NEUROLOGICAL INVESTIGATIONS

Investigation of muscle disease

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Various pathological processes, some genetically determined and others acquired, may affect the function of the skeletal muscles and may manifest in different ways. Some, such as the congenital myopathies, produce weakness and hypotonia at birth whereas others do not cause functional abnormalities until childhood, adolescence, or adult life. With the application of modern molecular biological techniques major advances have taken place in the identification of the genetic mutations responsible for many of the hereditary muscle diseases and new mutations in nuclear or mitochondrial DNA are being reported on a regular basis.^{1,2} These discoveries have had a major impact on the diagnostic approach to patients with these disorders and have led to the definition of new categories of myopathy

such as the *dystrophinopathies*, encompassing the Duchenne and Becker forms of muscular dystrophy, the *sarcoglycanopathies*, which include many cases of limb-girdle muscular dystrophy, and the *channelopathies* comprising the periodic paralyses and myotonic syndromes.

This review focuses on the modern approach to the clinical and laboratory investigation of patients with muscle diseases with particular emphasis on the application of molecular techniques in diagnosis.

Clinical evaluation

The investigation of a patient with muscle disease should always commence with a detailed history which, in the case of known hereditary disorders, may provide an immediate indication of the nature of the patient's condition. Moreover, a history of heavy alcohol consumption or administration of drugs with known myotoxic actions (table 1) may point to a toxic and therefore potentially reversible aetiology for the patient's symptoms.³⁻⁵ A history of a thyroidectomy or parathyroidectomy, or symptoms of hypothyroidism or hyperthyroidism, should alert the physician to the possibility of an endocrine cause whereas a history of chronic diarrhoea, purgative misuse, or excessive consumption of liquorice or other preparations containing glycyrrhizic acid such as snuff, chewing tobacco, and certain traditional Chinese medicines, should suggest the possibility of hypokalaemic myopathy. A history of malignancy, of a systemic connective tissue disease, other autoimmune disease, or immunodeficiency state may indicate a predisposition to an inflammatory myopathy.

When muscle pain is a feature, hypothyroid, osteomalacic or other metabolic myopathy, parasitic infestation (for example, trichinosis), or a toxic myopathy or neuromyopathy should be considered, (table 2), although in many patients no specific aetiology will be found even with complete investigation. Myalgia, muscle weakness, or fatigue developing after an acute viral infection also raises the possibility of an inflammatory myopathy but in many such patients when fatigue and reduced exercise tolerance are the major symptoms, a diagnosis of postviral chronic fatigue syndrome will usually be reached.

Table 1 Drug induced muscle disorders

Disorder	Inducing drug
Myalgia	Suxamethonium, danazol, clofibrate, salbutamol, lithium, captopril, colchicine, procainamide, metolazone, cytotoxics, zidovudine, isoetharine, zimeldine, labetalol, pindolol, cimetidine, penicillamine, gold, enalapril, rifampicin, L-tryptophan, nifedipine
Myotonia	Diazacholesterol, β -blockers,* β -agonists (fenoterol, ritodrine),* clofibrate,† diuretics (frusemide, ethacrynic acid, mersalyl, acetazolamide)†
Necrotising myopathy	Alcohol, gemfibrozil, lovastatin, simvastatin, clofibrate, ϵ -aminocaproic acid, cyclosporin, zidovudine, cocaine, emetine
Mitochondrial myopathy	Zidovudine
Inflammatory myopathy	D-penicillamine, L-tryptophan, others rarely
Autophagic myopathy	Chloroquine, vincristine, colchicine, amiodarone, perhexiline
Type 2 atrophy	Corticosteroids
Localised myopathy	Intramuscular antibiotics, narcotics

*May exacerbate myotonia.

†May cause myotonia in animals.

Table 2 Disorders in which muscle pain may be a prominent feature

Inflammatory	Hereditary
Viral myositis	Disorders of glycogen metabolism
Pyomyositis	Carnitine palmitoyl transferase deficiency
Parasitic myositis	Myoadenylate deaminase deficiency
Polymyositis/dermatomyositis	Mitochondrial myopathy
Granulomatous myositis	Dystrophinopathy
Interstitial myositis	Sodium channel myotonia
Localised nodular myositis	Malignant hyperthermia
Vasculitis	
Eosinophilic fasciitis	Others
	Fibromyalgia
Toxic	Polymyalgia rheumatica
Acute alcoholic myopathy	Postviral myalgia/fatigue
Acute/subacute drug induced myopathies	Muscle overuse syndromes
Myopathies due to envenomation	Myopathy with tubular aggregates
Endocrine	
Hypothyroidism	
Osteomalacia	
Hyperparathyroidism	

In certain instances it may be possible to reach a definitive diagnosis on the basis of the pattern of muscle involvement found on clinical examination or the finding of other distinctive features such as myotonia, fatigability, muscle contractures, or other systemic features. Although the distribution of muscle involvement in most of the acquired myopathies is relatively non-selective, in the genetic myopathies certain patterns of muscle weakness are distinctive and may be diagnostically helpful although it is being increasingly recognised that the phenotypic manifestations of specific genetic defects may be very variable (for example, the dystrophinopathies). Involvement of the extraocular and eyelid muscles is seen characteristically in oculopharyngeal muscular dystrophy, usually associated with dysphagia and often with limb muscle involvement. They are also involved in the syndrome of chronic progressive external ophthalmoplegia, which is usually due to a mitochondrial myopathy and may occur in isolation, or with a limb myopathy, or other systemic features such as pigmentary retinopathy, heart block, cerebellar ataxia, and sensorineural hearing loss as in the Kearns-Sayre syndrome. Involvement of the facial muscles is usually a prominent feature in facioscapulohumeral muscular dystrophy but may also occur in myasthenia gravis, when it is usually associated with involvement of the extraocular muscles, and often of the bulbar and limb muscles; fatigability is a prominent feature. In myotonic dystrophy there is often also involvement of the facial muscles and, characteristically, there is atrophy and weakness of the sternomastoids and of the distal limb muscles in the later stages of the disease. Other systemic features which point to the diagnosis include cataracts and, in men, frontal baldness and testicular atrophy. Severe weakness of the neck extensor muscles leading to the "dropped head syndrome"⁶ may occasionally be the presenting feature in patients with inflammatory myopathy and may also occur in motor neuron disease and longstanding myasthenia gravis. Weakness confined to or most severe in the distal limb muscles also occurs in the distal myopathies and scapulohumeral syndrome.

The limb-girdle syndrome, in which there is involvement of the girdle and proximal limb muscles, is relatively non-specific and may be seen in several genetic and acquired myopathies. Although in most cases of polymyositis and dermatomyositis there is a predominantly proximal pattern of muscle involvement, in inclusion body myositis the distribution of muscle weakness is characteristically selective, at least in the earlier stages of the condition, with involvement especially of the quadriceps femoris muscles in the lower limbs and the forearm flexors, particularly the flexor digitorum profundus, in the upper limbs.⁷ When present, the characteristic skin rash of dermatomyositis over the face and the extensor aspects of the metacarpophalangeal and interphalangeal joints is diagnostic of that condition.

Muscle hypertrophy, when confined to the calves, is seen most typically in Duchenne and Becker dystrophy, but occasionally also in

other types of muscular dystrophy or spinal muscular atrophy, whereas more generalised hypertrophy is common in myotonia congenita. A hypertrophic myopathy may occasionally occur in patients with amyloidosis, sarcoidosis, or cysticercosis. Muscle contractures occur, especially in Emery-Dreifuss muscular dystrophy, and are also a feature of the fibrosing myositis associated with scleromyxoedema.⁸ It is always worth looking for muscle tenderness, which, when confined to certain muscles such as those of the calves, may indicate a focal inflammatory or vasculitic process, whereas the characteristic pattern of myofascial tenderness in patients with fibromyalgia is virtually diagnostic of that condition.

Depressed deep tendon reflexes or sensory abnormalities in a patient with a myopathy suggest the presence of an associated peripheral neuropathy. This combination can occur in patients with drug induced neuromyopathy, connective tissue disease, inclusion body myositis, a paraneoplastic syndrome, or mitochondrial myopathy.

Biochemical studies

CREATINE KINASE

The serum concentration of creatine kinase is the most reliable biochemical indicator of muscle disease. The highest concentrations occur in patients with acute rhabdomyolysis, inflammatory or drug-induced myopathies, and Duchenne muscular dystrophy in the early stages when the patient is still ambulant. High concentrations may also occur in some metabolic myopathies such as hypokalaemic or hypothyroid myopathy. Moderately raised concentrations may also be found in patients with chronic neuropathic conditions such as spinal muscular atrophy or motor neuron disease, although it is rare for the creatine kinase to exceed 10 times the normal maximum concentration in these conditions.⁹ Raised creatine kinase concentrations may also be found in some people, without clinical evidence of neuromuscular disease¹⁰ and may be useful in detecting those with a genetic risk of malignant hyperthermia, presymptomatic muscular dystrophy, carriers of Duchenne and Becker muscular dystrophy, and early inflammatory myopathy (table 3). Serum creatine kinase concentrations are normal in most congenital myopathies, myotonic syndromes, and corticosteroid and thyrotoxic myopathy.

Slight increases in serum creatine kinase (up to about three times the normal maximum concentration) are not necessarily due to muscle disease and may occur transiently as a result of strenuous exercise, minor muscle trauma including intramuscular injections, and insertion of EMG needle electrodes, or viral illnesses.⁹ In a situation where the creatine kinase concentration is unexpectedly raised it is usual to repeat the test after an interval of a week during which the patient is advised not to engage in strenuous physical exercise, and to consider embarking on further investigations only if the concentration remains high or is rising.

Table 3 Causes of sustained increased serum creatine kinase concentrations in subjects without clinical signs of muscle disease

Physical exercise
Muscle trauma
Pressure
Falls
Injections
Acute psychosis/delirium
Dyskinesiae
Drugs
Alcohol
Others*
Hypothyroidism
Hypokalaemia
Presymptomatic
Malignant hyperthermia
Muscular dystrophies
Inflammatory myopathies
McArdle's disease
Carrier state
Duchenne, Becker dystrophy
Hereditary hyperCKaemia

*See table 1.

Serum concentrations of other enzymes such as aspartate and alanine aminotransferase, aldolase, and lactate dehydrogenase are of less value than the creatine kinase concentration but may provide a clue to the presence of muscle disease if they are found to be raised as part of an initial biochemical screen in a patient with no other indication of liver disease.

MYOGLOBIN

Myoglobinaemia and myoglobinuria may be due to various causes¹¹ and result in urinary pigmentation and a positive benzidine dip stick test on the urine as does haemoglobinuria and haematuria. Confirmation of myoglobinuria requires a specific myoglobin radioimmunoassay. Serum myoglobin concentrations are raised in patients with inflammatory¹² and other necrotising myopathies but are not of any additional value to the creatine kinase concentration in diagnosis or in monitoring the response to treatment.

LACTATE CONCENTRATIONS

Venous lactate concentrations may be raised at rest and after low levels of exercise in patients with mitochondrial myopathy and defects of the respiratory enzyme chain. Conversely, lactate production is absent or diminished in metabolic myopathies due to defects in glycogenolysis (for example, myophosphorylase or phosphorylase b kinase deficiency) or of the glycolytic pathway (for example, phosphofructokinase, phosphoglycerate mutase, phosphoglycerate kinase, or lactate dehydrogenase deficiency) and is the basis for the forearm exercise test. This was previously performed under ischaemic conditions but, because of the occurrence of severe rhabdomyolysis in some patients with glycogen metabolic defects, this is no longer recommended.⁹ Venous blood samples for estimation of lactate and ammonia are taken at rest and at 1, 2, 4, 6, and 10 minute intervals after a one minute period of repetitive maximum isometric contractions of the forearm flexor muscles. Whereas there is normally a twofold to threefold rise in the lactate concentration within the first two minutes after exercise, this response is absent or diminished in patients

with myophosphorylase deficiency or a glycolytic defect. The rise in venous ammonia concentrations that normally occurs after exercise is absent or reduced in patients with myoadenylate deaminase deficiency in which ammonia production during exercise is impaired.¹³

NUCLEAR MAGNETIC RESONANCE

SPECTROSCOPY

With NMR spectroscopy it is possible to monitor changes in muscle metabolite concentrations during exercise non-invasively (for example, inorganic phosphate, phosphocreatine, ATP, lactate concentrations, and intracellular pH).^{14,15} This may be useful in the evaluation of patients with disorders of glycolytic or mitochondrial metabolism. The technique is also useful in the evaluation of patients with fatigue and reduced exercise tolerance to determine whether there is evidence of an underlying defect of muscle energy metabolism.

