Splice mutations preserve myophosphorylase activity that ameliorates the phenotype in McArdle disease

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Over 100 mutations in the myophosphorylase gene, which cause McArdle disease, are known. All these mutations have resulted in a complete block of muscle glycogenolysis, and accordingly, no genotype-phenotype correlation has been identified in this condition. We evaluated physiologic and genetic features of two patients with a variant form of McArdle disease, associated with unusually high exercise capacity. Physiologic findings were compared to those in 47 patients with typical McArdle disease, and 17 healthy subjects. Subjects performed an ischaemic forearm exercise test to assess lactate and ammonia production. Peak oxidative capacity (VO2max) and cardiac output were determined, using cycle ergometry as the exercise modality. The two patients with atypical McArdle disease carried common mutations on one allele (R50X and G205S), and novel splice mutations in introns 3 [IVS3-26A→G (c.425-26A→G)] and 5 [IVS5-601G→A (c.856-601G→A)] on the other allele. Plasma lactate after ischaemic exercise decreased in all typical McArdle patients, but increased in the two atypical McArdle patients (10% of that in healthy subjects). Peak workload and oxidative capacity were 2-fold higher in patients with atypical McArdle disease compared to typical McArdle patients. Oxygen uptake, relative to cardiac output, was severely impaired in the 47 patients with typical McArdle disease, and partially normalized in the milder affected McArdle patients. These findings identify the first distinct genotype-phenotype relationship in McArdle disease, and indicate that minimal myophosphorylase activity ameliorates the typical McArdle disease phenotype by augmenting muscle oxidative capacity. The milder form of McArdle disease provides important clues to the level of functional myophosphorylase needed to support muscle oxidative metabolism.

Keywords: myophosphorylase deficiency; exercise capacity; glycogenosis V; McArdle phenotype; forearm exercise test

Introduction

Glycogen is an energy-rich macromolecule composed of chains of glycosyl-units and functions as an important fuel for many types of cells in the body. The dispersed presence of glycogen granules in cells, and its accompanying enzymes, secures a rapidly accessible source of cellular energy. The importance of glycogen as a source of energy for skeletal muscle includes the generation of ATP anaerobically from glycogenolysis at rates that are more than double that of oxidative phosphorylation. Thus, glycogen
metabolism is critical during muscle contraction at maximal effort. Less well recognized is the necessity of glycogenolysis for muscle oxidative metabolism. Glycogen is crucial for normal maximal rates of oxidative phosphorylation, and for supporting oxidative metabolism in the transition from rest to exercise by providing substrate and tricarboxylic cycle intermediates necessary for normal acceleration of oxidative phosphorylation.

McArdle disease (myophosphorylase deficiency; GSD V) is a recessively inherited disease, in which mutations of the myophosphorylase gene (PYGM), cause a block of muscle glycogen breakdown. McArdle disease was clinically defined in 1951 by Brian McArdle (McArdle, 1951). The enzyme defect was identified in 1959 (Mommaerts et al., 1959; Schmid and Mahler, 1959), and the gene was discovered in 1984 (Lebo et al., 1984). Typical symptoms are exercise intolerance with premature exertional fatigue, exercise-induced muscle contractures and myoglobinuria. The patient’s oxidative capacity is lowered to a third or half of normal, which illustrates the importance of muscle glycogen breakdown for muscle oxidative metabolism.

Variability among patients in frequency, severity and age of onset of symptoms, have been described (Martin et al., 2001; DiMauro et al., 2004). This variability in phenotype cannot be ascribed to differences in genotype among patients. All defined mutations to date result in complete loss of enzymatic activity. Although more than 100 disease-causing mutations of PYGM have been reported, it has never been possible to find a correlation between phenotype and genotype (Martin et al., 2001; Martinuzzi et al., 2003; Paradas et al., 2005; Aquaron et al., 2007; Deschauer et al., 2007). The alleged differences in phenotype have been based entirely on clinical history, but confirmation of physiological differences in exercise responses are lacking.

