Selected disorders of carbohydrate metabolism

Robert J. Beynon
Rosaline C. M. Quinlivan
Caroline A. Sewry

AM	acid maltase
CPT	carnitine palmitoyl transferase
DB	debrancher enzyme
GP	glycogen phosphorylase
LDH	lactate dehydrogenase
MDA	myoadenylate deaminase
OMIM	Online Mendelian Inheritance in Man (*www.ncbi.nlm.nih.gov/omim*)
PFK	phosphofructokinase
PGAM	phosphoglycerate mutase
PGM	phosphoglucomutase
PGK	phosphoglycerate kinase
SRCA	sarcoplasmic calcium ATPase

Definitions of entities

The breakdown of glucose (from blood) or intracellular glycogen yields pyruvate that can then enter the TCA cycle (via acetyl CoA in aerobic conditions) or be converted to lactate under ischaemic conditions. Failure in any step in glycogenolysis or glycolysis eliminates or diminishes carbon flux, leading to a rapid decline in high energy phosphates for muscular work, and causing rapid muscle fatigue, cramping, and sometimes rhabdomyolysis. In those conditions where there is a muscle-specific gene product, the symptoms may be restricted to muscle but in other conditions, where there is a single gene encoding the enzyme activity, the effects can be more widespread.

Inherited deficiencies in glyogenolytic/glycolytic enzymes are rare, and are usually autosomal recessive. They generally present as exercise intolerance, elevated serum creatine kinase, with rhabdomyolysis and exertion-induced myoglobinuria (3). One of the less well-understood features of this group of conditions relates to the marked variability in the severity of symptoms, even between individuals carrying the same mutations. This suggests that these enzyme deficiencies must be projected onto additional factors influencing muscle capabilities, whether genetic or environmental. In this respect, the anecdotal evidence of greater prevalence/severity in males may be relevant (24). In this chapter, we will consider three of the more common of these conditions: McArdle's disease, Tarui's disease, and debrancher deficiency. Some indication of the relative frequencies of these conditions may be gleaned from a survey conducted some years ago as part of the CARMEN programme, (*http://carmen.liv.ac.uk)*. Whilst not intended to be exhaustive, these figures (Figure 1) report the total cases from twenty different muscle clinics within the European community, although these data will reflect local interests.

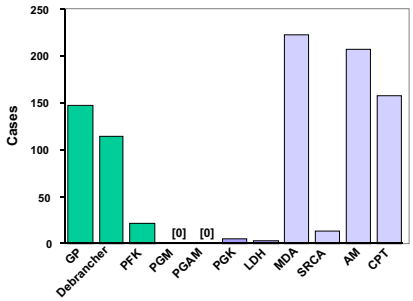

Figure 1. *Analysis of cases in 20 muscle centres.* As part of the background to CARMEN, we surveyed twenty specialist muscle centres throughout Europe and collated their cases for several metabolic myopathies. The conditions shaded in green are considered in this chapter, conditions shaded in pale blue are covered elsewhere in this volume.

McArdle's disease

McArdle's disease (myophosphorylase deficiency, Glycogen storage disease V, OMIM 232600 is a rare autosomal recessive disorder affecting approximately 1 of 100 000 persons (although precise epidemiological data are lacking). The condition was first identified in 1951 by Brian McArdle who reported a patient with myalgia coupled with a failure to produce lactate during ischaemic exercise (21). The condition results from the almost complete absence of functional glycogen phosphorylase in skeletal muscle (4, 8, 9). This enzyme is responsible for the breakdown of glycogen in the sarcoplasm, via phosphorolysis of the substrate, glycogen, and yielding glucose-1-phospate as the product. Of the several metabolic myopathies caused by deficiencies in sarcoplasmic enzymes, McArdle's disease is one of the most common (Figure 1).