OTHER STUDIES

Other biochemical studies which may be relevant in the investigation of some patients with muscle symptoms include serum potassium, calcium, phosphate, thyroxine, and cortisol concentrations, as well as urea and creatinine concentrations and urinary creatine and 3-methyl histidine excretion as indices of skeletal muscle mass and breakdown.¹⁶

Pharmacological testing

Although in some families with malignant hyperthermia mutations have been found in the ryanodine receptor gene and at risk subjects can therefore be detected using molecular genetic techniques, in many other families as well as in those without a known family history who are suspected of being at risk, it is still necessary to perform in vitro muscle contracture testing. This involves the exposure of muscle tissue obtained at biopsy to caffeine and halothane which, in at risk subjects, induce an exaggerated contractile response. Abnormal responses to caffeine and calcium ions have also been described using the skinned muscle fibre technique.¹⁷ Contracture testing should also be performed in those with unexplained episodes of muscle stiffness or rhabdomyolysis during or after anaesthesia, with infections, or after exercise or heat exposure, and in patients with central core or multi-core disease who are also at risk.

Electrodiagnostic studies

ELECTROMYOGRAPHY

Conventional EMG is an important investigative procedure in patients with suspected muscle disease. Firstly, it will often provide confirmation of a primary myopathic basis for the patient's condition and allow differentiation from a neurogenic disorder. Characteristically, the duration of motor unit action potentials (MUAPs) is diminished as is MUAP amplitude although this is often less

pronounced and more variable, and there is an increased number of polyphasic motor unit potentials. With voluntary contraction there is early recruitment of increased numbers of short duration MUAPs and an unduly full interference pattern which is often reduced in amplitude. Reduced motor unit recruitment and electrical excitability of the muscle are found during attacks of weakness in patients with periodic paralysis. Spontaneous fibrillation potentials, positive sharp waves, and complex repetitive discharges may be found in some myopathic disorders and are particularly prominent in active inflammatory myopathies and certain metabolic and toxic myopathies (for example, hypothyroid and chloroquine myopathy), in some cases of Duchenne muscular dystrophy and distal myopathy, and in myotonic disorders in which they are associated with a pronounced increase in insertional activity and the diagnostic waxing and waning ("dive bomber") myotonic discharges resulting from electrical instability of the muscle cell membrane.¹⁸ The occurrence of spontaneous potentials in non-myotonic myopathies has been attributed to functional denervation of muscle fibres which have been disconnected from their motor innervation as a result of segmental necrosis. Regenerating muscle fibres which have yet to be innervated may also be a source of spontaneous potentials.

The distribution of EMG changes within individual muscles and in representative proximal and distal limb and axial muscles provides an indication of the extent of the myopathic process and pattern of muscle involvement. This may be useful in selecting a muscle for biopsy, and may also indicate whether the disease process is one which is affecting the muscles in a patchy manner (often the case in the inflammatory myopathies) or more diffusely (as in the muscular dystrophies or metabolic myopathies). The coexistence in the same muscle of typical myopathic MUAPs and longer duration polyphasic potentials is a potentially confusing combination sometimes encountered in patients with very longstanding denervating conditions such as spinal muscular atrophy,¹⁸ and in inclusion body myositis in which there may also be prominent fibrillation potentials as well as increased fibre density and jitter.¹⁹

Although in many patients with well established myopathies the EMG changes are florid and unmistakable, in some patients with milder forms of myopathy the motor unit changes are less conspicuous. It is in the evaluation of such patients that quantitative techniques employing automated measurement of MUAP variables and motor unit recruitment for given levels of effort are useful.¹⁸

NEUROMUSCULAR TRANSMISSION STUDIES

Repetitive nerve stimulation studies should be performed if muscle fatigue is prominent or if there are other features suggestive of myasthenia gravis or of the Lambert-Eaton myasthenic syndrome or other neuromuscular junction disorder.

Single fibre electromyography (SFEMG),

which can be performed either with voluntary muscle activation or with nerve stimulation, characteristically shows increased jitter and intermittent blocking indicating failure of transmission at single motor endplates, and is more sensitive than repetitive nerve stimulation studies in the diagnosis of neuromuscular transmission disorders.

In myasthenia gravis SFEMG shows an abnormality in up to 95% of patients with limb involvement and a normal SFEMG study in a patient with clearcut limb weakness and fatigue is strongly against the diagnosis of myasthenia.¹⁸ However, similar abnormalities may be found in several myopathies and other neuromuscular disorders and it is therefore essential that the findings are interpreted in the context of the particular clinical situation.

NERVE CONDUCTION STUDIES

Motor and sensory nerve conduction studies as well as F wave and H reflex studies may be appropriate to exclude the possibility that the patient's symptoms are due to a peripheral neuropathy. Moreover, a peripheral neuropathy may coexist in some patients with myotonic dystrophy, mitochondrial myopathy, drug induced myopathy, inclusion body myositis, or inflammatory myopathy associated with connective tissue diseases or malignancy.

Muscle imaging

The techniques of CT and MRI can provide information on the cross sectional area of limb or axial muscles and may therefore be useful in detecting muscle atrophy or hypertrophy and defining selective patterns of muscle involvement in certain conditions such as the muscular dystrophies and inclusion body myositis.^{20,21} These techniques may also show differences in muscle properties between conditions such as the muscular dystrophies in which there is extensive fatty infiltration of muscle, and the inflammatory myopathies, but the changes are not sufficiently specific to be of diagnostic value.²² The techniques are, however, of use in detecting and defining the extent of suppurative lesions of muscle (pyomyositis). Radioisotopic techniques with muscle scanning after administration of technetium pyrophosphate can also be used to detect such lesions as well as areas of active inflammatory myopathy in patients with polymyositis or dermatomyositis, and areas of muscle infarction or pressure necrosis (crush syndrome). Isotopic scanning may also be used to show the extent of subcutaneous calcinosis in patients with dermatomyositis and to monitor changes with treatment.

Muscle biopsy

A biopsy is still required to provide a definitive diagnosis in the case of many muscle diseases. This is especially so in the case of the inflammatory myopathies, in sporadic cases of muscular dystrophy, suspected metabolic myopathies, and in patients in whom there is still uncertainty as to whether the condition is primarily neuropathic or myopathic after clinical and

EMG evaluation. In neuropathic disorders a biopsy from an affected muscle will show the characteristic changes of denervation including angulated fibres, grouped fibre atrophy, and, if the condition is protracted, fibre type grouping, but will not necessarily differentiate between anterior horn cell and peripheral nerve disorders. In the more chronic neurogenic disorders—such as the spinal muscular atrophies—histological changes resembling those found in chronic myopathic disorders may also be present (secondary myopathic change).

The effectiveness of muscle biopsy in establishing the diagnosis of myopathy is determined by the correct choice of muscle, the correct biopsy technique, and the application of the appropriate staining and other procedures to the biopsy.²³ In selecting a suitable biopsy site the ideal muscle is one which is only moderately affected (for example, with MRC grade 4 muscle power), and which has not been the site of previous intramuscular injections or needle EMG studies. Muscles which are too severely weakened or atrophic should be avoided as the histological changes in such muscles are often non-specific and difficult to interpret. Selection of a needle or open biopsy is determined by personal choice, experience, and availability. Whereas needle biopsy requires only a minor incision and the procedure can be repeated at multiple sites and on more than one occasion, the size of the tissue sample obtained is limited and histological interpretation may be difficult, particularly in diseases in which the muscle involvement is patchy. In many instances therefore, and especially in the case of suspected inflammatory myopathy or vasculitis, an open biopsy is preferable, having the advantage of providing a larger tissue sample and thereby reducing sampling error.

To maximise the information derived from the muscle biopsy a battery of histological and histochemical stains should be applied routinely to cryostat sections of frozen tissue. In addition, in some cases it will be necessary to apply other selected histochemical or immunohistochemical techniques using monoclonal antibodies for the diagnosis of specific enzyme deficiencies, storage disorders, or other hereditary defects such as the dystrophinopathies and sarcoglycanopathies. Electron microscopic examination of muscle tissue is also necessary especially in cases of suspected mitochondrial myopathy, inclusion body myositis, and in some congenital myopathies. Tissue should therefore be taken routinely for this purpose at the time of the biopsy and fixed in glutaraldehyde.²³ In metabolic and other inherited myopathies it is also prudent to obtain an additional tissue sample for quantitative biochemical analyses or molecular studies should these be required.

In the hereditary myopathies, immunohistochemistry of muscle biopsies should be used whenever possible to identify the patient or family muscle disease. For example, the autosomal recessive Duchenne-like muscular dystrophies and the limb-girdle dystrophies can be very difficult to distinguish from Duchenne

and Becker dystrophy clinically, whereas antibodies can make the distinction very easily. Up until recently these cases were among the residual of patients all muscle clinics have who are difficult to categorise. For example, Bonnemant *et al*²⁴ in their recent characterisation of mutations in β -sarcoglycan screened 62 patients with possible β -sarcoglycan involvement out of a bank of 2500 muscle biopsies to identify their one β -sarcoglycan patient. In our own clinic, screening nine families undiagnosed for years, with antibodies to adhalin, showed that three of the families were α -sarcoglycan (adhalin) negative (RD Johnson and BA Kakulas, unpublished observations) and thus are at risk from a sarcoglycanopathy. Immunohistochemistry or immunoblotting can also predict the severity of disease—for example, in both the dystrophinopathies^{25–27} and the sarcoglycanopathies^{24,28}. On the other hand, molecular analysis cannot always predict severity of disease, as people with apparently the same mutation can have different severities of disease²⁹ perhaps due to alternate splicing around the mutation.^{30,31}

Molecular diagnosis

Molecular biology, particularly positional cloning, has led to the recent major increase in understanding of the causes of inherited diseases, through identifying the genes mutated in these diseases and thus the faulty proteins.³² In the muscle clinic, similar molecular techniques can be used for four major purposes:

- Identifying the precise disease affecting an individual patient and family.
- Accurate, simple prenatal diagnosis
- Identifying asymptomatic members of families who are at risk from having more severely affected offspring
- Presymptomatic diagnosis.

All of these lead to more accurate and appropriate genetic counselling.

IDENTIFYING THE PRECISE DISEASE AFFECTING AN INDIVIDUAL PATIENT AND FAMILY

Molecular diagnosis is especially useful for conditions which are difficult to distinguish clinically or by other laboratory techniques. The sarcoglycanopathies,³³ in which mutations in adhalin, β -sarcoglycan, and γ -sarcoglycan all look similar by immunohistochemistry, are a good example of diseases only distinguishable by molecular analysis.³⁴

ACCURATE, SIMPLE PRENATAL DIAGNOSIS

Identifying the precise mutation or mutations causing the disease in a family also provides the only really practical route to prenatal diagnosis where that is appropriate. The only current alternative for inherited muscle diseases would be fetal muscle biopsy.³⁵

IDENTIFYING ASYMPTOMATIC MEMBERS OF FAMILIES WHO ARE AT RISK FROM HAVING MORE SEVERELY AFFECTED OFFSPRING

Molecular diagnosis allows the identification of minimally affected or asymptomatic persons. This is important for identifying carriers

of X linked diseases such as Duchenne and Becker muscular dystrophy, Emery-Dreifuss dystrophy, and X linked centronuclear myopathy who are at risk of having affected male offspring. It is also important for tracing at risk subjects—for example, in myotonic dystrophy pedigrees—through cascade screening.

PRESYMPTOMATIC DIAGNOSIS

Presymptomatic diagnosis using molecular techniques has been applied, for example, to diagnose the cause of the raised creatine kinase and to screen other family members after neonatal or infant screening for Duchenne muscular dystrophy.^{36 37} Presymptomatic diagnosis is also possible by molecular techniques in any of the later onset diseases, such as hypokalaemic periodic paralysis.³⁸

The polymerase chain reaction (PCR),³⁹ which amplifies specific regions of DNA, has played a major part in the molecular revolution and is being manipulated for ever more purposes—such as reverse transcriptase PCR (RT-PCR) which allows PCR to amplify specific cDNA messages⁴⁰ or the creation of restriction enzyme sites to discriminate between normal and mutant alleles.⁴¹

DEGREES OF EASE OF MOLECULAR DIAGNOSIS

There are perhaps three degrees of difficulty for molecular diagnosis: easy, difficult, and average. Easy diseases to diagnose are those in which most cases are caused by a single mutation, or a few mutations, or mutations which are simple to detect. Difficult diseases to diagnose are: (a) those in which most cases are caused by many different missense mutations which alter only a single amino acid and thus do not truncate the protein; (b) diseases in which linkage to a particular locus has been identified but the disease gene has not been identified; or (c) diseases with significant genetic heterogeneity—that is, where the same clinical phenotype may be caused by mutations in a number of different genes. Average diseases to diagnose are those caused in most cases by nonsense mutations which produce a premature stop codon and therefore truncated proteins.