In the present study we report two unrelated, unusual patients diagnosed with McArdle disease, who can perform regular aerobic exercise at an intensity that is far greater than typical patients with McArdle disease. Genetic testing of these patients revealed novel splice mutations of PYGM, which preserve minimal amounts of myophosphorylase activity. When we compared these patient’s responses to cycle and handgrip exercise with those in 47 patients with ‘classical’ McArdle disease and 17 healthy subjects, it was evident that they had an intermediate phenotype between these two comparators. Besides describing the first phenotype–genotype relationship in McArdle disease, the study provides clues to the very small levels of functional myophosphorylase and glycogenolysis, which are needed to support carbohydrate-dependent oxidative metabolism in skeletal muscle. This understanding may have important implications for therapy of McArdle disease, because even a minimal increase in myophosphorylase activity may rescue the phenotype. Since patients with McArdle disease carry many nonsense mutations, this could potentially be achieved by readthrough drug therapy.

**Methods**

**Subjects**

An unrelated Swedish woman and North American man, aged 30 and 39 years, respectively, with McArdle disease and unusually high capacity for aerobic exercise were studied. Both are ethnic Caucasian. Their results were compared to 47 patients with typical McArdle disease, and 17 healthy, age-matched subjects. The exercise results and clinical history from the 47 patients with typical McArdle disease were pulled from medical reports on patients studied by us in past years.

All McArdle patients, including the two with atypical McArdle disease, had experienced muscle cramps and pain provoked by sudden vigorous exercise many times. Furthermore, both atypical McArdle patients and most patients with typical McArdle disease had experienced post-exercise episodes of rhabdomyolysis and myoglobinuria. In the woman with atypical McArdle disease, myoglobinuria had only occurred two to three times with no episodes of renal failure, whereas the man had experienced more than 10 episodes of myoglobinuria, and one case of renal failure. Both recalled experiencing exercise-induced cramps and pain in their early teens, but were not diagnosed until their twenties, based on episodes of myoglobinuria and exercise-induced symptoms. Neither of the two atypical McArdle patients had permanent weakness. Muscle morphology showed myopathic changes and plasma creatine kinase levels were increased, with no obvious difference between atypical and typical McArdle patients. The great divider between atypical and typical patients, however, was the big difference in capacity for sustained, aerobic exercise. All 47 patients with typical McArdle disease complained of not being able to keep up with their peers when engaging in any kind of physical activity, whereas the two variant McArdle patients lived more normal lives. The woman with the mild form of McArdle disease played hockey regularly on a team, and the man frequently cycled without experiencing difficulties in keeping up with friends and family.

The study was approved by the ethics committees of Copenhagen, and the University of Texas Southwestern Medical Centre, Dallas, Texas. Each subject was informed about the nature of the studies, and potential risks, and gave written consent to participate.

**Forearm exercise testing**

To evaluate the glycogenolytic potential in muscle, the two atypical McArdle patients, 41 patients with typical McArdle disease, and 15 of the healthy controls performed an ischaemic forearm exercise test, in which plasma lactate from a cubital vein in the exercising arm was measured before and after 1 min of exercise. Exercise was performed with intended maximal handgrips on a dynamometer (19117 Smedley Hand Dynamometer; modified by Stoelting, Wood Dale, IL, USA). The subjects performed 30 grips, lasting 1 s each, with intervals of 1-s rests between contractions. Maximal handgrip force was determined as the highest of three maximal grips prior to the ischaemic forearm test. The force of each handgrip during the ischaemic forearm test was recorded and expressed as a percentage of maximal handgrip.

**Cycle ergometry testing**

Cycle testing was performed in all subjects, but not all patients with typical McArdle disease performed all exercise tests (see below, and figure legends of Figs 5 and 6 for explanation). All subjects were tested in the fed state, after having eaten a carbohydrate-rich breakfast 2–3 h before the test. The maximal oxidative capacity, and the ratio between oxygen uptake and cardiac output were determined on a stationary ergometer (MedGraphics CPE, St. Paul, MN) operated via a cardiopulmonary exercise test system that measured gas exchanges, workload, and heart rate. Cardiac output was measured by the acetylene-rebreathing technique (Triebwasser et al., 1977).
VO$_{2\text{max}}$ was determined by increasing the workload by 5–10W increments every other minute until exhaustion. Increments were adjusted so that the duration of the test was 12–15 min.