McArdle's disease is generally held to be an adult-onset condition although, rarely, cases have been confirmed in childhood. Typically, however, diagnosis is made in the third decade of life and in retrospect, affected individuals are virtually united in their memory, for example, of extreme difficulty in school-day physical education. The condition normally presents with exertion-induced fatigue and myalgia, and under extreme exertion, painful muscle cramps and myoglobinuria with the attendant risk of renal failure. However, there is marked heterogeneity in the presentation of this condition, such that some patients maintain a high level of muscle performance whilst others of the same age and with the same genotype are severely disabled with exercise intolerance restricted to a few yards of walking. The disorder is progressive with muscle wasting and weakness occurring from middle age.

McArdle's disease should be suspected by the pattern of symptoms described above. Wasting of the upper body, especially latissimus dorsi, may be seen in contrast with a tendency for bulky lower limb muscles. A raised serum creatine kinase (three to twenty times normal) is common, as in many other conditions where generalised muscle weakness is apparent. An absence of lactate production in an ischaemic forearm test increases suspicion of the diagnosis.

The large number of mutations in this condition, most of them rare, precludes mutation analysis as a uniformly definitive test although the most

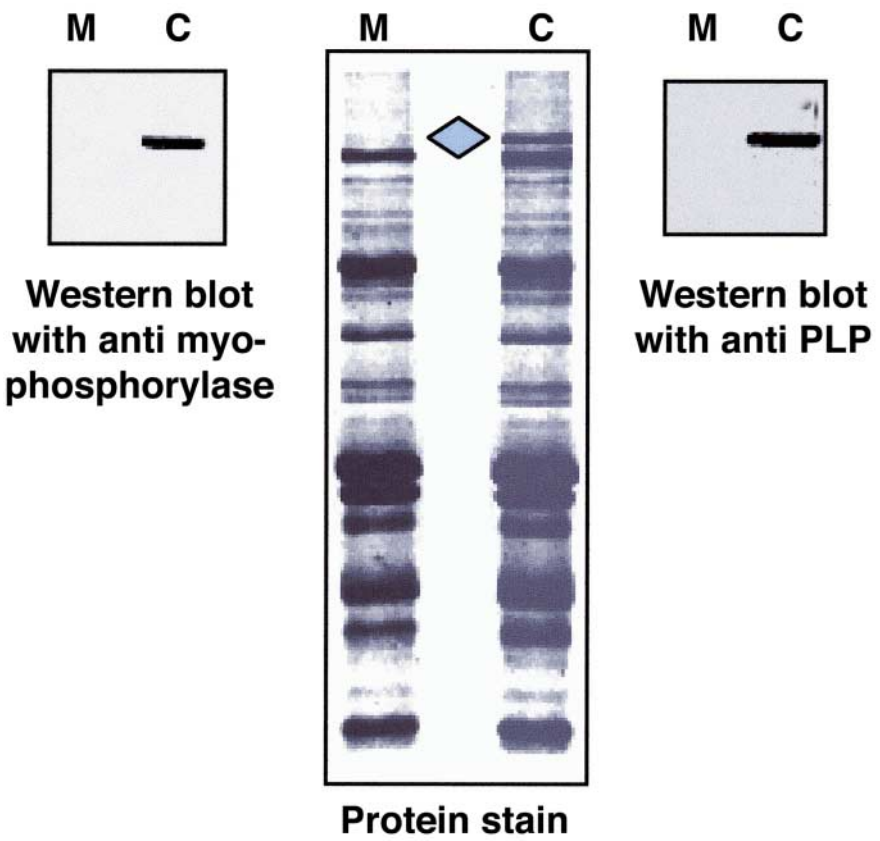

Figure 2. *Protein expression in McArdle's disease.* Soluble proteins from muscle biopsies of a normal and a McArdle's patients were separated on linear 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis. The absence of the myophosphorylase band is readily apparent (diamond), as is the lack of immunoreactivity in western blots using an antibody to muscle phosphorylase or to the cofactor, pyridoxal phosphate.

common mutations in certain ethnic groups, R50X in particular (see below), can provide a conclusive diagnosis in a significant number of cases.