Easy diseases to diagnose by molecular analysis

Diseases which currently fall into this category are:

- (1) Myotonic dystrophy, in which the triplet repeat expansion can be detected and sized by a PCR reaction, and/or Southern blotting using EcoRI, BglII, or BamHI digestion.⁴²
- (2) Duchenne and Becker muscular dystrophy (for most patients) as most disease causing mutations are large deletions of the dystrophin gene. These deletions can be detected by multiplex PCR analysis of DNA from affected boys to identify the deleted exons.^{25 43–45}
- (3) Spinal muscular atrophy (autosomal recessive), in which the common deletion can be detected by two single strand conformation polymorphism (SSCP) analyses, one for exon 7 of the survival motor neuron gene and one for exon 8.^{46 47}

- (4) Hypokalaemic periodic paralysis, in which mutations to two residues in the dihydropyridine receptor calcium channel (CACNL1A3) account for all cases so far described.⁴⁸

- (5) Mitochondrial myopathies, in which the common point mutations can be tested for using PCR followed by enzyme digestion^{41 49} or SSCP analysis.⁵⁰

Difficult diseases to diagnose by molecular analysis

Diseases in which most cases are caused by many different missense mutations—Finding the mutations causing disease in such conditions is the most difficult task for molecular analysis. This is because the entire gene sequence has to be screened, and the difficulty is therefore increased if the gene message is large, which is the case for many of the structural proteins of muscle. The difficulty of identifying missense mutations was reinforced again in a recent summary of current methods of screening genes for unknown mutations,⁵¹ which states that, “Detection of mutations, particularly at the 100% level is time consuming and expensive” and “The ‘one best method’ still remains elusive”. Indeed, the plethora of techniques currently on the market for identifying unknown mutations in genes indicates that none is ideal. A previous review⁵² summarised the techniques then current such as the simple single strand conformation analysis (SSCA), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis (HA), RNAase A cleavage, chemical mismatch cleavage (CMC), and direct sequencing. Techniques which should perhaps be added to the list include restriction endonuclease fingerprinting (REF),⁵³ dideoxyfingerprinting,⁵⁴ and enzymatic mismatch cleavage (EMC).^{55 56}

The heroic way to look for mutations is to search genomic DNA. This will often involve synthesising PCR primers for regions flanking each of the exons of the gene followed by, for example, SSCP analysis of each exon. This would necessitate synthesising 80 primers for a gene like the cardiac β -myosin heavy chain gene (MYH7) involved in familial hypertrophic cardiomyopathy—as MYH7 has 40 exons—and then performing 40 separate SSCPs. The short cut alternative is to examine the cDNA for mutations.⁵² This reduces the number of base pairs that have to be screened compared with examining the genomic sequence of the gene. Analysis of cDNA will also instantly disclose any deletions or duplications of the message and splice site mutations which also significantly alter the size of the transcript, leading to rapid identification of one type of single base mutation.^{57 58} To examine the cDNA however, a source of RNA is required from the patient in whom the candidate gene is expressed. This should be relatively simple, as nearly all patients with a muscle disease should have had a muscle biopsy, and RNA can easily be extracted from frozen muscle tissue and cDNA synthesised for examination. Analysis of cDNA made from illegitimate transcription messages⁵⁹ should always be considered as a possible means of

obtaining cDNA for muscle disease genes. However, in some cases it may be more appropriate to analyse cDNA from muscle, (for example, the mitochondrial myopathies), as the mutations in skeletal muscle may be different and more relevant than those in circulating lymphocytes.⁶⁰

Suggesting that a missense mutation in any message is responsible for disease—especially in a giant message like the dystrophin message⁶¹—is fraught with difficulties.⁶² The criteria normally used to decide that an identified alteration in a DNA sequence is a disease causing mutation include a major change of an amino acid, change of an amino acid conserved in many species, the change not being seen in a large number of controls, and the change being in a candidate gene. However, even when all these criteria are fulfilled, the change may still not be disease causing.⁶² A missense mutation found only in a single patient, or a small family, probably requires a functional assay (not necessarily a transgenic mouse) or at least characterisation in an expression system to be certain that the missense mutation causes the disease.⁶³ Unless there is this degree of certainty about the status of the identified alteration in DNA, it should not be used for prenatal diagnosis.

Diseases in which there is only linkage but the precise disease gene has not been identified—Distal myopathy,^{64,65} dominant limb-girdle muscular dystrophy,^{66,67} X linked centronuclear (myotubular) myopathy,⁶⁸ oculopharyngeal muscular dystrophy,⁶⁹ and recessive nemaline myopathy⁷⁰ are all diseases in which there is at present linkage but no identified disease gene. When there is only linkage for a disease, the use of linkage for diagnosis should perhaps be restricted to individual families in which the significant LOD score of 3 has been obtained, especially if there is evidence of genetic heterogeneity for the disease.

Diseases with significant genetic heterogeneity—Perhaps the best example of this at present is malignant hyperthermia, in which although many families show linkage to the ryanodine receptor gene on chromosome 19⁷¹ many others apparently do not^{72,73} and virtually only single families have shown linkage to other regions of the genome.⁷⁴ The limb-girdle mus-

cular dystrophies may also be included in this category. Some of the different types can now be identified as sarcoglycanopathies or dystrophinopathies by immunohistochemistry, but it is still a large task to precisely identify the gene involved by molecular diagnosis.

Average diseases to diagnose by molecular analysis Average diseases are those in which most mutations causing disease are nonsense mutations, as these can be detected using the protein truncation test (PTT).^{75,76} This test uses an in vitro translation system to synthesise protein from cDNA. If there is a mutation in the protein coding region leading to a premature stop codon, the protein synthesised in vitro will be shorter than normal and can be identified by electrophoresis. The PTT was first applied widely to the identification of nonsense, premature stop codon producing, mutations in familial adenomatous polyposis coli⁷⁵ and Duchenne muscular dystrophy,⁷⁶ (fig 1), but it has also been applied to neurofibromatosis.^{77,78} The PTT should perhaps be used for all recessive disorders, especially severe ones, as these are often caused by nonsense mutations. For example, the PTT would have detected many of the mutations so far described in the sarcoglycanopathies, especially the severe cases,^{24,28,33,79} in which prenatal diagnosis would be most appropriate and there would be most likelihood of pressure to rapidly find the family mutation.

Obviously some diseases fit into both the easy and difficult categories or both the easy and average categories. Duchenne muscular dystrophy is both easy for most mutations and average for the minority. Mitochondrial myopathies are easy for most cases and difficult for the others. The common mtDNA point mutations can be tested relatively easily, but all the other possible mutations are difficult to screen for and to separate from polymorphisms not causing disease.⁸⁰ This is also true for other diseases. However, testing only for the common known mutations becomes a self fulfilling prophecy and unless the rest of the transcript is screened for mutations, many mutations causing disease may be missed.

As emphasised by Forrest *et al*⁵¹ it is essential that comprehensive databases of gene mutations and perhaps, even more importantly, databases of normal polymorphisms should be created and widely disseminated, so that individual diagnostic laboratories can cross check whether a base change identified in a patient is a known mutation causing disease, a known polymorphism, or entirely new.

Muscular dystrophies

The classification of the muscular dystrophies has undergone considerable change with the recognition of the disease genes and molecular basis for some of these conditions—in particular, the X linked and the limb-girdle dystrophies (table 4).¹ The availability of genetic or molecular markers for many of these conditions has meant that the diagnosis can now be established with greater precision and that diseases manifesting with similar phenotypes can

Figure 1 Protein truncation for the deletion prone part of the dystrophin gene carried out on mRNA from muscle biopsies. Lanes 1 and 2 are from control muscle biopsies. Lanes 3, 4, 5, and 6 show variously truncated proteins in four patients with Duchenne muscular dystrophy.

Table 4 Muscular dystrophies

Dystrophy type	Locus	Gene
X linked:		
Dystrophinopathies	Xp21·2	Dystrophin
Duchenne		
Becker		
Quadriceps myopathy		
Myalgia and cramps		
HyperCKaemia		
Emery-Dreifuss	Xq28	Emerin
Centronuclear myopathy	Xq28	?
Autosomal dominant:		
Facioscapulohumeral		
FSHD	4q35	?
FSHD2	?	?
Limb-girdle*		
LGMD1A	5q22-q34	?
LGMD1B	?	?
Distal*		
MPD1	14q	?
Oculopharyngeal*		
OPMD	14q11·2-q13	?
Autosomal recessive		
Limb-girdle		
LGMD2A	15q15·1-q21·1	Calpain
LGMD2B	2q13-p16	?
LGMD2C (SCARMD)	13q12	γ -sarcoglycan
LGMD2D	17q12-q21·33	Adhalin (α -sarcoglycan)
LGMD2E	4q12	β -sarcoglycan
Congenital		
LAMA2	6q2	Merosin
Fukuyama	9q31-q33	?
Distal (Myoshi)*	2p	?
Oculopharyngeal*	?	?

*Autosomal dominant or recessive.

be readily distinguished. For example, in some patients with Becker dystrophy who have a dystrophin mutation the clinical phenotype may closely resemble that of limb-girdle muscular dystrophy, which is now known to have at least seven different genetic varieties (table 4). Other examples include the differentiation of merosin deficiency from a dystrophinopathy in infants with congenital muscular dystrophy and the differentiation of adhalin deficiency from dystrophinopathy in the severe childhood autosomal recessive form of muscular dystrophy (now classified as LGMD2C). The molecular definition of these conditions has also had important implications for the identification of heterozygote carriers and for prenatal diagnosis, especially in the X linked dystrophies. It has also led to the recognition that there is a wide range of phenotypic expression and clinical severity, particularly in the dystrophinopathies and sarcoglycanopathies.^{79 81}

DIAGNOSTIC APPROACH

The diagnostic approach to the patient with a suspected muscular dystrophy initially involves a detailed clinical assessment of the affected patient and family history which, when positive, may point to an X linked disorder or to one of the distinctive forms of dominantly inherited myopathy such as facioscapulohumeral dystrophy or distal myopathy. Electromyography is helpful in confirming the myopathic nature of the condition and excluding myotonic or chronic neurogenic conditions. A raised serum creatine kinase concentration, although not specific, may also be a diagnostic pointer particularly if the concentration is very high as is usually the case in the early stages of Duchenne or Becker muscular dystrophy when concentrations of up to 300 times normal may occur. However, in the other dystrophies less pronounced increases or

normal concentrations may be found. The high serum creatine kinase in affected boys with Duchenne dystrophy at birth has been the basis for the development of neonatal screening programmes for newborn males in some countries.⁸² The detection of isolated cases by neonatal screening makes it possible to prevent the birth of secondary cases in families and it has been estimated that it is possible to prevent 15 to 20% of new cases of Duchenne dystrophy with this type of screening.

In most instances definitive diagnosis of a muscular dystrophy and recognition of the particular type still requires a muscle biopsy for routine histology and immunohistochemical staining for dystrophin and for the other proteins of the dystrophin-glycoprotein complex such as the sarcoglycans. The immunohistochemical demonstration of complete or partial dystrophin deficiency is the gold standard for establishing the diagnosis of Duchenne and Becker muscular dystrophy. Complete or virtually complete absence of dystrophin is characteristic of Duchenne dystrophy whereas in Becker dystrophy there is usually patchy preservation of the protein on the sarcolemma of muscle fibres. Dystrophin immunohistochemistry may therefore be of prognostic as well as diagnostic value in differentiating Duchenne and Becker dystrophy in young boys. The importance of using a panel of antibodies to different regions of the dystrophin molecule from the N to the C terminus has been emphasised.⁸³

Immunoblotting for dystrophin in muscle tissue is a more sensitive technique, which may show more subtle changes in the molecular size or amount of dystrophin. Hoffman *et al*²⁶ showed that patients with Duchenne muscular dystrophy had less than 3% of the normal quantity of dystrophin, intermediate patients had up to 60% of normal concentrations, whereas patients with Becker dystrophy most often had abnormal sized dystrophin rather than abnormal quantities.

MOLECULAR DIAGNOSIS

Dystrophinopathies

The main applications of molecular diagnosis are accurate diagnosis if other methods have failed or are inappropriate, accurate diagnosis of asymptomatic subjects (in the case of these X linked diseases this largely means carriers), and prenatal diagnosis.