On a separate day, 25 patients with typical McArdle disease and the two McArdle patients with the milder phenotype were tested on the cycle ergometer at a constant workload, corresponding to approximately 65% of the post-second wind VO$_{2\text{max}}$ of each patient. This was done to determine the magnitude of the second wind phenomenon in atypical versus typical McArdle patients.

**Myophosphorylase activity and molecular genetic testing**

Myophosphorylase activity was first screened for by histochemical staining of frozen muscle sections, and then by biochemical determination of myophosphorylase activity, where whole muscle was buffered and homogenized as previously described (Wagner et al., 1976). The biochemical assay was performed as described (Mendicino et al., 1975), except: 1% proteamine sulphate was not used. In addition, the assay was run at 30°C instead of 25°C, to enhance activity readings. About 50mg of flash frozen muscle was thawed in ice-cold 0.15M KCl before weighing, and a 10% homogenate was prepared, using a TenBroeck micro-homogenizer in Imidazole buffer (Wagner et al., 1976).

Muscle phosphorylase activity is determined in a spectrophotometric assay in which enzymatic activity is linked to the production of NADPH, which is monitored as a change in absorbance with time. When phosphorylase activity is present, a consistent linear increase in absorbance occurs with the rate of change in absorbance determined by the level of enzyme activity. In most typical McArdle patients, there is no change or an actual decrease in absorbance with time, making the judgement of absolutely no enzyme activity easy. In other cases, there may be some fluctuation in absorbance that may be mistaken for residual enzymatic activity. We defined significant residual enzyme activity as an increase in absorbance as recorded at 5-s intervals for 3 min of observation that is highly significant by linear regression analysis ($R^2 \geq 0.95$), and reproducible in multiple aliquots of the patient samples. In this way, we differentiated all typical McArdle patients from our two patients who had significant residual activity.

Genomic DNA from the two patients with the mild form of McArdle disease was isolated from blood samples by standard methods. Initially, exonic and flanking intronic sequences of $PYGM$ were PCR amplified, purified (NucleoFast, Machery-Nagel), sequenced directly (BigDye Terminator V1.1) and resolved on an ABI 3130XL. After cDNA analysis, the entire sequence of introns 3 and 5 were PCR amplified, and sequenced. Identified mutations were confirmed in a new PCR and sequencing reaction. NM_0015609 was used as the reference sequence for $PYGM$.

Total muscle RNA was extracted from a needle biopsy using RNeasy (Qiagen), and cDNA was generated using Superscript II TM (Invitrogen, Paisley, UK) in combination with random hexamer primers, both according to the manufacturer’s description. The entire cDNA sequence was amplified in overlapping fragments using cDNA specific primers. cDNA fragments of abnormal size were gel-purified, re-amplified and sequenced (primers and PCR conditions are available on request).

Allele specific PCR [amplifying the wild type allele c.613G (p.G205)] was carried out in the man, but not the woman with atypical McArdle disease. The analysis was not performed in her due to technical reasons, related to the distance between the two mutations found in $PYGM$.

PCR conditions were: 1 × AmpliTaq Gold Buffer, 1 U AmpliTaqGold, 1.5 mM MgCl$_2$, 0.2 mM dNTP’s and 0.125 μM of the two primers $PYGM_{ex3}F$: CACCTACCAAGGGTGTGTG and $PYGM_{ex5R_{ARMS}}$: GTGTGCACATGGACG in a reaction volume of 20 μl. Cycling conditions: 95°C; 5 min (94°C; 20 s, 58°C; 30 s 72°C; 45 s) × 30, 72°C; 10 min. The PCR fragment was subsequently purified and sequenced by standard methods.

**Analyses and statistics**

Venous blood was sampled in syringes containing 10μl 0.33 M EDTA/ ml blood as anticoagulants. The blood was immediately spun at 4°C and analysed for lactate on an YSI model 2300 STAT plus.

Values are expressed as mean±SD unless otherwise stated. A $P<0.05$ (two-tailed testing) was considered significant. Differences between typical McArdle patients and healthy controls were assessed by an unpaired Student’s $t$-test.

**Results**

**Biochemical and molecular genetic analyses**

Myophosphorylase was absent on histochemical staining in all McArdle patients, including the two atypical cases. Myophosphorylase activity determined biochemically on whole muscle specimens was absent in the 47 patients with typical McArdle disease, but significant residual amounts of myophosphorylase were found in the two McArdle patients with a mild phenotype (1 and 2.5% of normal).