Molecular genetics and pathogenesis. There are three isoenzymic forms of glycogen phosphorylase: *i)* the liver form, encoded on chromosome 14, *ii)* the brain/foetal form, encoded on chromosome 20, and *iii)* the muscle form, encoded on chromosome 11 (proximal part of 11q13). It is only the last of these that is affected in McArdle's disease, and with few exceptions affected patients have virtually no detectable enzyme activity in muscle biopsy. In contrast to other glycogenoses, the restriction to the muscle specific isoform means that there is no liver involvement. In the few patients that have been studied in detail, the lack of enzyme activity is coincident with a lack of immunoreactive protein (Figure 2) or of mRNA (22). Lack of mRNA is a feature of the most common mutation, R50X, which although a nonsense mutation, seems to bring about rapid degradation of mRNA as a consequence of early termination of translation (5). By contrast, a compound het-

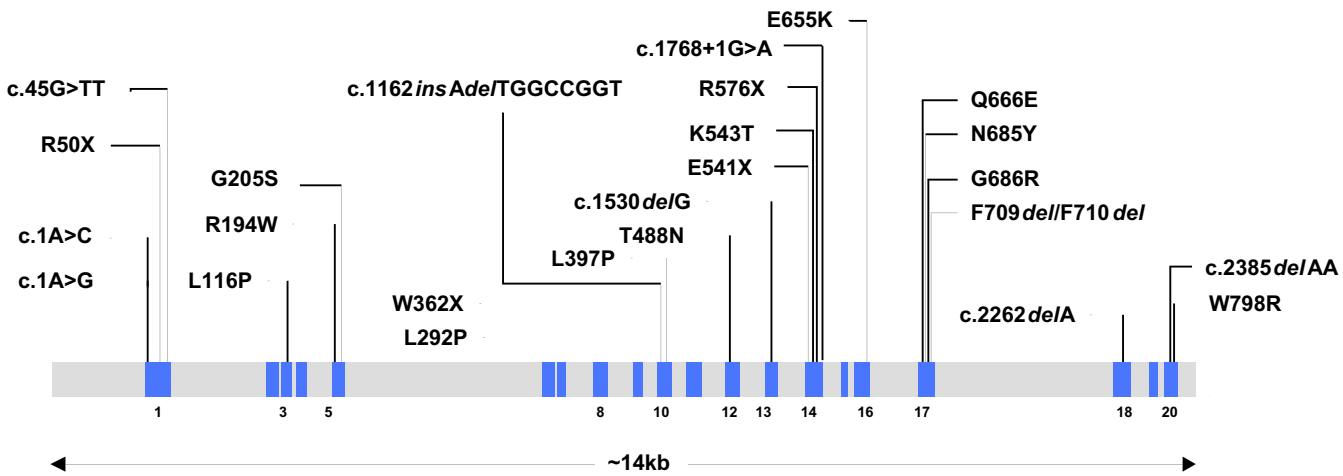

Figure 3. *Mutation map of the myophosphorylase gene.* The figure summarises current information on the known mutations in the myophosphorylase gene. Note that the exact sizes of the two largest introns (5 and 17) are unknown, which precludes a mutation labelling scheme based on the genomic sequence. Accordingly, mutations have been named with reference to the protein sequence, where the initiation methionine residue is numbered 1, or the cDNA sequence, where the first base of the ATG codon for that methionine is numbered 1. Because the protein sequence was previously counted from the second residue (the N-terminal methionine residue is removed from the mature protein), most mutation designations in the literature in the literature are displaced by one residue. Thus, the most common Caucasian mutation, R50X has previously been known as R49X or R49TER. Mutations given with reference to the cDNA sequence are prefaced with "c." In the absence of the full genome sequence, there is no simple way to define a mutation in the first base of intron 14, and the label c.1768+1G>A is used to indicate the mutation that is also known as 1844+1G>A. The detailed bibliography defining these mutations will be presented elsewhere (Quinlivan et al, unpublished).

erozygote of R50X/G205S revealed a strong mRNA signal on northern blotting (unpublished data). In other groups of patients, a similar heterogeneity in myophosphorylase mRNA levels was observed (13, 30), but these studies predated the discovery of mutations in the gene, and it is not possible to infer anything further about the relationship between genotype and molecular phenotype.