The dystrophin gene was the first major disease gene to be identified by positional cloning. The dystrophin message and the distribution of deletion mutations which cause about 70% of cases of Duchenne and Becker muscular dystrophy were first described in 1987.⁸⁴ However, pulsed field gel electrophoresis had already indicated that most mutations causing Duchenne dystrophy were large deletions.⁸⁵ The identification of the dystrophin gene immediately allowed much more accurate differential diagnosis of Duchenne and Becker muscular dystrophy using the available cDNA probes.⁸⁶⁻⁸⁸ Molecular diagnosis of boys affected with dystrophinopathies is now carried out by multiplex PCR of genomic DNA (fig 2),

Figure 2 Duchenne muscular dystrophy multiplex PCR. Lane 1: size standard; lanes 2, 4, 6, 8: patients with Duchenne muscular dystrophy; lanes 3, 5, 7, 9: control: lanes 2 and 3 (from top down) promotor, exons 3, 8, 50, 53; lanes 4 and 5: exons 13, 16, 74; lanes 6 and 7: exons 48, 43, 47, 60; lanes 8 and 9: exons 19, 42, 4. Patient is deleted for at least exons 48 and 50 (arrows).

which gives a diagnosis in hours rather than the previous days or weeks.^{25 43-45} Prenatal diagnosis is also achieved with multiplex PCR when a family deletion has been identified, and by linkage using microsatellites⁸⁹ for the dystrophin gene in the large minority of families in which deletions have not been identified. Splice site mutations in the gene can be identified by RT-PCR.^{57 58}

Identification of carriers of dystrophinopathies has been achieved with dosage Southern blotting,⁹⁰⁻⁹² presence of junction fragments,⁸⁵ and non-inheritance of alleles.⁹³ However, as with all aspects of molecular diagnosis, carrier detection has moved to PCR; PCR dosage has been used with great accuracy to identify carriers^{94 95} as has RT-PCR from illegitimate transcription of the dystrophin message in circulating lymphocytes.⁴⁰

The increasing diversity of dystrophinopathy phenotypes has been reviewed by Beggs *et al*⁹⁶ and includes quadriceps myopathy,⁹⁷ cramps and myalgia,^{98 99} hyperCKaemia,¹⁰⁰ and X linked cardiomyopathy.^{101 102}

Limb-girdle muscular dystrophies

The first recessive limb-girdle muscular dystrophy gene to be localised (LGMD2A) was on chromosome 15^{103 104} through linkage in families from the island of Reunion. Mutations in the calcium activated protease gene calpain were subsequently identified both in the families from Reunion and in the Amish families which showed linkage to the chromosome 15 region.¹⁰⁵ (Two different autosomal recessive limb-girdle muscular dystrophies segregate in the Amish community—the other is LGMD2E.³⁴) The identification of calpain as the mutant gene in LGMD2A was the first implication of a non-structural protein in a muscular dystrophy.¹⁰⁵ No abnormalities in the dystrophin associated glycoproteins were

detected in patients with LGMD2A.¹⁰⁵⁻¹⁰⁷

There are four other localised or identified recessive limb-girdle muscular dystrophy genes: LGMD2C, LGMD2D, and LGMD2E are the sarcoglycanopathies whereas LGMD2B, localised to the short arm of 2p,¹⁰⁸ is an as yet unidentified gene.

There are at least two genes for autosomal dominant limb-girdle muscular dystrophy, one (LGMD1A) localised on chromosome 5,^{66 67} The other (LGMD1B) is not yet localised.¹⁰⁹

Sarcoglycanopathies

Adhalinopathy (α -sarcoglycanopathy) was the first sarcoglycanopathy to be identified, initially by deficiency in immunohistochemical staining¹¹⁰ and later by mutations in the adhalin gene.^{28 111 112} Adhalinopathy is also known as recessive limb-girdle muscular dystrophy type D (LGMD2D). Mutations in the other two sarcoglycans have now been demonstrated. Mutations in the β -sarcoglycan gene on chromosome 4 are associated with LGMD2E, the second limb-girdle muscular dystrophy found among the Amish³⁴ and also other communities.²⁴ Mutations in the γ -sarcoglycan gene on chromosome 13 are associated with severe congenital autosomal recessive muscular dystrophy (SCARMD, LGMD2C).³³ In each of these diseases there is a reduction of immunohistochemical staining for all three sarcoglycans. Thus reduction of immunostaining for any of the sarcoglycans in the absence of reduction in dystrophin staining indicates mutation in one of, at present, three genes. As Lim *et al*³⁴ state in the discussion of their paper, only molecular investigation of all the candidate genes can provide a definitive diagnosis.

Congenital muscular dystrophy

Congenital muscular dystrophies are charac-

terised by severe dystrophic changes of muscle from birth.¹¹³ Tomé *et al*¹⁰⁷ were the first to show merosin (LAMA2) deficiency in cases of congenital muscular dystrophy of the occidental type. However, not all of the clinically indistinguishable cases showed merosin deficiency, and this heterogeneity is yet to be explained on a molecular basis. It is estimated that 40% of occidental congenital muscular dystrophy cases show LAMA2 deficiency.¹¹³ Subsequently mutations of the LAMA2 gene were identified in patients with congenital muscular dystrophy.¹¹⁴

The LAMA2 gene maps to the long arm of chromosome 6, the Japanese form of congenital muscular dystrophy, Fukuyama muscular dystrophy to the long arm of chromosome 9,¹¹⁵ and thus a different as yet unidentified gene must be involved. Fukuyama muscular dystrophy and Walker-Wallberg syndrome may be genetically identical.¹¹⁶

Facioscapulohumeral muscular dystrophy

A gene for facioscapulohumeral muscular dystrophy (4q35) was first mapped in 1990 to chromosome 4 near the telomere.¹¹⁷ Subsequently, altered sized fragments involving a repeated sequence with homeodomain homology were identified in familial and sporadic cases of facioscapulohumeral muscular dystrophy.^{118,119} The precise gene or genes affected by the chromosomal rearrangements have not, however, been identified and thus the mechanism of pathogenesis remains a mystery. In addition, recombination with the altered sized fragments has been documented¹²⁰ and not all facioscapulohumeral muscular dystrophy families show linkage to 4q35.¹²¹ Facioscapulohumeral muscular dystrophy is thus still a problematic disease for molecular diagnosis.

Oculopharyngeal muscular dystrophy

A gene for oculopharyngeal muscular dystrophy among French Canadians has been linked to the proximal long arm of chromosome 14 close to the genes for cardiac α -myosin and β -myosin⁶⁹ and in the same region as a gene for dominant distal myopathy.⁶⁵

Emery-Dreifuss muscular dystrophy

The Emery-Dreifuss muscular dystrophy gene has been identified and its protein product named emerin.¹²² Emerin seems to be a transmembrane protein but its function is uncertain.

Distal myopathy

Two genes for distal muscular dystrophy have been localised. One (MPD1) with a phenotype similar to that originally described by Gowers⁶⁵ and the other for the recessive Miyoshi myopathy.⁶⁴ It is interesting that the MPD1 gene maps to a similar region of chromosome 14 as a gene for oculopharyngeal muscular dystrophy⁶⁹ and that the Miyoshi myopathy gene is in a similar region of chromosome 2 to a gene for autosomal recessive limb-girdle muscular dystrophy (LGMD2B)¹⁰⁸ raising the possibility that the two conditions may be caused by mutations in the same gene.¹²³

Myotonic syndromes

Table 5 gives a classification of these conditions, based on the underlying molecular defects. Differentiation of the various types is usually possible on the basis of clinical features such as the age of onset, pattern of inheritance, provocative factors for the myotonia, and the presence or absence of dystrophic muscle weakness or other systemic features.

MYOTONIC DYSTROPHY

This is the most common of the genetic forms of myotonia and is transmitted as an autosomal dominant trait with considerable variability in the degree of phenotypic expression and age of onset of symptoms. The congenital form, in which there is generalised hypotonia and weakness, often with facial diplegia and a distinctive tent shaped mouth, may occur in the offspring of either affected males or females but the more severe cases are usually the offspring of female heterozygotes who may themselves be only very mildly affected.^{124,125} Mental retardation and delay in motor and speech development are common in early onset cases. Myotonia is not present in affected infants but is usually readily demonstrable in the tongue, hand, and forearm muscles in patients presenting during adolescence or early adult life, and tends to become progressively less severe with the passage of time. Progressive weakness and atrophy of the forearm, calf, and sternomastoid muscles occur as the condition progresses and in some cases weakness of the facial, bulbar, and respiratory muscles also develops and may be associated with irregular breathing patterns, sleep apnoea, and respiratory failure.¹²⁶ Ventilatory function studies as well as monitoring of breathing and arterial blood gases during sleep should be performed if these problems are suspected.

Other distinctive features in the adult include frontal baldness and testicular atrophy in men and distinctive subcapsular cataracts, which are best seen with slit lamp examination.¹²⁵ Cardiac involvement also occurs and electrocardiographic abnormalities which include atrioventricular and intraventricular conduction defects and atrial or ventricular arrhythmias, are common and may cause Stokes-Adams attacks or even sudden death.^{125,127} The incidence of mitral valve prolapse is also increased in some families.¹²⁸ Investigation of cardiac function, including ECG and echocardiography, should therefore be performed routinely and, when indicated, ECG monitoring and radionuclide

Table 5 Classification of hereditary myotonias

Myotonia	Gene
Chloride channel myotonias:	
Autosomal dominant (Thomsen)	CLCN1
Autosomal recessive (Becker)	CLCN1
Sodium channel myotonias:	
Paramyotonia congenita	SCN4A
Myotonia fluctuans	SCN4A
Myotonia permanens	SCN4A
Dystrophic myotonias:	
Myotonic dystrophy	Myotonin protein kinase
Proximal myotonic myopathy	?

Figure 3 Analysis of the myotonic dystrophy triplet repeat expansion by PCR. Lane 1: grandmother showing two normal sized alleles; lane 2: asymptomatic grandfather showing one normal sized allele and a small expansion; lane 3: uncle of proband (son) of lanes 1 and 2 showing non-inheritance of grandfather's allele (expansion too big for PCR); lane 4: mother of proband (daughter) of lanes 1 and 2 showing non-inheritance of grandfather's allele (expansion too big for PCR); lane 5: proband (daughter of lane 4) showing non-inheritance of mother's allele, expansion too large for PCR.

angiocardiology may also be indicated. Other systemic manifestations which may require investigation in their own right include diabetes mellitus, disorders of the thyroid or immune function, and gastrointestinal and genitourinary abnormalities.¹²⁵

Myotonic dystrophy is one of the easy muscle diseases to diagnose by molecular techniques, as virtually all cases have variations of the same mutation: the expansion of the CTG triplet repeat in the myotonin protein kinase gene on chromosome 19.¹²⁹⁻¹³¹ The large triplet repeat expansions causing severe disease can be identified by Southern blotting after digestion of genomic DNA with EcoRI, and smaller expansions can be identified by digestion with BglI or BamHI.⁴¹ The PCR can identify normal subjects by the presence of two alleles within the normal size range and the very small expansions in minimally affected or asymptomatic subjects (fig 3).¹³¹⁻¹³² Often, this identifies where the disease came from in a family with someone other than the person the family has always suspected. Thus by cascade screening it is possible to detect and warn those family members at risk from possible cardiac complications and sudden death.

A dominantly inherited myotonic myopathy with proximal muscle weakness and cataracts without the characteristic trinucleotide repeat expansion has recently been described¹³³⁻¹³⁴ and has been shown not to be allelic with the genes for myotonic dystrophy or for the chloride or sodium channelopathies.¹³³

CHLORIDE CHANNEL MYOTONIA

It is now known that both the autosomal dominant (Thomsen) and recessive (Becker) forms of myotonia congenita are caused by mutations in the muscle chloride channel (CLCN1) gene on chromosome 7.⁴⁸ Symptoms may be present from birth in the dominant form but often do not develop until early childhood or adolescence. The myotonia is widespread, usually painless, and more severe in the recessive form being accentuated by rest, cold, and emotion and improving with repeated muscle contraction. It is commonly associated with diffuse muscular hypertrophy, particularly in affected males. Muscle weakness may develop after exercise, particularly in cases of the recessive form of the condition, and is associated with a reduction in the amplitude of the evoked CMAP and twitch tension.¹³⁵ In addition, some degree of fixed weakness and atrophy with EMG evidence of myopathy develops, particularly in the forearm and sternomastoid muscles, in about two thirds of patients with the recessive condition.¹³⁶

To date, a total of 19 separate mutations in the CLCN1 gene have been identified, six being associated with dominant disease and 13 with the recessive disease.⁴⁸ Most (four out of five) of the truncating mutations cause recessive disease. The mutations are spread throughout the coding region of the gene and many have been found in single families, indicating that other mutations are highly likely to be found in other families. Myotonia congenita is therefore one of the difficult diseases

to diagnose, with molecular techniques requiring screening of the entire coding region of the gene.

SODIUM CHANNEL MYOTONIA

These varieties of myotonia have all been localised to the same locus and have been associated with different mutations in the α subunit of the sodium muscle channel gene (SCN4A) on chromosome 17.^{48, 137} In paramyotonia congenita myotonia is characteristically induced by cold exposure, particularly in the facial and hand muscles, and may be associated with attacks of weakness (cold paresis) which improve with warming. In most cases the myotonia is increased by repeated muscle contraction (paradoxical myotonia) rather than improving as in most other myotonic disorders. In potassium sensitive paramyotonia (paralysis periodica paramyotonica) paradoxical myotonia and episodes of weakness are precipitated by potassium administration. Such cases account for the overlap between paramyotonia and hyperkalaemic periodic paralysis in some families.