The male McArdle patient with a mild phenotype carried the known G205S mutation and the novel IVS3-26A>G mutation. Standard exonic sequencing identified the G205S mutation. RT-PCR and subsequent cDNA sequencing showed the existence of a normal length transcript carrying the G205S mutation, and one abnormally spliced product, missing an exon (Fig. 1). The IVS3-26A>G mutation, responsible for this exon skipping, was subsequently identified in intron 3 (Fig. 2). Allele specific PCR showed that the two mutations were located on separate alleles.
The female McArdle patient with a mild phenotype carried the common R50X mutation on one allele, and a novel IVS5-601G>A (c.856-601G>A) mutation in intron 5. Again, RT-PCR and subsequent sequencing showed that there was one normal length transcript carrying the R50X mutation (not shown), and one abnormally spliced fragment (Fig. 1), in which 175 bp from intron 5 were spliced in between exons 5 and 6 (r.855_856ins856-602_776). In both patients, trace amounts of additional alternatively spliced products could be seen.

**Forearm exercise**

After ischaemic forearm exercise, plasma lactate concentration decreased (by on average 0.1 mmol/l; \( P \leq 0.005 \)) from rest in 41 patients with typical McArdle disease, but increased by 0.4 mmol/l 1 min after exercise in both atypical McArdle patients (Fig. 3). The increase in the two atypical McArdle patients corresponded to about 10% of the increase seen in healthy subjects. The rate of muscle fatigue during ischaemic forearm exercise was similar in patients with classical and mild McArdle disease, and was greatly exaggerated compared to healthy subjects (Fig. 4). However, unlike all other McArdle patients, the atypical McArdle patient with the highest residual phosphorylase activity could complete 60 minutes of ischemic exercise without getting contractures of forearm muscles.

**Cycle exercise testing**

Peak oxidative capacity (\( \text{VO}_{2\text{max}} \)) was almost two-fold higher in the two patients with a mild form of McArdle disease (23 and 24 ml min\(^{-1}\)kg\(^{-1}\)) compared with typical McArdle patients (13 ± 3 ml min\(^{-1}\)kg\(^{-1}\)) (Fig. 5). Peak workload on the bike was also two-fold higher in the two atypical McArdle patients (70 and 105 W) compared with typical McArdle patients (34 ± 7 W). Still, the oxidative capacity was not fully normal in the two atypical McArdle patients. They had a \( \text{VO}_{2\text{max}} \) which was 65% and 55% of the normal mean for healthy, age-matched subjects, and deviated more than two standard deviations from the normal mean.

There was a close linear relationship between cardiac output and \( \text{VO}_{2} \) for healthy subjects (\( r^2 = 0.78, \ P < 0.0001 \)) and patients with typical McArdle disease (\( r^2 = 0.55, \ P < 0.0001 \)), but the slope of increase in \( \text{VO}_{2} \) relative to cardiac output was almost 3-fold greater in healthy subjects (0.1522) compared to patients with typical McArdle disease (0.0545), (\( P < 0.0001 \), Fig. 6). This is consistent with a marked impairment of oxygen utilization relative to oxygen delivery in typical McArdle patients (Haller and Vissing, 2004b). In contrast, preservation of a minor level of glycogenolysis in patients with the mild form of McArdle disease was associated with a relationship between \( \text{VO}_{2} \) and cardiac output, which was
intermediate of that in typical patients and healthy subjects (Fig. 6), consistent with enhanced substrate available to support oxidative phosphorylation in atypical versus typical McArdle patients.

The magnitude of the second wind phenomenon (spontaneous drop in heart rate after seven minutes of exercise at the constant workload) was blunted in the two patients with atypical McArdle, as an indication of a better supply of energy to muscle in the pre-second wind exercise period. Thus, heart rate dropped spontaneously by 36 ± 3 beats per minute (range of drop: 21–70) in the 25 typical McArdle patients studied with this protocol, while the drop was only 17 and 13 beats per minute in the two atypical patients.