The first report of point mutations in the myophosphorylase gene were made independently by Tsujino et al (38) and Bartram et al (5). These papers reported the identification of the most common mutation R50X (previously known as R49X) in North American and UK patients respectively, The paper by Tsujino et al (38) reported two additional mutations (G205S and K543T), and provided a clear analysis of the apparent pseudodominance of the condition, showing that it was attributable to one parent being a compound heterozygote and the other parent carrying a third mutation. In another early study, the R50X mutation was observed to be the most frequent in a group of German patients (42). However, in southern European countries, the preponderance of R50X is lower and this mutation has never been seen in Japanese patients. In contrast, one common mutation in the last group is F709del/F710del (39), which has never been observed in non-Japanese patients.

Since these early reports, a total of 25 mutations have been identified as of May 2001 (Figure 3). The most common mutation is R50X, but G205S has also been observed in patients from several countries and in different family lines. Many other private mutations are seen in single patients or in isolated family lineages. Of 102 European patients entered in the CARMEN database (*http://carmen.liv.ac.uk*), 46 were homozygous for R50X, and a further 41 were heterozygotes with the same mutation. One patient was homozygous for G205S and a further 5 were heterozygotes that included this mutation.

Structural changes. A definitive diagnosis can only be obtained by skeletal muscle histochemistry (Figure 4). The histochemical method is specific for the isoform present in mature muscle fibres (see below). Thus, regenerating fibres, the immature fibres in spindles, and some smooth muscle fibres will stain but all mature extrafusal fibres will be negative. There is no other known condition where this occurs.

Myophosphorylase is the key enzyme in sarcoplasmic glycogen degradation, but deficiency of this enzyme is not always associated with a marked accumulation of glycogen although there may be some sub-sarcolemmal accumulation of glycogen. The commonly used method relies on activation of nascent enzyme in muscle, and utilises the enzyme in the reverse direction (glycogen synthesis), monitoring the accumulation of glycogen as iodine-reactive material. The colour development is not stable, and it is essential to include a control sample in parallel with the test sample, and observe the outcome as soon as possible in order to avoid a false positive diagnosis.

Future perspectives. Although McArdle's disease is rarely considered to be life threatening, there is a need for treatments that reduce muscle fatiguability and pain, which in turn may diminish the progressive loss of muscle bulk as patients get older. Nutritional and pharmaceutical therapies have not yielded a marked improvement, and no treatment has been universally adopted for this condition (Quinlivan and Beynon, unpublished). The most promising study that has been conducted recently has been a double-blind, placebo-controlled crossover study of oral creatine, which enhanced anaerobic exercise capability although there was no improvement in aerobic exercise ability (41). After a single patient reported improvements with oral vitamin B6 (26), a random, double blind, placebo controlled crossover study failed to confirm a general enhancement of muscle performance (Beynon et al, unpublished).