A third variety of sodium channel myotonia has been designated myotonia fluctuans. In this condition, which is dominantly inherited, myotonia is of variable severity at different times, may be associated with painful muscle spasms, with a tendency to increase in severity after exercise, ingestion of potassium, and administration of depolarizing agents, and is responsive to treatment with acetazolamide¹³⁷ or, in some families, mexilitine.¹³⁸ A fourth variety of sodium channel myotonia with continuous severe myotonia, muscle stiffness, and hypertrophy has also been described and designated myotonia permanens.⁴⁸

The mutations in these conditions are spread through a large part of the coding region of the SCN4A gene making molecular diagnosis difficult, although two mutations, Thr1313Met and Gly1306Val, predominate in paramyotonia congenita and should be initially screened for.

Periodic paralysis

Some conditions may cause episodic weakness or paralysis of the limb muscles (table 6). It is difficult to evaluate the importance of a complaint of episodic weakness unless the patient is watched during an attack. Most patients who complain of episodes of weakness are in fact referring to fatigue. In those patients who do have episodes of documented weakness it is first necessary to exclude disorders of neuromuscular transmission such as myasthenia gravis, demyelinating disorders of the peripheral or central nervous system, transient ischaemic episodes, or attacks of hysterical weakness. If these conditions can be excluded, the possibility of one of the primary periodic paralyses arises, particularly if there are other affected family members (table 6).

Differentiation between the main varieties of primary periodic paralysis is usually possible on the basis of the duration of the attacks of weakness, provocative factors, and serum

Table 6 Classification of periodic paralyses

Hereditary:	
Hypokalaemic (DHP receptor; 1q31–32)	
Potassium sensitive (SCN4A; 17q23–25)	
Hyperkalaemic	
Paramyotonia congenita	
Paralysis periodica paramyotonica	
Andersen's syndrome	
Acquired:	
Hypokalaemic	
Hyperkalaemic	
Thyrotoxic	

potassium concentrations during attacks. In hypokalaemic periodic paralysis the attacks are often precipitated by carbohydrate ingestion and tend to be less frequent and of longer duration (up to 24 hours) than in the potassium sensitive periodic paralyses (for example, hyperkalaemic periodic paralysis) in which the attacks are often precipitated by fasting or by resting after exercise.¹³ Although the serum potassium concentration during an attack of weakness may help to distinguish the hypokalaemic from the hyperkalaemic form, potassium concentrations are often normal in the second and may occasionally also be normal in the hypokalaemic form.⁹ The serum creatine kinase concentration is often raised in severe attacks of both forms and may be somewhat raised even between attacks. Myotonia, when present clinically or evident on EMG, is more suggestive of hyperkalaemic periodic paralysis or paramyotonia but may occasionally occur in the eyelid muscles in hypokalaemic periodic paralysis.⁹

When carried out during an attack of weakness EMG shows a progressive reduction in motor unit recruitment and in the amplitude of the compound muscle action potential evoked by motor nerve stimulation, whereas repetitive nerve stimulation shows a transient potentiation of the compound muscle action potential.¹³⁹ A myopathic EMG pattern may be found even during the attack free interval particularly in patients who develop fixed muscle weakness, as often occurs in those who have had the condition for several years. Changes in the evoked compound muscle action potential also occur with exercise and are the basis for the hand exercise test described by McManis *et al.*¹⁴⁰ Serial measurements of compound muscle action potential amplitudes show a greater than normal increase during exercise and decrease in the post-exercise period in patients with periodic paralysis. The test was found to be abnormal in 70% of cases of periodic paralysis but did not distinguish between the different forms.

Another form of exercise testing, which is useful in detecting some cases of hypokalaemic periodic paralysis, including asymptomatic cases, is that described by Kantola and Tarssanen,¹⁴¹ which involves a 30 minute period of bicycle ergometry. Affected persons do not show the normal rise in plasma potassium concentrations after the exercise period.

Muscle biopsy shows a characteristic vacuolar change in muscle fibres, and sometimes necrotic muscle fibres, particularly during an attack of weakness, but sometimes even during attack free intervals, in both major forms of

periodic paralysis. Vacuolar change may be found even in some family members without definite attacks of weakness.¹⁴¹ Although the biopsy changes are distinctive, a biopsy is seldom necessary as the results of provocative tests and molecular genetic analysis are usually diagnostic.

As the plasma potassium concentrations during an attack of weakness are sometimes misleading, provocative tests remain important in evaluating people with a negative family history or those from families in which the mutation is not known. In hypokalaemic periodic paralysis, attacks of weakness may be induced by giving an oral glucose load (1.5 gm/kg over three minutes; maximum 100 g).⁹ Muscle strength in selected groups, serum electrolytes, and ECG should be monitored every 30 minutes for three to five hours. If weakness does not develop, an intravenous glucose load (3 g/kg in water) is given over a period of one hour. If weakness does not develop after 30 minutes, intravenous insulin (0.1 U/kg) is given and repeated at 60 minutes if weakness fails to develop. Regular measurements of muscle strength and of plasma electrolyte and glucose concentrations, and ECG should be performed throughout the test and the period of induced weakness.^{9 13}

In the potassium sensitive forms of periodic paralysis attacks of weakness may be induced by oral potassium loading.^{9 13} A widely used protocol involves giving 0.05 g/kg of potassium chloride in a glucose free solution over a period of three minutes. Muscle strength, serum electrolytes, and ECG are monitored every 15 minutes in the first two hours and every 30 minutes in the next two hours and weakness usually develops after 90 to 180 minutes. If the initial test is negative, higher potassium loads of 0.1 to 0.15 g/kg may be used. The test is contraindicated in patients with insulin dependent diabetes mellitus or renal insufficiency. Cold testing, involving immersion of the arm in water at 10°C for 30 minutes, may be useful in confirming the diagnosis of paramyotonia in patients with a history of episodes of cold induced weakness or myotonia.⁹

The molecular basis for both the hypokalaemic and hyperkalaemic forms of periodic paralysis have now been defined. Hypokalaemic periodic paralysis has been shown to be caused by three mutations in the dihydropyridine receptor calcium channel gene (CACNLIA3) on chromosome 1q31–32 affecting two amino acid residues.^{38 48 142 143} The mutations can be detected by PCR followed by enzyme digestion as they either create or destroy restriction enzyme sites¹⁴² or by SSCP.³⁸ Mutations in the gene encoding the α subunit of the skeletal muscle sodium channel (SCN4A) causing hyperkalaemic periodic paralysis were first described in 1991.^{144 145} A recent review by Lehmann-Horn and Rüdel¹⁴⁸ catalogues the five mutations of SCN4A now known to be associated with hyperkalaemic periodic paralysis. Two of the mutations, Thr704Met and Met1592Val, predominate and can be screened for first. There is perhaps

a degree of genetic heterogeneity in hyperkalaemic periodic paralysis as one family with a convincing clinical diagnosis of the disease did not map to the SCN4A gene.⁶² Mutations in the same gene have also been found in some families with paramyotonia congenita⁴⁸ and in some cases of hyperkalaemic periodic paralysis associated with cardiac arrhythmias (Andersen's syndrome).^{146 147}

Congenital myopathies

The congenital myopathies are a heterogeneous group of disorders which may result in hypotonia in infancy or may present later in childhood with weakness and delayed motor milestones (table 7). Some, such as central core disease, are benign and relatively non-progressive whereas others such as X linked centronuclear myopathy are usually fatal in infancy. Skeletal abnormalities such as talipes, scoliosis, and hip dislocation as well as dysmorphic features are not uncommon. Ptosis and ophthalmoplegia are often a feature in centronuclear myopathy and may also occur in minicore disease. Respiratory insufficiency may occur, particularly in nemaline myopathy, centronuclear myopathy, and cytoplasmic body myopathy. The congenital myopathies should be considered in the differential diagnosis of the floppy infant and need to be distinguished from other neuromuscular and CNS disorders which may produce infantile hypotonia.

In all of these conditions a definitive diagnosis relies on a muscle biopsy with full histochemical and electron microscopic examination of muscle tissue. These will identify specific morphological changes such as central cores, nemaline rods, central nucleation, and abnormalities of myofibre differentiation and fibre type proportions or distinctive inclusion bodies (for example, cytoplasmic bodies, finger print bodies, spheroid bodies, hyaline bodies, reducing bodies, zebra bodies, and tubular aggregates). This will also exclude other conditions such as congenital muscular dystrophy, metabolic myopathies, and neurogenic disorders such as spinal muscular atrophy and congenital peripheral neuropathies.^{9 148}

The genetic basis for some of the congenital myopathies such as central core disease, nemaline myopathy, and the neonatal form of centronuclear myopathy have now been defined. To date, one gene for autosomal dominant nemaline myopathy has been linked

to chromosome 1¹⁴⁹ and a mutation in the α -tropomyosin gene TPM3 has been shown to segregate with the disease.¹⁵⁰ A gene for recessive nemaline myopathy has been localised to chromosome 2.⁷⁰ Neither of the loci map to the known locations for the α -actinin genes.¹⁵¹ This and the mutation in tropomyosin may indicate that nemaline myopathy is a disease of the thin filament rather than of the Z disc.¹²³ Central core disease was first linked to the same region of chromosome 19 as malignant hyperthermia^{152 153} and then mutations associated with central core disease were identified in the RYR1 gene.^{154 156} Thus both malignant hyperthermia and central core disease are caused by mutations in the RYR1 gene and whether central cores manifest or not may depend on the degree of calcium loading of the individual muscle fibres.¹⁵⁵

Mitochondrial myopathies

Since the discovery of the first mitochondrial DNA mutations in 1988^{157 158} it has become apparent that the clinical spectrum of mitochondrial diseases is extremely diverse, ranging from relatively mild and slowly progressive myopathies confined to the extraocular muscles (chronic progressive external ophthalmoplegia) to severe fatal infantile myopathies (for example, cytochrome c oxidase deficiency) and multisystem encephalomyopathies (such as the Kearns-Sayre, MELAS, and MERFF syndromes^{9 159-162}) (table 8). In addition, deficiencies of mitochondrial enzymes encoded by nuclear DNA have been identified (for example, succinate dehydrogenase deficiency).

CLINICAL PHENOTYPES AND GENOTYPES

Table 9 shows that mitochondrial defects may be associated not only with myopathic disorders but with a wide variety of other neurological and non-neurological manifestations.

Although some classic syndromes have been defined (table 8), there is a certain amount of overlap between these and new phenotypic combinations are still being identified. Moreover, there is considerable variability in the age of onset, rate of progression, and extent of phenotypic expression in different tissues even within the major syndromes. This

Table 7 Congenital myopathies

Central core disease*
Nemaline myopathy
Centronuclear myopathy
Minicore disease*
Congenital fibre type disproportion
Myopathies with cytoplasmic inclusions
Fingerprint body myopathy
Reducing body myopathy
Cytoplasmic body myopathy
Hyaline body myopathy
Myopathy with tubular aggregates
Zebra body myopathy
Sarcotubular myopathy

*Predisposition to malignant hyperthermia.

Table 8 Mitochondrial myopathies and encephalomyopathies

Progressive external ophthalmoplegia
Sporadic
Familial
Kearns-Sayre syndrome
Ophthalmoplegia
Pigmentary retinopathy
Cardiac conduction defects
Cerebellar ataxia
Sensorineural deafness
Limb-girdle myopathy
Infantile myopathy
Benign reversible
Severe fatal
MERFF syndrome
Myoclonus
Epilepsy
Myopathy
MELAS syndrome
Myopathy
Lactic acidosis
Stroke-like episodes

Table 9 Clinical manifestations of mitochondrial disease

Neurological	Non-neurological
External ophthalmoplegia	Short stature
Limb myopathy	Cardiac conduction defects
Fatigability and poor exercise tolerance	Cardiomyopathy
Cerebellar ataxia	Pigmentary retinopathy
Epilepsy	Cataracts
Myoclonus	Lactic acidosis
Sensorineural deafness	Diabetes mellitus
Peripheral neuropathy	Hypoparathyroidism
Dementia	Renal tubular defects
Stroke	Episodic nausea and vomiting
Vascular headache	Pancytopenia
Dystonia	Intestinal pseudo-obstruction
Basal ganglion calcification	Multiple lipomas

heterogeneity is characteristic of this group of disorders¹⁶³ and is attributable to the random distribution of mitochondria containing the mutated DNA within different tissues and to the admixture of normal and abnormal populations of mitochondria within the cells of these tissues (heteroplasmy) as a result of which different tissues in the same patient and different members of the same family may be involved to different degrees.

