeletal and cardiac muscle [6].

An even most unusual observation linked homozygous and compound heterozygous mutations in the *GYG1* gene in seven patients of various ethnic background presenting with late-onset weakness and polyglucosan accumulation – rather than lack of glycogen – in their muscle biopsies and no cardiopathy [7]. It was further noted that the severe mutations resulted in depletion of glycogenin-1 or impairment of glycogenin-1 interaction with glycogen synthase, but the cause of polyglucosan accumulation remains difficult to understand. More recently, Colombo et al. [8] reported two sisters presenting adult onset limb girdle weakness associated with an intronic GYG1 variant previously encountered in 4/7 cases of the seminal paper [7], and Luo et al. described a woman manifesting proximal limb weakness associated a homozygous missense variants [9]

Here, we report on five Sardinian patients presenting late-onset polyglucosan body myopathy associated with a single *GYG1* homozygous intronic mutation.

2. Materials and methods

2.1. Patients

Five adult patients with polyglucosan myopathy from four unrelated Sardinian families were included in this study. After informed consent, all five patients underwent open biopsy of the quadriceps muscle for histochemical studies.

2.2. Muscle pathology and biochemistry

Proximal muscle biopsies (quadriceps) were studied extensively with traditional

histochemical battery, including periodic acid-Schiff (PAS) stain before and after digestion with α-amylase (diastase). Detailed electron microscopy analysis was performed in four patients as described by Malfatti et al [10].

Biochemical analyses were performed in frozen muscle specimens in various laboratories, including values for the following enzymes: acid α-glucosidase, glycogen branching enzyme, and all glycogenolytic and glycolytic enzymes.

2.3. Molecular genetic analysis

Genomic DNA was extracted from blood and frozen skeletal muscle by standard methods and Sanger sequencing was used for the *GYG1* gene. Briefly, exon 2 was amplified by PCR using primers hGYG1-Ex2F 5'-CCA AAG GGC TAC AGC TTG AT and hGYG1-Ex2R 5'CTC TAC CCG GTG CTC AAT TC. Amplified exon 2 fragment was sequenced with forward or reverse primer.

3. Results

3.1. Clinical findings

Clinical data for all 5 patients are summarized in Table 1. Onset of their weakness started at age 40 to age 60. Their initial complaints included progressive muscles weakness affecting both girdles, causing waddling gait, difficulty in climbing stairs and lifting arms. Notably, two patients, P1 and P5, also had exercise intolerance or myalgia. All patients showed slow progression, with impaired walking, inability to climbing stairs and rising from the floor with positive Gowers sign.

Their cardiac examinations revealed decreased global systolic function due to concomitant ischemic cardiomyopathy in patient 1 and only mild changes compatible

with their late ages in the other patients (slight left atrial dilation in patient 2, mild mitral and tricuspid valve insufficiency in patients 3, 4 and 5 revealed by echocardiography).

Serum CK values were invariably modestly increased. Electromyography (EMG) showed a mixed myopathic and neurogenic pattern in patient 2, but a clear myopathic pattern in patient 4. Muscle MRI studies were performed and a representative image of lower limbs in patient 5 shows fatty acid replacement of the major gluteus (Figure 1A) and of both vastus lateralis and adductor magnus muscles (Figure 1B).

3.2. Morphological and biochemical analysis

Light and electron microscopic investigations of muscle revealed characteristic alterations. Thirty percent of the fibers harbored vacuoles in the central and subsarcolemmal areas, containing intense PAS-positive inclusions, often surrounded by a halo of less intense PAS-positive material: treatment of muscle fibers with extensive digestion by α-amylase resulted in residual PAS-positive polyglucosan whereas the less intense PAS-positive normal glycogen disappeared (Figure 2 A, B). Electron microscopy revealed the typical polyglucosan structure, consisting of ovoid structure composed of partly filamentous material. A thin rim of normal glycogen particles and normal-looking mitochondria surrounded the polyglucosan bodies (Figure 3 A, B). Muscle biopsy from Patient 5 failed to show inclusions both in light and electron microscopy.

Biochemical analyses in frozen muscle biopsy from patient 1 found normal activities of the following enzymes: acid α-glucosidase, glycogen branching, all glycogen breakdown and glycolytic enzymes (including phosphofructokinase).

2.4. Molecular genetic analysis

The presence of abundant normal glycogen in addition to polyglucosan suggested us to sequence the gene (*AGL*) encoding glycogen debranching enzyme, which resulted normal.

Next we analyzed glycogenin, which has been shown to cause polyglucosan in muscle [7]. A single homozygous pathogenic variant of *GYG1* is identified in all five patients (Table1) and consists of an intronic (c,143+3G>C) change (Figure 4). This intronic change on a splice donor site has altered proper splicing according to the data reported in a previous paper [7].