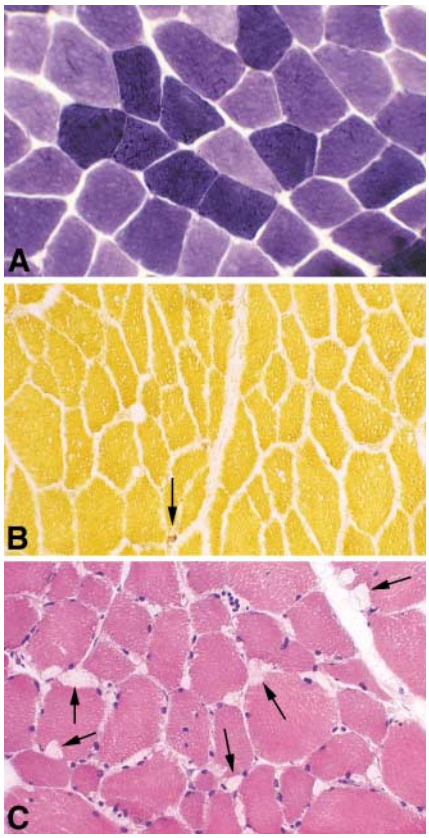

Figure 4. *Histochemical diagnosis of McArdle's disease.* Histochemical demonstration of phosphorylase in **A**. a control and **B**. a case of McArdle's disease. Note the normal fibre typing pattern in the control and absence of the enzyme in most fibres in the McArdle case. Activity is only seen in one very small, regenerating fibre (arrow). The extent of activity staining in regenerating fibres, and the origin of the activity has been discussed recently (20). **C**. In this H & E stain there are subsarcolemmal unstained regions corresponding to deposits of glycogen. Examples indicated by arrows.

Gene therapy for McArdle's disease remains a possibility. The availability of animal models, notably in Charolais cattle and Merino sheep, has made future trials feasible. In Charolais cattle, the mutation is R490W, and the resultant molecular phenotype was determined to be zero protein, unknown mRNA (40). In the Merino sheep, the molecular phenotype is unknown, other than for lack of myophosphorylase activity, but the mutation, at the 3′ end of introns 19 invokes a cryptic splice site within exon 20, causing a frame shift and premature termination of translation (35).

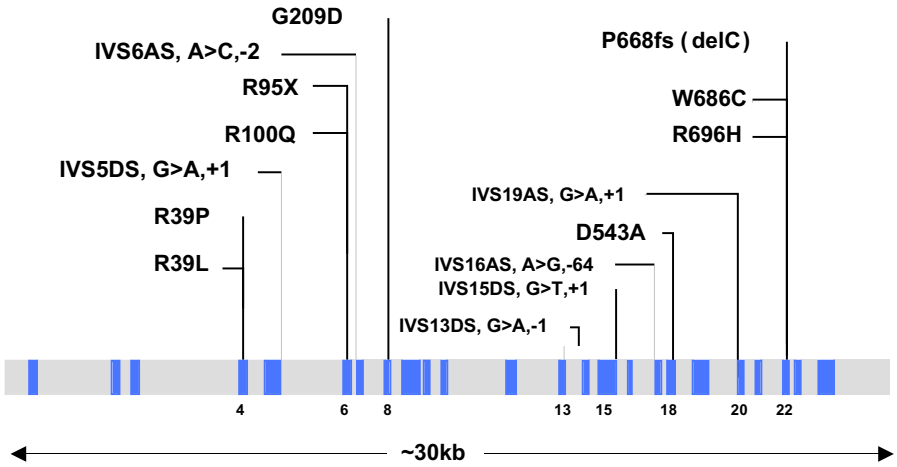

Figure 5. *Pathological mutations in the human PFK gene.* This map is based on that published by Fujii & Miwa (12) but contains additional mutations.

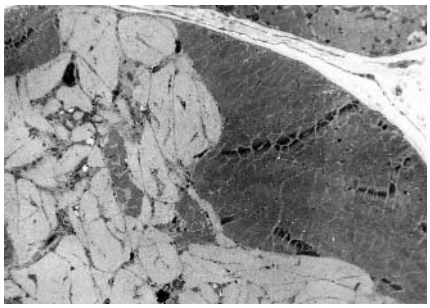

Figure 6. *Section of muscle from GSD VII patient.* Large areas in this muscle fibre are devoid of myofibrils and contain quasi compartments which, on higher power (not shown), contain granular as well as filamentous material and cellular organelles. This picture is from a patient with a metabolic myopathy and hemolytic anemia due to phosphofructokinase defiency. Histochemical reaction for phosphophructokinase activity showed complete negativity (not shown). Electron micrograph.×2500. Image kindly provided by Dr George Karpati, Montreal.