The classic syndromes have been associated with different types of genomic defect. For example, patients with the pure form of chronic progressive external ophthalmoplegia (CPEO) usually have single major deletions^{159 160} but point mutations have been found in some patients.² Similarly, those patients with the Kearns-Sayre syndrome have single large mtDNA deletions which occur sporadically.^{157 164} In patients with familial CPEO two distinct syndromes have been identified. In the first, which is maternally inherited, deletions are not found, whereas in the second, which is dominantly inherited, large scale multiple mtDNA deletions are usually present and are thought to be secondary to a mutation in a nuclear gene encoding a factor which controls mtDNA replication.¹⁶⁵ Such a gene has recently been linked to chromosome 10q23.3–24.3 in a Finnish family with autosomal dominant CPEO.⁵⁰ Cases presenting purely with a proximal myopathy may also be dominantly inherited¹⁶⁶ but are uncommon and the genotypic basis for these cases has not been defined. In the encephalomyopathy syndromes different point mutations have been found in tRNA-lys (nucleotides 8344 or 8356) in the MERRF syndrome¹⁶⁷ and tRNA-leu (nucleotides 3243, 3251, 3252, 3271 or 3291) in the MELAS syndrome.^{2 160}

DIAGNOSTIC APPROACH

In patients with CPEO, Kearns-Sayre syndrome, or one of the other classic syndromes, the clinical features are often sufficiently distinctive to strongly suggest the diagnosis of a mitochondrial disorder, and in familial cases, a maternal pattern of transmission would also raise the possibility of such a diagnosis. A raised fasting venous lactate concentration is an additional diagnostic pointer. Lactate concentrations are usually normal in cases of CPEO, but venous and CSF concentrations

are often raised in the MELAS syndrome and may also be raised in Kearns-Sayre syndrome and in patients with a proximal myopathy or encephalomyopathy.¹⁵⁹ A muscle biopsy is the most important diagnostic investigation, the characteristic findings being of ragged red fibres with the modified Gomori trichrome stain, with abnormally prominent subsarcolemmal mitochondrial aggregates with the oxidative enzyme stains (NADH dehydrogenase, succinate dehydrogenase) and abnormal mitochondrial morphology with mitochondrial inclusions on electron microscopy. In addition, histochemical staining for cytochrome oxidase activity shows a characteristic mosaic pattern of fibres lacking enzyme activity in most cases. Immunohistochemical techniques may be applied to determine whether nuclear or mtDNA encoded subunits of cytochrome oxidase are absent. In situ hybridisation with probes to different parts of the mtDNA molecule may also be used to study the distribution of mutant mitochondria in muscle fibres.

The identified common mutations in the mitochondrial genome associated with MERRF or MELAS can be tested using PCR followed by enzyme digestion^{41 49} or SSCP analysis.¹⁶⁸ The major deletions of the mtDNA associated with Kearns-Sayre syndrome or CPEO may be identified either using Southern blotting⁸⁰ or PCR¹⁶⁹ including long range PCR, which can amplify the entire 16 569 base pairs of the mtDNA in one reaction.¹⁷⁰ The severity of the disease relates to the percentage of mutant mitochondria,⁶⁰ which can be estimated either after Southern blotting, or enzyme digestion after PCR, or from SSCP.¹⁶⁷

Whether all the base changes shown to be associated with mitochondrial related disease actually cause disease may be debatable; some may merely be linked to other disease causing mutations.¹⁷¹ On the other hand, the polymorphisms of mtDNA may contribute to disease.¹⁷² The mitochondrial genome is thus problematic for molecular diagnosis, the relevance of some mutations to disease being unclear.

Acute rhabdomyolysis and myoglobinuria

Various hereditary and acquired disorders may lead to episodes of severe widespread muscle fibre destruction and myoglobinuria.¹¹ Clinically, there is widespread muscle weakness which may be profound and in severe cases there is often severe muscle pain, tenderness, and depression of the deep tendon reflexes. When myoglobinuria is severe it may lead to the development of acute renal failure. In patients with malignant hyperthermia muscle rigidity, hyperpyrexia, and rhabdomyolysis usually develop during anaesthesia when susceptible subjects are exposed to halothane and some other inhalational anaesthetic agents, but episodes of rhabdomyolysis may also be precipitated by stress, strenuous physical activity, or systemic infective illnesses.

Patients presenting in this way for the first time should undergo a detailed clinical assessment for a drug induced, toxic, or infective

cause and hypokalaemia, hypothyroidism, and other metabolic disorders should be excluded. Patients in whom no obvious cause can be identified—especially those in whom there is a history of previous episodes—should have a muscle biopsy and appropriate biochemical investigations for an underlying genetic disorder such as malignant hyperpyrexia or a disorder of glycolysis or fatty acid metabolism.^{11 173}

Susceptibility to malignant hyperthermia in humans was first linked to the region containing the ryanodine receptor calcium release channel on chromosome 19^{71 174} after the mutation for swine malignant hyperthermia was linked to the glucose phosphate isomerase gene which maps in humans to chromosome 19. A few mutations have now been identified in the relatively large (15 kb) RYR1 gene message¹⁷⁵ in a minority of families with malignant hyperthermia.⁷⁴ The aim of molecular diagnosis in this condition must be to reduce the reliance on the extremely invasive current gold standard of the muscle contracture test, with which the molecular diagnosis is not always in agreement.^{72 74} However, until a clearer and more comprehensive list of mutations in the RYR1 gene causing malignant hyperthermia is available, including resolution of the apparent genetic heterogeneity, and the discrepancies between molecular diagnosis and the contracture test are resolved, molecular diagnosis for susceptibility to malignant hyperthermia will remain problematical.

Painful muscle conditions

Muscle pain may be a feature of some myopathies and other conditions (table 2). The pain may be focal or diffuse and may occur at rest, during, or after exercise. When myalgia develops after an infective illness, an alcoholic binge, exposure to a myotoxic drug, or after an episode of envenomation, the cause is usually apparent. A wide variety of drugs may cause myalgia and muscle cramps without significant muscular weakness and the symptoms usually resolve promptly after withdrawal of the offending agent (table 1). The finding of significant muscular weakness, tenderness, and increase in serum creatine kinase suggests a necrotising myopathy and a muscle biopsy is usually warranted to confirm the diagnosis. A biopsy is also indicated when an inflammatory myopathy is suspected and should include the overlying fascia, if there is a possibility of fasciitis. Muscle pain and stiffness are common symptoms in hypothyroidism and in patients with metabolic bone disease. The diagnosis in such patients can usually be confirmed by appropriate biochemical and other studies without resorting to muscle biopsy.

Muscle pain and cramping which develop during exercise suggest a disorder of muscle energy metabolism, such as deficiency of myophosphorylase, phosphofructokinase, or other glycolytic enzyme defect, or of carnitine palmityl transferase, especially if there is a history of discolouration of the urine after exercise, suggesting myoglobinuria. Such patients warrant further investigation including the

forearm exercise test (see above) and assays of carnitine and carnitine palmityl transferase activity as well as a muscle biopsy for histochemical and biochemical studies to confirm the diagnosis of these metabolic disorders. With molecular studies mutations causing disease have been identified in some of these conditions but such studies are not usually necessary for the diagnosis. A muscle biopsy is also necessary for the diagnosis of mitochondrial myopathy, malignant hyperthermia, dystrophin deficiency, and tubular aggregates, which may sometimes present with unexplained myalgia, cramps, or exercise intolerance.

The vast majority of patients with complaints of muscle pain, stiffness, or cramping do not have any significant muscular weakness or other evidence of muscle disease. It is important in such patients to look for evidence of muscular and myofascial tenderness. The finding of point tenderness in the typical sites is diagnostic of the condition of fibromyalgia and invasive investigations such as EMG and muscle biopsy can usually be avoided. Similarly, a pattern of proximal or axial muscle pain and stiffness with malaise in elderly patients with preserved muscle strength and a raised erythrocyte sedimentation rate is diagnostic of polymyalgia rheumatica and warrants a therapeutic trial of prednisone without muscle biopsy, although the possibility of an underlying connective tissue or metabolic disorder or malignancy should always be considered and investigated appropriately. In a third group of patients, with the postviral myalgia/fatigue syndrome, there are no diagnostic clinical or laboratory abnormalities. Muscle biopsy and EMG may, however, be indicated in such patients to exclude an inflammatory or metabolic myopathy, particularly when symptoms are disabling and if the serum creatine kinase concentration is raised.

Inflammatory myopathies

The diagnosis of an inflammatory myopathy may be readily made on clinical grounds in patients with the characteristic skin changes of dermatomyositis or with the typical pattern of muscle weakness seen in inclusion body myositis and may also be suspected in patients with a connective tissue disorder such as systemic lupus erythematosus, progressive systemic sclerosis, mixed connective tissue disease, Sjögren's syndrome, or other autoimmune disease who develop a proximal myopathy and would be supported by the finding of raised serum creatine kinase and an abnormal EMG (see above). However, it is important that the diagnosis should be firmly established on histological grounds and a muscle biopsy should always be performed before commencing treatment. An open biopsy, usually from the deltoid or vastus lateralis muscle, is preferable for the diagnosis of inflammatory myopathy. In addition to routine histological preparations, mononuclear cell populations, immune complex deposition, MHC, and intercellular adhesion molecule expression can

be assessed with immunohistochemical techniques.^{176 177}

Other investigations are directed towards identifying any underlying immune or infective disorder or malignancy. These should include an autoantibody screen for antinuclear factor, rheumatoid factor, anti-RNP, and, when appropriate, other autoantibodies such as PM-Scl and Ku, which are frequently associated with the polymyositis/scleroderma overlap syndrome, and antibodies to the aminoacyl-tRNA synthetases such as Jo-1 which are found in patients with myositis and associated interstitial lung disease and other overlap features.¹⁷⁸ As patients with HIV and HTLV-1 infection may develop an inflammatory myopathy similar to polymyositis,¹⁷⁹ serological testing for HIV and HTLV-1 infection as well as studies of lymphocyte subsets are also indicated, especially in areas where infection with these agents is prevalent. The possibility of an underlying malignancy should be considered, particularly in adults presenting with dermatomyositis but it is rare in patients with juvenile dermatomyositis, connective tissue disease, or inclusion body myositis.¹⁸⁰ The search for a malignancy should include a detailed clinical evaluation, including pelvic and rectal examination, routine haematological and biochemical studies, chest radiography, and mammography in females. In addition, in patients with dysphagia a barium swallow or upper gastrointestinal endoscopy should be performed to exclude carcinoma of the oesophagus or gastro-oesophageal junction before attributing the dysphagia to the myositis.

Cardiac involvement, resulting in arrhythmias, heart block, or congestive cardiac failure may sometimes occur even in patients with isolated polymyositis or dermatomyositis.¹⁸⁰ Electrocardiography, and when appropriate other investigations of cardiac function, should therefore be performed during the active phase of the disease. In addition, assessment of respiratory function should be performed, especially in patients with severe muscle disease or suspected interstitial lung disease.^{180 181}

Quantitative assessment of muscle strength in representative upper and lower limb muscle groups using a myometer or isokinetic dynamometer is important in assessing the patient's response to treatment and makes it possible to recognise an upward or downward trend in muscle performance even before there is a change in the patient's functional state or MRC grading.¹⁸¹ Serial measurements of the serum creatine kinase concentration are also of value in assessing the response to treatment and a rising creatine kinase concentration, even within the normal range, may be the first indication of reactivation of the myositis in a patient who has previously been in remission.¹⁸¹ Measurements of serum concentrations of myoglobin and of soluble interleukin 2 receptor concentrations in blood have been reported to be sensitive indicators of disease activity^{12 182} but are not widely used.