4. Discussion

Our findings in the five patients confirm that a common autosomal recessive intronic *GYG1* mutation causes (or is associated with) late-onset polyglucosan myopathy as recently reported in ten unrelated patients with different ethnic backgrounds and harboring pathogenic *GYG1* variants [7-9]

The clinical phenotype is homogenous, consisting in late-onset mildly progressive limb-girdle weakness. Patient 3 showed distal involvement, which contrasts with that reported in a previous paper [7], where two patients (P2,P7) showed distal leg involvement, and one (P6) showed uniquely hands and fingers muscle weakness. Of note, a revealing symptom in P1 and P5 was exercise intolerance. P5 differed from the other patients also because she had a mildly myopathic muscle biopsy without inclusions, but MRI axial views of her pelvic and proximal thighs clearly showed fatty replacement of muscles as indicated in recently reported patients [8, 9]. Exercise

intolerance is a relatively new clinical finding of GYG1-related polyglucosan body myopathy, reported also in a recent paper [9].

By contrast, the firstly described GYG1 patient with glycogenosis type 0 illustrated a different clinical phenotype, characterized by young-onset cardiomyopathy and exercise intolerance, without PAS-positive inclusions but profound glycogen depletion in his muscle biopsy and accumulation of abnormal glycogen in his heart. In further contrast to the patients with *GYG1*-related polyglucosan body myopathy, the patient with glycogenosis type 0 had more abundant residual glycogenin-1 [6].

Very interestingly, we have identified the same mutation in all the patients. This intronic variant affecting the splice donor site and blocking glycogenin-1 biosynthesis segregated with the disease.

Polyglucosan body myopathy is but one example of several polyglucosan storage diseases, some of which are well known, including fatal infantile GSD IV or adult polyglucosan body disease (APBD) [2, 11], some cases of GSD VII [12], teen age-onset myoclonus epilepsy (Lafora disease) [13], and a new form of polyglucosan myopathy with cardiomyopathy due to mutations in *RBCK1* gene [14].

Several pathogenic mechanisms have been associated with polyglucosan accumulation. An obvious pathogenic mechanism was associated with the imbalanced ratio of the two glycogen synthetic enzymes, glycogen synthase (GYS) and glycogen branching (GBE), favoring the advantage of GYS over GBE activity. An obvious polyglucosan deposit in multiple tissues is due to loss of GBE activity due to the polymorphic phenotypes associated with GSD IV. However, a subtler GYS/GBE imbalance was identified by excessive activity of GYS-1, which caused less

conspicuous polyglucosan deposit in skeletal muscle from patients with phosphofructokinase (PFK) deficiency (GSD VII, Tarui disease) [15]. This was due to accumulation in muscle of glucose-6-phosphate (G6P), an upstream substrate of PFK deficiency and a physiologic activator of GYS-1. This phenomenon was documented later by serendipitously generating transgenic mice with deficient acid maltase and with upregulation of GYS-1, resulting in abundant polyglucosan generation in skeletal muscle [16]. A final discovery of a gain-of-function mutation in equine GYS-1 revealed the polyglucosan myopathy in some breeds of horses [17].

Different pathogenic mechanisms were involved in two very similar disorders. APBD and Lafora disease, which both cause severe polyglucosan deposition mostly in central and peripheral nervous systems, but also in other tissues, including skeletal muscle. APBD is due to two common *GBE1* founder mutations in Ashkenazi Jewish patients[18], but the most common mutations due to Lafora disease affect two distinct genes, one (*EPM2A*) encoding the laforin protein, a glycogen phosphatase, the other (*EPM2B*) encoding the malin protein, a ubiquitin 3 ligase [13]. The pathogenesis of polyglucosan accumulation in Lafora disease is not completely clear but there is good evidence that mutation in the glucan phosphatase laforin leads to hyperphosphorylated glycogen, which, in turn, leads to polyglucosan formation. Mutations in malin, a ubiquitin ligase, may impede the normal removal of laforin and excess laforin also causes polyglucosan formation [19].

Interestingly, a new form of polyglucosan storage disease, either limited to muscle or involving heart and muscle, was defined genetically by mutations in another ubiquitin ligase gene, *RBCK1*, affecting ten patients from eight families [14]. The ten

patients were characterized by a juvenile-onset myopathy but eight of them had a rapidly progressive cardiomyopathy, which required cardiac transplantation in four. Notably, some patients with mutations in the same gene had a different phenotype, with failure to thrive, chronic autoinflammation, and recurrent episodes of sepsis [20]. They also accumulated polyglucosan in muscle, heart, and liver, but a careful genotype-phenotype correlation analysis will be needed to clarify the different tissue involvement in these allelic disorders.