Adenoviral-mediated delivery of a myophosphorylase cDNA into myoblasts from McArdle's patients, or myophosphorylase-deficient sheep brought about restoration of activity above normal values. Any toxicity due to over-expression was thought unlikely given the tight controls on the activity of the enzyme (25).

Tarui's disease

Tarui's disease (phosphofructokinase deficiency, Glycogen storage disease VII, OMIM) is a deficiency of the enzyme (phosphofructokinase, PFK) that is a key regulator of glycolysis (12, 36). There are three isozymic forms of PFK, the muscle form is encoded at chromosome 12q13 (not on chromosome 1 as originally reported), the liver form at chromosome 21q, and the platelet form at chromosome 10p. The enzyme is tetrameric and in skeletal muscle consists exclusively of M_4 homotetramers. Liver contains exclusively the L_4 homotetramer. The erythrocyte PFK consists of a series of isoenzymic forms: M_4, M_3L, M_2L_2, ML_3, and L_4. The M-type transcript exists in a number of splice variants, but in skeletal muscle the predominant form comprises exons 2 to 24, and intron 2 is retained within the mature transcript. The start codon is located within exon 3 (23).

Deficiency of PFK is a heterogeneous condition, characterised by a myopathy defined by exercise intolerance, muscle cramps, and myoglobinuria. Further, the importance of glycolysis in erythrocytes and the loss of the M-type subunit in these cells means that PFK activity drops to about 50% of normal values, eliciting an associated haemolytic anaemia. There seems to be a particularly high prevalence of the condition among Japanese and those of Ashkenazi Jewish descent (27) although it has been observed in other ethnic groups. A severe fatal infantile form of the condition seems to manifest as a reduction in PFK levels in all tissues examined, which is unlikely to be attributable to single gene mutations and may reflect some *trans* acting factor (1).

Molecular genetics and pathogenesis. Muscle sections may show some accumulation of glycogen (Figure 5) although this need not be pronounced (6). There is some indication of accumulation of glycogen in abnormal structures in older patients (16, 32). A total of 15 disease-causing mutations in the PFK-M gene have been identified (Figure 6). These range from missense and nonsense mutations to splice variants and single base deletions causing a frameshift and premature termination of translation. In Ashkenazi Jews, the most common mutations are a G to A substitution at the 5′ splice donor site of intron 5, and the deletion of a single base (C) at position 2079 in the mRNA (P688fs in protein-based nomenclature). Together, these two mutations account for over 90% of alleles in this group of patients, making feasible a diagnostic strategy based on DNA testing.

The sole reported animal model of PFK deficiency is the dog, specifically, English Springer spaniels (14, 15), which present with classic symptoms of anaemia and exercise intolerance. The PFK-M protein is completely absent by SDS-PAGE or western blotting, and the common causative mutation observed in dogs from the United States and Europe is W742X (33). Some dogs presenting with similar symptoms were heterozygous for this mutation, and it is likely that other mutations exist.

Structural changes. Muscle sections may show some accumulation of glycogen although this need not be pronounced (6). There is some indication of accumulation of glycogen in abnormal structures in older patients (16, 32). Other changes are illustrated in Figure 6.

Future perspectives. PFK deficiency is rare, and gene-mediated therapies are likely to be consequential to devel-