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- 1 Neuromuscular disorders: gene location. *Neuromuscul Disord* 1995;6:1-III.
- 2 Mitochondrial encephalomyopathies: gene mutation. *Neuromuscul Disord* 1995;6:V-VI.
- 3 Argov Z, Mastaglia FL. Drug-induced neuromuscular disorders in man. In: Walton JN, Karpatis G, Hilton-Jones D, eds. *Disorders of voluntary muscle*. 6th ed. Edinburgh: Churchill Livingstone, 1994:898-1029.
- 4 Lane RJM, Mastaglia FL. Drug-induced myopathies in man. *Lancet* 1978;ii:562-5.
- 5 Mastaglia FL. Toxic myopathies. In: Rowland LP, DiMauro S, eds. *Handbook of clinical neurology*. Amsterdam: Elsevier, 1992:595-622.
- 6 Suarez GA, Kelly JJ. The dropped head syndrome. *Neurology* 1992;42:1625-7.
- 7 Garlepp MJ, Mastaglia FL. Inclusion body myositis. *J Neurol Neurosurg Psychiatry* 1996;60:251-5.
- 8 Harvey JM, Mastaglia FL, Zilko PJ, Ojeda VJ, Cheah PS. Scleromyxedema and inflammatory myopathy: a clinicopathological study of three patients. *Aust NZ J Med* 1986;16:329-35.
- 9 Griggs RC, Mendell JR, Miller RG. Evaluation and treatment of myopathies. *Contemporary neurology series*. Philadelphia: FA Davis, 1995.
- 10 Sunohara N, Takagi A, Nonaka I, Sugita H, Satoyoshi E. Idiopathic hyperCKemia. *Neurology* 1984;34:544-7.
- 11 Tien I, DiMauro S, Rowland LP. Myoglobinuria. In: Rowland LP, DiMauro S, eds. *Handbook of clinical neurology*. Amsterdam: Elsevier, 1992:553-93.
- 12 Kagen LJ. Myoglobinaemia in inflammatory myopathies. *JAMA* 1977;237:1448-52.
- 13 Moxley RT. Metabolic and endocrine myopathies. In: Walton JN, Karpatis G, Hilton-Jones D, eds. *Disorders of voluntary muscle*. 6th ed. Edinburgh: Churchill Livingstone, 1994:647-716.
- 14 Argov Z, Bank WJ. Phosphorus magnetic resonance spectroscopy (³¹P-MRS) in neuromuscular disorders. *Ann Neurol* 1991;30:90-7.
- 15 Moussavi RS, Carson PJ, Boska MD, Weiner MW, Miller RG. Nonmetabolic fatigue in exercising human muscle. *Neurology* 1989;39:1222-6.
- 16 McKernan RO, Hallidan D, Purkiss P, Royston P. 3-methylhistidine excretion as an index of myofibrillar protein catabolism in neuromuscular disease. *J Neurol Neurosurg Psychiatry* 1979;42:536-41.
- 17 Ohkoshi N, Yoshizawa T, Mizusawa H, et al. Malignant hyperthermia in a patient with Becker muscular dystrophy: dystrophin analysis and caffeine contracture study. *Neuromusc Disord* 1995;5:53-8.
- 18 Fawcett PRW, Barwick DD. The clinical neurophysiology of neuromuscular disease. In: Walton JN, Karpatis G, Hilton-Jones D, eds. *Disorders of voluntary muscle*. 6th ed. Edinburgh: Churchill Livingstone, 1994:1033-104.
- 19 Eisen A, Berry K, Gibson G. Inclusion body myositis: myopathy or neuropathy? *Neurology* 1983;33:1109-14.
- 20 Grindrod S, Tofts P, Edwards R. Investigation of human skeletal muscle structure and composition by X-ray computerised tomography. *Eur J Clin Invest* 1983;13:465-8.
- 21 Kiers L. Diabetic muscle infarction: magnetic resonance imaging (MRI) avoids the need for biopsy. *Muscle Nerve* 1995;18:129-30.
- 22 De Visser M, Verbeeten B. Computed tomography of the skeletal musculature in Becker-type muscular dystrophy and benign infantile spinal muscular atrophy. *Muscle Nerve* 1985;8:435-44.
- 23 Pamphlett R. Muscle biopsy. In: Mastaglia FL, Walton JN, eds. *Skeletal muscle pathology*. 2nd ed. Edinburgh: Churchill Livingstone, 1992:95-121.
- 24 Bönnemann CG, Modi R, Noguchi S, et al. β -sarcoglycan (A3b) mutations cause autosomal recessive muscular dystrophy with loss of sarcoglycan complex. *Nature Genet* 1995;11:266-73.
- 25 Beggs AH, Kunkel LM. Improved diagnosis of Duchenne/Becker muscular dystrophy. *J Clin Invest* 1990;85:613-9.
- 26 Hoffman EP, Fischbeck KH, Brown RH, et al. Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. *N Engl J Med* 1988;318:1363-8.
- 27 Hoffman EP, Kunkel LM, Angelini C, Clarke A, Johnson M, Harris JB. Improved diagnosis of Becker muscular dystrophy by dystrophin testing. *Neurology* 1989;39:1011-7.
- 28 Piccolo F, Roberds SL, Jeanpierre M, et al. Primary adhalinopathy: a common cause of autosomal recessive muscular dystrophy of variable severity. *Nature Genet* 1995;10:243-5.
- 29 Malhotra SB, Hart KA, Klamut HJ, et al. Frame-shift deletions in patients with Duchenne and Becker muscular dystrophy. *Science* 1988;242:755-9.
- 30 Chelly J, Gilgenkrantz H, Lambert M, et al. Effect of dystrophin gene deletions on mRNA levels and processing in Duchenne and Becker muscular dystrophies. *Cell* 1990;63:1239-48.
- 31 Morisaki H, Morisaki T, Newby LK, Holmes EW. Alternative splicing: a mechanism for phenotypic rescue of a common inherited defect. *J Clin Invest* 1993;91:2275-80.
- 32 Collins FS. Positional cloning moves from perdictional to traditional. *Nature Genet* 1995;9:347-50.
- 33 Noguchi S, McNally EM, Othmane KB, et al. Mutations in

- the dystrophin-associated protein γ -sarcoglycan in chromosome 13 muscular dystrophy. *Science* 1995;270:819-22.
- 34 Lim LE, Duclos F, Broux O, et al. β -sarcoglycan: characterization and role in limb-girdle muscular dystrophy linked to 4q12. *Nature Genet* 1995;11:257-65.
 - 35 Evans MJ, Greb A, Kunkel LM, et al. In utero fetal muscle biopsy for the diagnosis of Duchenne muscular dystrophy. *Am J Obstet Gynecol* 1991;165:728-32.
 - 36 Bradley DM, Parsons EP, Clarke AJ. Experience with screening newborns for Duchenne muscular dystrophy in Wales. *BMJ* 1993;306:357-60.
 - 37 Greenberg CR, Jacobs HK, Halliday W, Wrogmann K. Three year's experience with neonatal screening for Duchenne/Becker muscular dystrophy: gene analysis, gene expression and phenotype prediction. *Am J Med Genet* 1991;39:68-75.
 - 38 Ptacek LJ, Tawil R, Griggs RC, et al. Dihydropyridine receptor mutations cause hypokalaemic periodic paralysis. *Cell* 1994;77:863-8.
 - 39 Mullis KB, Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. *Meth Enzymol* 1987;155:335-50.
 - 40 Roberts RG, Bentley DR, Barby TFM, et al. Direct diagnosis of carriers of Duchenne and Becker muscular dystrophy by amplification of lymphocyte RNA. *Lancet* 1990; 336:1523-6.
 - 41 Zeviani M, Amati P, Bresolin N, et al. Rapid detection of the A-G mutation of mtDNA in Italian families with myoclonus epilepsy and ragged red fibers. *Am J Hum Genet* 1991;48:203-11.
 - 42 Shelbourne P, Winquist R, Kunert E, et al. Unstable DNA may be responsible for the incomplete penetrance of the myotonic dystrophy phenotype. *Human Molecular Genetics* 1992;1:467-73.
 - 43 Abbs S, Yau SC, Clark S, Mathew CG, Bobrow M. A convenient multiplex PCR system for the detection of dystrophin gene deletions: a comparative analysis with cDNA hybridisation shows mistypings by both methods. *J Med Genet* 1991;28:304-11.
 - 44 Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic Acids Res* 1988;16:11141-56.
 - 45 Chamberlain JS, Gibbs RA, Ranier JE, Caskey CT. Multiplex PCR for the diagnosis of Duchenne muscular dystrophy. In Innis M, Gelfand D, Sninsky J, White T, eds. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press, 1990:272-81.
 - 46 Lefebvre S, Burglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995;80:155-65.
 - 47 Rodrigues NR, Owen N, Talbot K, Ignatius J, Dubowitz V, Davies KE. Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. *Human Molecular Genetics* 1995;4:631-4.
 - 48 Lehmann-Horn F, Rüdell R. Hereditary nondystrophic myotonias and periodic paralyses. *Curr Opin Neurol Neurosurg* 1995;8:402-10.
 - 49 Wallace DC. Mitochondrial DNA mutations and neuromuscular disease. *TIGS* 1989;5:9-13.
 - 50 Suomalainen A, Kaukonen J, Amati P, et al. An autosomal locus predisposing to deletions of mitochondrial DNA. *Nature Genet* 1995;5:146-51.
 - 51 Forrest S, Cotton R, Landegren U, Southern E. How to find all those mutations. *Nature Genet* 1995;10:375-6.
 - 52 Grompe M. The rapid detection of unknown mutations in nucleic acids. *Nature Genet* 1993;5:111-7.
 - 53 Liu Q, Sommer SS. Restriction endonuclease fingerprinting (REF): a sensitive method for screening for mutations in long, contiguous segments of DNA. *Biotechniques* 1995;18:470-7.
 - 54 Sarkar G, Yoon H-S, Sommer SS. Dideoxy fingerprinting (ddf): a rapid and efficient screen for the presence of mutations. *Genomics* 1992;13:441-3.
 - 55 Mashal RD, Koontz J, Sklar J. Detection of mutations by cleavage of DNA heteroduplexes with bacteriophage resolvases. *Nature Genet* 1995;9:177-83.
 - 56 Youil R, Kemper BW, Cotton RGH. Screening for mutations by enzyme mismatch cleavage with T4 endonuclease VII. *Proc Natl Acad Sci USA* 1995;92:87-91.
 - 57 Wilton SD, Johnsen RD, Pedretti JR, Laing NG. Two distinct mutations in a single dystrophin gene: identification of an altered splice-site as the primary Becker muscular dystrophy mutation. *Am J Med Genet* 1993;46:563-9.
 - 58 Wilton SD, Chandler DC, Kakulas BA, Laing NG. Identification of a point mutation and germinal mosaicism in a Duchenne muscular dystrophy family. *Human Mutation* 1994;3:133-40.
 - 59 Chelly J, Gilgenkrantz H, Hugnot JP, et al. Illegitimate transcription: application to the analysis of truncated transcripts of the dystrophin gene in nonmuscle cultured cells from Duchenne and Becker patients. *J Clin Invest* 1991;88:1161-6.
 - 60 Wallace DC. Mitochondrial diseases: genotype versus phenotype. *TIGS* 1993;9:128-33.
 - 61 Prior TW, Papp AC, Snyder PJ, et al. A missense mutation in the dystrophin gene in a Duchenne muscular dystrophy patient. *Nature Genet* 1993;4:357-60.
 - 62 Wang J, Zhou J, Todorovic SM, et al. Molecular genetic and genetic correlations in sodium channelopathies: lack of founder effect and evidence for a second gene. *Am J Hum Genet* 1993;52:1074-84.
 - 63 Ohno K, Hutchinson DO, Milone M, et al. Congenital myasthenic syndrome caused by prolonged acetylcholine receptor channel openings due to a mutation in the M2 domain of the ϵ subunit. *Proc Natl Acad Sci USA* 1995;92:758-62.
 - 64 Bejaoui K, Hirabayashi K, Hentati F, et al. Linkage of Miyoshi myopathy (distal autosomal recessive muscular dystrophy) locus to chromosome 2p12-14. *Neurology* 1995;45:768-72.
 - 65 Laing NG, Laing BA, Meredith C, et al. Autosomal dominant distal myopathy: linkage to chromosome 14. *Am J Hum Genet* 1995;56:422-7.
 - 66 Speer MC, Yamaoka LH, Gilchrist JH, et al. Confirmation of genetic heterogeneity in limb-girdle muscular dystrophy: linkage of an autosomal dominant form to chromosome 5q. *Am J Hum Genet* 1992;50:1211-7.
 - 67 Yamaoka LH, Westbrook CA, Speer MC, et al. Development of a microsatellite genetic map spanning 5q31-33 and subsequent placement of the LGMD1A locus between D5S178 and IL9. *Neuromuscul Disord* 1994;4:471-5.
 - 68 Janssen EAM, Hensels GW, van Oost BA, et al. The gene for X-linked myotubular myopathy is located in an 8 Mb region at the border of Xq27.3 and Xq28. *Neuromuscul Disord* 1994;4:455-61.
 - 69 Brais B, Xie Y-G, Sanson M, et al. The oculopharyngeal muscular dystrophy locus maps to the region of the cardiac α and β myosin heavy chain genes on chromosome 14q11.2-q13. *Human Molecular Genetics* 1995;4:429-34.
 - 70 Wallgren-Pettersson C, Avela K, Marchand S, et al. A gene for autosomal recessive nemaline myopathy assigned to chromosome 2q by linkage analysis. *Neuromuscul Disord* 1995;5:441-3.
 - 71 McCarthy TV, Healy JMS, Heffron JJA, et al. Localization of malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2. *Nature* 1990; 343:562-4.
 - 72 Deufel T, Golla A, Iles D, et al. Evidence for genetic heterogeneity of malignant hyperthermia susceptibility. *Am J Hum Genet* 1992;50:1151-61.
 - 73 Levitt RC, Nouri N, Jedlicka AE, et al. Evidence for genetic heterogeneity in malignant hyperthermia susceptibility. *Genomics* 1991;11:543-7.
 - 74 MacLennan DH. Discordance between phenotype and genotype in malignant hyperthermia. *Curr Opin Neurol Neurosurg* 1995;8:397-401.
 - 75 Powell SM, Petersen GM, Krush AJ, et al. Molecular diagnosis of familial adenomatous polyposis. *N Engl J Med* 1993;329:1982-7.
 - 76 Roest PAM, Roberts RG, Sugino S, van Ommen G-JB, den Dunnen JT. Protein truncation test (PTT) for rapid detection of translation-terminating mutations. *Human Molecular Genetics* 1993;2:1719-21.
 - 77 Heim RA, Silverman LM, Kam-Morgan LNW, Luce MC. Screening for truncated NF1 proteins. *Nature Genet* 1994;8:318-9.
 - 78 Heim RA, Kam-Morgan LNW, Binnie CG, et al. Distribution of 13 truncating mutations in the neurofibromatosis 1 gene. *Human Molecular Genetics* 1995;4:975-81.
 - 79 Campbell KP. Adhalin gene mutations and autosomal recessive limb-girdle muscular dystrophy. *Ann Neurol* 1995;38:353-4.
 - 80 Byrne E. Mitochondrial DNA abnormalities in human disease. *Med J Aust* 1991;154:646-7.
 - 81 Kakulas BA. The spectrum of dystrophinopathies. In: Lane RJM, ed. *Handbook of muscle disease*. New York: Marcel Dekker 1996 (in press).
 - 82 Van Ommen GJB, Scheuerbrandt G. Neonatal screening for muscular dystrophy. Consensus recommendation of the 14th workshop sponsored by the European Neuromuscular Center (ENMC). *Neuromuscul Disord* 1993;3:231-9.
 - 83 Muntoni F, Mateddu A, Cianchetti C, et al. Dystrophin analysis using a panel of anti-dystrophin antibodies in Duchenne and Becker muscular dystrophy. *J Neurol Neurosurg Psychiatry* 1993;56:26-31.
 - 84 Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell* 1987;50:509-17.
 - 85 den Dunnen J, Bakker E, Klein-Breteler E, Pearson P, van Ommen G. Direct detection of more than 50% of the Duchenne muscular dystrophy mutations by field inversion gels. *Nature* 1987;329:640-2.
 - 86 Laing NG, Mears ME, Thomas HE, et al. Differentiation of Becker muscular dystrophy from limb-girdle muscular dystrophy and Kugelberg-Welander disease using a cDNA probe. *Med J Aust* 1990;152:270-1.
 - 87 Lunt PW, Cumming WJK, Kingston H, et al. DNA probes in differential diagnosis of Becker muscular dystrophy and spinal muscular atrophy. *Lancet* 1989;i:46-7.
 - 88 Norman A, Thomas N, Coakley J, Harper P. Distinction of Becker from limb-girdle muscular dystrophy by means of dystrophin cDNA probes. *Lancet* 1989;i:466-8.
 - 89 Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed by the polymerase chain reaction. *Am J Hum Genet* 1989;44:388-96.
 - 90 Hejtmancik J, Harris S, Tsao C, Ward P, Caskey C. Carrier diagnosis of Duchenne muscular dystrophy