Discussion

We studied two unusual patients with McArdle disease, and a large cohort of typical McArdle patients, from whom several insights about McArdle disease and muscle glycogen metabolism in general emerge: (i) The study is the first to demonstrate a phenotype–genotype relationship in McArdle disease; (ii) The minimal residual myophosphorylase activity in the two mildly affected McArdle patients, which were associated with unusual novel mutations interfering with splicing, moderates the McArdle phenotype by augmenting oxidative capacity; and (iii) the shift in phenotype towards normal demonstrates that very low levels of myophosphorylase are needed to sustain rates of glycogenolysis that support muscle oxidative metabolism. This finding opens new avenues for treatment of McArdle disease, since minimal expression of myophosphorylase, induced by readthrough, nonsense mutation drug therapy, could potentially normalize the metabolic state of the muscle.

The most striking difference between mildly affected and typical McArdle patients is the large difference in oxidative capacity. This increased metabolic capacity allows atypical McArdle patients to perform sustained aerobic activities that would otherwise cause rapid muscle fatigue and risk of muscle contractures, and rhabdomyolysis in patients with typical McArdle disease. Thus, they are able to live more normal lives, being able to keep up physically with their peers in activities of daily living. The increased muscle oxidative capacity conferred by minimal residual levels of myophosphorylase emphasizes the crucial requirement for glycogen to support muscle oxidative phosphorylation. The mildly affected patients still had quite limited maximal anaerobic capacity, as suggested by the low lactate production with ischaemic exercise, episodes of exertional myoglobinuria, and a fatigability of muscles during ischaemic exercise, which was comparable to typical McArdle patients. These findings illustrate that the amount of glycolytic flux needed to support normal oxidative metabolism is far less than that needed to support peak rates of anaerobic glycolysis. In this respect, the mildly affected McArdle patients resemble other disorders of muscle glycolysis with some preserved enzyme activity, such as phosphoglycerate mutase deficiency (Vissing et al., 2005) and phosphorylase b kinase deficiency (Orngreen et al., 2008).

A key feature of limited oxidative substrate availability when glycolysis is blocked is the second wind phenomenon, which is unique/pathognomonic to McArdle disease (Vissing and Haller, 2003a; Haller and Vissing, 2004a). It is attributable to a severe energy crisis early in exercise in patients with McArdle disease, when the need for muscle glycogenolysis is high. It is associated with a hyperkinetic circulatory response, in which cardiac output is high relative to the ability of working muscle to extract available oxygen (Fig. 6). The increased tolerance to exercise after the second wind is attributable to an enhanced delivery of extramuscular fuels (hepatic glucose and fat) to exercising muscle (Vissing et al., 1992; Haller and Vissing, 2002; Nielsen et al., 2002; Orngreen et al., 2009), which can partially compensate for the blocked muscle glycogenolysis. That oxidative phosphorylation in McArdle disease is dependent upon extra-muscular
fueled is also illustrated by the virtual abolition of the second wind when McArdle patients ingest sucrose before exercise (Vissing and Haller, 2003b; Andersen et al., 2008). A second wind has been reported anecdotally in other metabolic myopathies, but has never been demonstrated physiologically, and all available evidence point to a unique occurrence of this phenomenon in McArdle disease (Vissing and Haller, 2003a; Haller and Vissing, 2004a; Vissing et al., 2005; Orngreen et al., 2008). The fact that patients with atypical McArdle disease have a blunted second wind and a normalization of the oxygen uptake/cardiac output ratio compared to typical McArdle patients, implies that the residual glycogenolysis in these patients reduces, but does not eliminate, dependence upon blood borne fuels to support muscle oxidative phosphorylation.

It is a general notion that most enzymes of glycogenolysis and glycolysis are abundantly present in muscle. In line with this, no solid proof of symptomatic carriers of single glycolytic gene mutations has been reported. Phosphofructokinase, is the rate-limiting enzyme for muscle glycolysis, but no persons heterozygous for mutations causing phosphofructokinase deficiency have been reported to be symptomatic (Vora et al., 1983). Symptomatic carriers in McArdle disease have been reported several times (Schmidt et al., 1987; Manfredi et al., 1993), but have always been based on anecdotal rather than physiological evidence of limitations in exercise capacity. In one study, in which symptomatic carriers were in fact tested with an ischaemic forearm exercise test, the lactate response to exercise of carriers of single PYGM mutations could not be distinguished from subjects with no mutations (Taylor et al., 1987). We have recently shown that persons carrying single PYGM mutations have preserved maximal oxidative capacity and lactate responses to maximal exercise, even when residual myophosphorylase in the carriers was below a third of normal (Andersen et al., 2006). In accordance with the dramatic change in phenotype with just 1–2% residual myophosphorylase activity in the atypical McArdle patients, it can be calculated that at maximal rates of lactate production in contracting muscle, only 5% functional myophosphorylase is needed (Fischer et al., 1971). This calculation is supported by in vivo investigations of rates of glycogen breakdown and phosphorylase activity in healthy humans (Gollnick et al., 1978). The McArdle patients with a mild phenotype, investigated in the present study, provide important human in vivo insights into the minimal levels of functional myophosphorylase needed to support maximal rates of glycogenolysis.