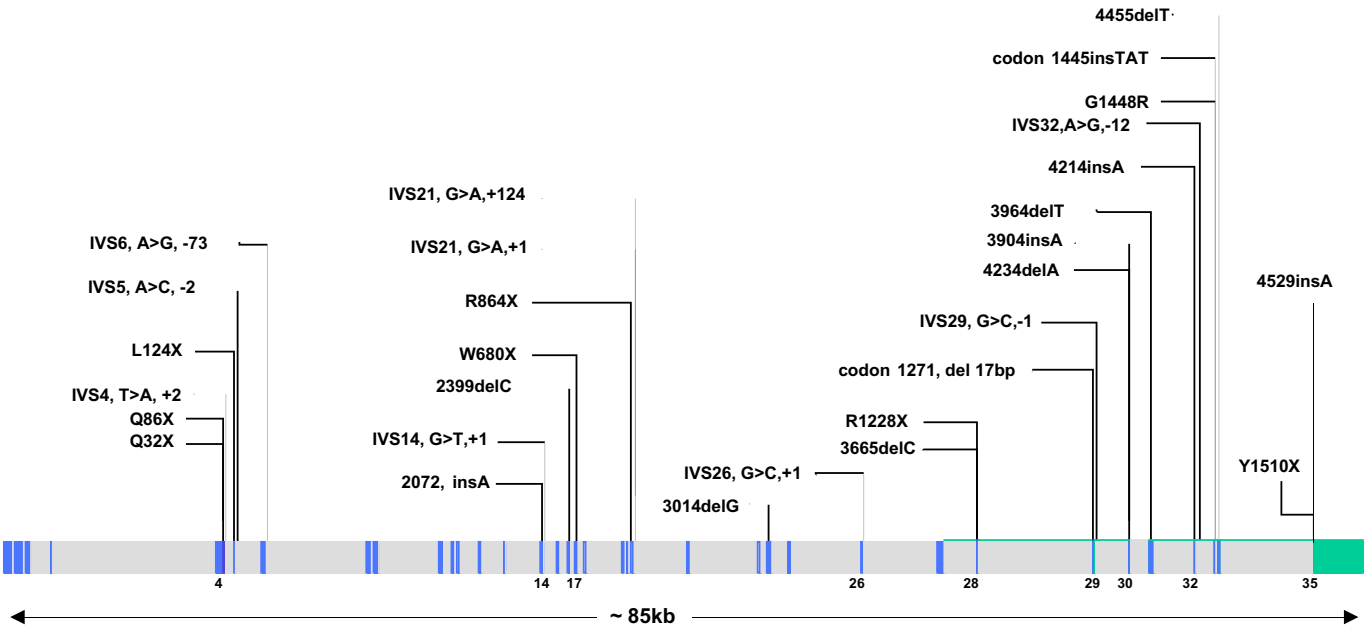

Figure 7. *Pathological mutations in the human debrancher gene (AGL).* The mutations indicated are those that have been specifically associated with GSD IIIa or for which no association may yet be made, as the affected individual was a child. There is some disparity in relation to cDNA base numbering and the mutations have mostly been cited in the same nomenclature as used by the original authors.

opments in the treatment of other myopathies. At present, a managed lifestyle seems to offer the best option for optimal adjustment to daily life, but there is one report of a ketogenic diet that seemed effective in a severely affected infant (34).

Debrancher deficiency

Debrancher deficiency (Cori-Forbes disease, Glycogen storage disease III, OMIM 232400) is a complex set of conditions of variable severity (11, 17). Most patients have liver and muscle involvement (Type IIIa), but approximately 15% of all affected individuals manifest liver involvement without any muscle-specific symptoms (Type IIIb). There may be an associated cardiomyopathy.

The enzyme affected is the product of a single gene (gene name AGL, chromosome 1p21), which encodes a large protein (1,532 amino acids, 175kDa). The enzyme, in concert with glycogen phosphorylase, effects glycogenolysis by removing the 1,6 branch points in the glycogen structure (amylo-1,6-glucosidase activity) and by maintaining the lengths of the 1,4 linked glucose chains for effective phosphorylase action (1,4,glucan-1,4 glycosyl transferase activity). The catalytic sites for these two activities are located at different domains of the protein, and in rare cases, either one or the other activity can be selectively disabled (Type IIIc or Type IIId respectively).