Yet another problem remains to be clarified to explain the distinct clinical presentations between two allelic disorders, both due to mutations in the *GYG1* gene: (i) a juvenile glycogen-depleted myopathy and a severe cardiopathy; and (ii) a late-onset myopathy with polyglucosan storage in skeletal muscle but without cardiomyopathy. The hypothesis suggested by Malfatti is based on the finding of different residual amounts of glycogenin-1 in the two conditions [7]. Abundant residual presence of glycogenin-1 would explain the complete early loss of muscle glycogen and early accumulation of abnormal glycogen in the heart. However, mixed components of normal glycogen and abundant polyglucosan are present in muscle from patients with almost absent residual glycogenin-1, which is not mandatory as a primer for glycogen synthase, although alternative primers remain to be investigated. The histopathological comparisons of polyglucosan body myopathies with different etiologies could shed some light on the comprehension of mechanisms leading to polyglucosan formation and how they contribute to muscle weakness [21].

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Legends to Table and Figures

Figure 1. Muscle MRI from Patient 5 shows fatty replacement at hip level (A), of notably the major gluteus (1) and vastus lateralis (2), and at thigh level (B), of notably the vastus lateralis (2) and the adductor magnus (3).

Figure 2. Serial sections of vastus lateralis muscle stained strongly with PAS reaction but incompletely digested by alpha-amylase.

Figure 3. Electron microscopy shows presence of subsarcolemmal abnormal glycogen material corresponding to polyglucosan bodies (A). At higher magnification, polyglucosan bodies are composed of poorly organized filamentous material (B).

Figure 4. Intronic mutation (c.143+3G>C) indicated by arrow causes aberrant splicing of the *GYG1* mRNA.

Table 1. Clinical and laboratory findings in the five patients

	Pt 1	Pt2	Pt 3	Pt 4	Pt 5
Gender	M	M	F	F	F
Age at onset	40	53	55	60	45
Initial symptoms	Exercise intolerance, myalgia; progressive weakness in scapular and pelvic girdle	Difficulty walking and climbing stairs	Weakness in cervical girdle; slow progression of weakness in pelvic girdle	Weakness in scapular girdle, slow progression of weakness in pelvic girdle	Difficulty walking and climbing stairs. Exercise intolerance, progressive weakness in scapular and pelvic girdle
Age at examination	63	65	77	80	65
Clinical features	• Waddling gait, can walk only 50 m without aid, for greater distances needs a walker. • Hyperlordosis. • Cannot climb stairs, rise from ground or lift his arms.	• Waddling gait for unlimited distances. • Hyperlordosis. • Right winging scapula • Gowers sign.	• Waddling gait for few meters at home; uses walker outside. • Hyperlordosis; • Cervical and dorsal kyphosis • Cannot climb stairs, rise from ground or lift her arms.	• Waddling gait for small distances using a cane. • Hyperlordosis. • Cannot climb stairs, rise from ground or lift her arms.	•Waddling gait for few meters at home;. •Hyperlordosis. Cannot climb stairs, rise from ground.
Serum CK	116 U/l (n.v. 55-170)	254 (n.v. 55-170)	78 U/L (n.v. 55-170)	155 U/l (n.v. 55-170)	95 (n.v. 55-170)
Cardiac examination	ECG: bradychardia:53/min EcoCG: global systolic function at lower limits (ischemic cardiomyopathy)	ECG: normal EcoCG: dilation of aortic root; slight left atrial dilation	ECG: normal EcoCG: sclerosis of aortic valve, slight mitral and tricuspid insufficiency	ECG: normal EcoCG: slight mitral and tricuspid insufficiency, hypertensive cardiomyopathy.	ECG: normal EcoCG: slight mitral and tricuspid insufficiency.
EMG	Myopathic pattern proximal; mixed pattern (myopathic and	Neurogenic findings indicating radiculopathy.	Myopathic finding with rare fibrillation potentials on deltoid muscle	Clear myopathic pattern more proximal	Clear myopathic pattern more proximal

	neurogenic indicating radiculopathy) distal.				
Muscle pathology: light microscopy	Partially α-amylase resistant PAS-positive inclusions	Partially α-amylase resistant PAS-positive but not abundant inclusions	Partially α-resistant PAS-positive inclusions visible in scattered fibers	Numerous vacuoles in many fibers. α-amylase-resistant PAS-positive inclusions	Nonspecific myopathic changes without vacuoles in two different biopsy(2009-2015)