The functionality of the residual myophosphorylase levels in the two atypical McArdle patients was clearly demonstrated, not only by the much higher oxidative capacity of the patients, but also by the production of small amounts of lactate during ischaemic forearm exercise, and the lack of exercise-induced contractures of forearm muscle in the atypical patient with the highest residual phosphorylase level in muscle. This is very different from the consistent development of contractures and drop in plasma lactate during ischaemic forearm exercise, observed in patients with typical McArdle disease. It is well known that healthy muscle not only produces lactate from anaerobic glycolysis during exercise, but also oxidizes huge amounts of lactate (van Hall et al., 2003). In McArdle patients with complete blocks in glycogenolysis, production of lactate is severely limited, whereas the capacity to oxidize is intact. The drop in plasma lactate during exercise is a direct consequence of this mismatch between production and oxidation of lactate in myophosphorylase deficient muscle.

Myophosphorylase is a homodimer that associates into a tetramer to form the enzymatically active phosphorylase A. It is in this form that the activity of phosphorylase is assessed. Phosphorylase b kinase is believed to be the main activator of phosphorylase from the inactive (B) to the active (A) form. In a patient with phosphorylase b kinase deficiency, we have recently shown that at moderate exercise, myophosphorylase is almost exclusively activated by phosphorylase b kinase, since lactate responses to exercise were abolished in the patient (Orngreen et al., 2008). During maximal exercise, lactate responses were normalized in the patient, indicating that other regulatory mechanisms, such as AMP and Ca$^{2+}$, take over the activation of phosphorylase (Orngreen et al., 2008). In the rare mild form of McArdle disease, it remains to be seen whether a higher fraction of the residual myophosphorylase is in the active form, and if the pattern of myophosphorylase activation differs from that in healthy muscles.

In our experience, clinicians may judge and McArdle patients may perceive their functional limitation with great variance, but on functional testing, the limitation in exercise capacity is very uniform. This is in accordance with the low variance of oxidative capacity among patients with typical McArdle disease found in this study, which is one of the largest cohorts of McArdle patients studied to date. Several studies have suggested mutations or polymorphisms in other genes that can modulate the phenotype in McArdle disease. One such gene is the gene for adenosine monophosphate deaminase (AMPD1), in which heterozygosity for the C34T mutation in AMPD1 has been reported to confer reduced aerobic capacity, but only in female McArdle patients (Rubio et al., 2007a). The same authors were unable to find a correlation between clinical severity and AMPD1 genotype in another study of McArdle disease (Rubio et al., 2007c). A McArdle patient, homozygous for the C34T mutation, has been reported to have an exercise capacity identical to other McArdle patients, which seems to negate any association between AMPD1 genotype and McArdle phenotype (Heller et al., 1987). We have observed the same lack of influence of heterozygosity for C34T on McArdle phenotype (Haller and Vissing, unpublished observations).