Expression of debrancher enzyme is controlled differentially in liver and muscle. There are six splice variants involving exons 1 to 3. Variants 1, 5, and 6 are present in liver and muscle, whereas variants 2, 3, and 4 occur in muscle but not liver (2). The start codon is located within exon 3, and variants 1 to 4 encode identical proteins that have an additional 27 amino acids that are absent from variants 5 and 6. In turn, variants 5 and 6 differ at the N-terminal from variant 1 by 10 and 11 amino acids, respectively (2). Tissue specific mRNA or protein variants may explain in part the distinction between Type IIIa and Type IIIb cases—in the latter, enzyme activity is absent in liver but present in muscle, whereas in Type IIIa the enzyme activity is impaired in both tissues. Western blotting of samples from 41 patients with GSD III showed that only patients with defective transferase activity (Type IIId) had cross-reactive material. In all other cases (31 Type IIIa, four with type IIIb and three unknown), the cross-reactive material was greatly reduced or absent (10).

In Type IIIa cases, the liver symptoms (hepatomegaly, hypoglycaemia, and hyperlipidaemia) are present in childhood but usually disappear in adulthood. Muscle symptoms may be absent in childhood but appear progressively with age. Because glycogen breakdown is not fully impaired (glycogen phosphorylase activity would be expected to be normal) plasma creatine kinase may be less markedly elevated, and episodes of myoglobinuria are less common than in McArdle's disease or Tarui's disease.

Molecular genetics and pathogenesis. Over 20 mutations have been described for debrancher deficiency Type IIIa (Figure 7). These range from missense and nonsense mutations to splice variations and short deletions within the gene. Whilst the differentially expressed mRNA isoforms may explain some differences between Type IIIa and Type IIIb (31), debrancher

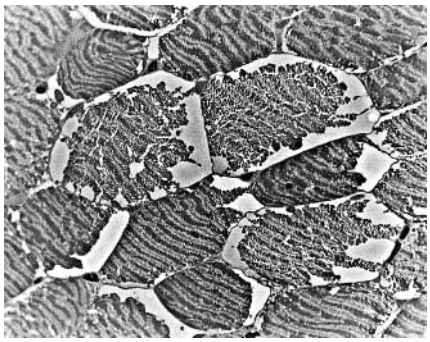

Figure 8. Large sarcolemmal lakes filled with material of groundglass staining appearence (glycogen) are present in many muscle fibres in a case of myopathy caused by glycogen debrancher enzyme deficiency. Semithin resin section. ×550. Image kindly provided by Dr George Karpati, Montreal.

deficiency, in common with the other myopathies discussed here, demonstrates clinical heterogeneity even within a group of patients homozygous for the same mutation (4455delT). It has been suggested that even in Type IIIa cases, the clinical phenotypes may be further subdivided (18, 19).

Structural changes. Muscle pathology is mild and sections may show glycogen accumulation (Figure 8), often near the periphery of the fibre.

Future perspectives. The explanation for the different clinical phenotypes will undoubtedly extend beyond simple descriptions of the mutations in the gene of interest, and unravelling genetic and environmental factors is a major challenge for debrancher deficiency, just as it is for the other myopathies. There are no specific treatments, although a high-protein diet proved effective in a single case, where failure of respiratory muscles had been precipitated by extreme dieting (18, 19).

A note on "Double Trouble"

The frequency of myoadenylate deaminase deficiency (Chapter 10.4) in the general population is sufficiently high (1-2%) such that is it possible to encounter patients lacking this enzyme and a second enzyme, such as myophosphorylase or phosphofructokinase. This coincidence, often termed "double trouble," has been advanced to explain more severe forms of the conditions (7, 28, 37). However, the pattern of clinical phenotypes again remains elusive, and there are cases where muscle dysfunction seems no worse than others lacking the glycolytic or glycogenolytic enzyme alone (29), or other cases where the conditions are severe. It seems unlikely that "double trouble," where the second component is a deficiency of myoadenylate deaminase, can explain the range of clinical phenotypes observed in these conditions.

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