Polymorphisms in the gene for angiotensin-converting enzyme (ACE) have also been suggested to modulate the phenotype of McArdle disease (Martinuzzi et al., 2003), but again this has only been demonstrated for female McArdle patients, and the difference in oxidative capacity or clinical score among groups was minimal (Rubio et al., 2007b; Gómez-Galliego et al., 2008). In keeping with this, treatment with the angiotensin-converting enzyme inhibitor, ramipril, only had minimal influence on oxidative capacity in McArdle disease (Martinuzzi et al., 2008). Much larger variations in exercise capacity can be observed with aerobic conditioning and changes in dietary intake of carbohydrate. Thus, it has been shown that peak work capacity can be increased by 50% with regular aerobic training (Haller et al., 2006), and that a diet high in carbohydrates can boost peak oxidative capacity by 25% in McArdle disease (Andersen and Vissing, 2008). Considering that almost all genotypes in McArdle disease, except those reported in
the two atypical McArdle patients in this study, result in no resid-
ual myophosphorylase activity, it is not surprising that no study,
looking for genotype-phenotype correlations in McArdle disease,
has ever been able to find such a relationship (Martin et al., 2001;
Martinuzzi et al., 2003; Paradas et al., 2005; Aquaron et al.,
2007; Deschauer et al., 2007). The current study is, therefore,
the first demonstration of a phenotype-genotype correlation in
McArdle disease.

An intriguing question remains concerning variability in the phe-
notype of McArdle disease. Why do approximately one third of
patients with McArdle disease develop fixed proximal weakness,
preferentially of the shoulder girdle, after age 40? (DiMauro et al.,
2004). Development of the weakness is unrelated to sex, profes-
sion, level of physical activity or genotype. Although static symp-
toms (muscle weakness and atrophy) are unrelated to the
dominating dynamic symptoms of exercise-related pain and cramps,
and thus the primary effects of energy deficiency in
McArdle disease, the muscle weakness shows that there is still
some unexplained phenotypic variability, which cannot readily be
explained.

Deep intronic mutations affecting PYGM, like the two described
in this study, have not been reported before. Deep intronic muta-
tions are often very ‘leaky’, allowing some normally spliced pro-
ducts to be generated (Beck et al., 1999). The ‘leakiness’ is
evidenced by the residual myophosphorylase activity found in
our patients, which on physiological testing proved to be func-
tional, as evidenced by small, but significant, increases in plasma
lactate with ischaemic exercise. It is inconceivable that the two
intronic mutations should confer any residual functionality of myo-
phosphorylase per se, since the IVS5-601G>A mutation results in
a 175 bp insertion, creating a frame-shift and the IVS3-26A>G
mutation results in skipping of the entire exon 4 of PYGM. That
the IVS5-601G>A mutation is pathogenic is further suggested by
the location of the mutation right after the 175 inserted bp. The
new mutations reported in this study can easily be overlooked
during routine sequencing of PYGM, and it is quite possible that
sporadic, symptomatic persons, in whom only one mutation in
PYGM can be found after conventional sequencing, may in fact
carry similar type mutations. Interestingly, in a recent study of
55 patients with McArdle disease, direct genomic exon sequencing
failed to identify the second mutation allele in five patients (Rubio
et al., 2007a).

The finding in the present study of a great moderation in phe-
notype with just minimal residual myophosphorylase is promising
for therapies aimed at boosting functional enzyme levels in
muscle. One such therapy is the introduction of PYGM to
muscle cells, using an adenovirus as a vector (Baque et al.,
1994; Pari et al., 1999). However, this approach is far from
being implemented as therapy for humans due to technical and
safety issues. Another, perhaps more promising approach, is ther-
apy with drugs that can read through premature stop mutations
and which may prove particularly useful in McArdle disease
because the vast majority of patients carry nonsense mutations
(Quinlivan and Vissing, 2007). Attempts at readthrough using
gentamicin have been unsuccessful in McArdle disease (Schroers
et al., 2006). Another drug, which may potentially prove success-
ful, is PTC124, which has shown promising results in animal
studies of Duchenne muscular dystrophy (Welch et al., 2007),
and is currently being tested in humans with Duchenne muscular
dystrophy, carrying nonsense mutations. Considering that most
patients carry nonsense mutations in McArdle disease and, as
demonstrated by the present study, that only minimal amounts
of functional myophosphorylase is necessary to dramatically
change the phenotype, readthrough drugs may be a promising
avenue for treatment of McArdle disease. Our results indicate
that a similar effect of PTC124 on myophosphorylase activity, as
the increase to 5% of normal cystic fibrosis transmembrane con-
ductance regulator seen with PTC124 treatment of nonsense-
mediated cystic fibrosis (Kerem, 2004; Kerem et al., 2008),
could significantly ameliorate or even eliminate the principal man-
ifestations of McArdle disease.

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