- using restriction fragment length polymorphisms. *Neurology* 1986;36:1553-62.
- 91 Laing NG, Siddique T, Bartlett RJ, et al. Duchenne muscular dystrophy: detection of deletion carriers by spectrophotometric densitometry. *Clin Genet* 1989;35:393-8.
 - 92 Mao Y, Cremer M. Detection of Duchenne muscular dystrophy carriers by dosage analysis using the DMD cDNA clone 8. *Hum Genet* 1989;81:193-5.
 - 93 Bartlett RJ, Pericak-Vance MA, Lanman JT, et al. Prenatal detection of an inherited Duchenne muscular dystrophy deletion allele. *Neurology* 1987;37:355-6.
 - 94 Abbs S, Bobrow M. Analysis of quantitative PCR for the diagnosis of deletion and duplication carriers in the dystrophin gene. *J Med Genet* 1992;29:191-6.
 - 95 Ioannou P, Christopoulos G, Panayides K, Kleanthous M, Middleton L. Detection of Duchenne and Becker muscular dystrophy carriers by quantitative multiplex polymerase chain reaction analysis. *Neurology* 1992;42:1783-90.
 - 96 Beggs AH, Hoffman EP, Snyder JR, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. *Am J Hum Genet* 1991;49:54-7.
 - 97 Sunohara N, Arahata K, Hoffman EP, et al. Quadriceps myopathy: forme fruste of Becker muscular dystrophy. *Ann Neurol* 1990;28:634-9.
 - 98 Gospe SM, Lazaro RP, Lava NS, Grootscholten BS, Scott MO, Fischbeck KH. Familial X-linked myalgia and cramps: a non-progressive myopathy associated with a deletion in the dystrophin gene. *Neurology* 1989;39:1277-80.
 - 99 Malapert D, Recan D, Leturcq F, Degos JD, Gherardi RK. Sporadic lower limb hypertrophy and exercise induced myalgia in a woman with dystrophin gene deletion. *J Neurol Neurosurg Psychiatry* 1995;59:552-4.
 - 100 Servidei S, Manfredi G, Mirabella M, et al. Familial hyperCKemia can be a variant of Becker muscular dystrophy. *Neurology* 1993;43:A293.
 - 101 Piccolo G, Azan G, Tonin P, et al. Dilated cardiomyopathy requiring cardiac transplantation as initial manifestation of Xp21 Becker type muscular dystrophy. *Neuromuscul Disord* 1994;4:143-6.
 - 102 Yoshida K, Ikeda S-I, Nakamura A, et al. Molecular analysis of the Duchenne muscular dystrophy gene in patients with Becker muscular dystrophy presenting with dilated cardiomyopathy. *Muscle Nerve* 1993;16:1161-6.
 - 103 Beckmann JS, Richard I, Hillaire D, et al. A gene for limb-girdle muscular dystrophy maps to chromosome 15 by linkage. *C R Acad Sci (Paris)* 1991;312:141-8.
 - 104 Fougere F, Broux O, Richard I, et al. Mapping of a chromosome 15 region involved in limb girdle muscular dystrophy. *Human Molecular Genetics* 1994;3:285-93.
 - 105 Richard I, Broux O, Allamand V, et al. Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. *Cell* 1995;81:27-40.
 - 106 Matsumura K, Nonaka I, Campbell KP. Abnormal expression of dystrophin-associated proteins in Fukuyama-type congenital muscular dystrophy. *Lancet* 1993;341:521-2.
 - 107 Tome FMS, Evangelista T, Leclerc A, et al. Congenital muscular dystrophy with merosin deficiency. *C R Acad Sci (Paris)* 1994;317:351-7.
 - 108 Bashir R, Strachan T, Keers S, et al. A gene for autosomal recessive limb-girdle muscular dystrophy, maps to chromosome 2p. *Human Molecular Genetics* 1994;3:455-7.
 - 109 Speer MC, Yamaoka LH, Gilchrist JM, et al. Evidence for genetic heterogeneity in the dominant form of limb-girdle muscular dystrophy. *Am J Hum Genet* 1993;53:A1082.
 - 110 Matsumura K, Tome FMS, Collin H, et al. Deficiency of the 50K dystrophin-associated glycoprotein in severe childhood autosomal recessive muscular dystrophy. *Nature* 1992;359:320-2.
 - 111 Ljunggren A, Duggan D, McNally E, et al. Primary adhalin deficiency as a cause of muscular dystrophy in patients with normal dystrophin. *Ann Neurol* 1995;38:367-72.
 - 112 Roberds SL, Leturcq F, Allamand V, et al. Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. *Cell* 1994;78:625-33.
 - 113 Arahata K, Ishii H, Hayashi YK. Congenital muscular dystrophies. *Curr Opin Neurol Neurosurg* 1995;8:385-90.
 - 114 Helbling-Leclerc A, Zhang X, Topaloglu H, et al. Mutations in the laminin α 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. *Nature Genet* 1995;11:216-8.
 - 115 Toda T, Segawa M, Nomura Y, et al. Localization of a gene for Fukuyama type congenital muscular dystrophy to chromosome 9q31-33. *Nature Genet* 1993;5:283-6.
 - 116 Toda T, Yoshioka M, Nakahori Y, et al. Genetic identity of Fukuyama-type congenital muscular dystrophy and Walker-Warburg syndrome. *Ann Neurol* 1995;37:99-101.
 - 117 Wijmenga C, Frants RR, Brouwer OF, Moerer P, Weber JL, Padberg GW. Localisation of facioscapulohumeral muscular dystrophy gene on chromosome 4. *Lancet* 1990;336:651-3.
 - 118 Wijmenga C, Brouwer OF, Padberg GW, Frants RR. Transmission of de novo mutation associated with facioscapulohumeral muscular dystrophy. *Lancet* 1992;340:985-6.
 - 119 Wijmenga C, Hewitt JE, Sandkuijl LA, et al. Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. *Nature Genet* 1992;2:26-30.
 - 120 Weiffenbach B, Dubois J, Storvick D, et al. Mapping the facioscapulohumeral muscular dystrophy gene is complicated by chromosome 4q35 recombination events. *Nature Genet* 1993;4:165-9.
 - 121 Gilbert JR, Stajich JM, Wall S, et al. Evidence for heterogeneity in facioscapulohumeral muscular dystrophy (FSHD). *Am J Hum Genet* 1993;53:401-8.
 - 122 Bione S, Maestri E, Rivella S, et al. Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. *Nature Genet* 1994;8:323-7.
 - 123 Laing NG. Inherited disorders of contractile proteins in skeletal and cardiac muscle. *Curr Opin Neurol Neurosurg* 1995;8:391-6.
 - 124 Hageman ATM, Gabreëls, Liem KD, Renkawek K, Boon JM. Congenital myotonic dystrophy; a report on thirteen cases and a review of the literature. *J Neurol Sci* 1993;115:95-101.
 - 125 Harper PS. *Myotonic dystrophy. Major problems in neurology*. Vol 9. Philadelphia: WB Saunders, 1979.
 - 126 Serisier DE, Mastaglia FL, Gibson GF. Respiratory muscle function and ventilatory control I in patients with motor neurone disease, II in patients with myotonic dystrophy. *Q J Med* 1982;202:205-26.
 - 127 Viitasalo MT, Kala R, Karli P, Eisalo A. Ambulatory electro-cardiographic recording in mild or moderate myotonic dystrophy and myotonia congenita (Thomsen's disease). *J Neurol Sci* 1983;62:181-90.
 - 128 Winters SJ, Schreiner B, Griggs RC, Rowley P, Nanda NC. Familial mitral valve prolapse and myotonic dystrophy. *Ann Intern Med* 1976;85:19-22.
 - 129 Buxton J, Shelbourne P, Davies J, et al. Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. *Nature* 1992;355:547-8.
 - 130 Fu Y-H, Pizzuti A, Fenwick Jr RG, et al. An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science* 1992;255:1256-8.
 - 131 Mahadevan M, Tsiflidis C, Sabourin L, et al. Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. *Science* 1992;255:1253-5.
 - 132 Reardon W, Harley HG, Brook JD, et al. Minimal expression of myotonic dystrophy: a clinical and molecular analysis. *J Med Genet* 1992;29:770-3.
 - 133 Ricker K, Koch MC, Lehmann-Horn F, et al. Proximal myotonic myopathy. *Neurology* 1994;44:1448-52.
 - 134 Thornton CA, Griggs RC, Moxley RT. Myotonic dystrophy with no trinucleotide repeat expansion. *Ann Neurol* 1994;35:269-72.
 - 135 Sun SF, Streib EW. Autosomal recessive generalised myotonia. *Muscle Nerve* 1983;6:143-7.
 - 136 Becker PE. Syndromes associated with myotonia: clinical-genetic classification. In: Rowland LP, ed. *Pathogenesis of human muscular dystrophies*. Amsterdam: Excerpta Medica 1977:699-705.
 - 137 Hudson AJ, Ebers GC, Bulman DE. *Brain* 1995;118:547-63.
 - 138 Ricker K, Moxley RT, Heine R, Lehmann-Horn F. Myotonia fluctuans. A third type of muscle sodium channel disease. *Arch Neurol* 1994;51:1095-102.
 - 139 Campa JA, Sanders DB. Familial hypokalaemic periodic paralysis. *Arch Neurol* 1974;31:110-5.
 - 140 McManis PG, Lambert EH, Daube JR. The exercise test in periodic paralysis. *Muscle Nerve* 1986;9:704-10.
 - 141 Kantola IM, Tarssanen LT. Diagnosis of familial hypokalaemic periodic paralysis: role of the potassium exercise test. *Neurology* 1992;42:2158-61.
 - 142 Elbaz A, Vale-Santos J, Jurkat-Rott K, et al. Hypokalaemic periodic paralysis and the dihydropyridine receptor (CACNL1A3): genotype/phenotype correlations for two predominant mutations and evidence for the absence of a founder effect in 16 Caucasian families. *Am J Hum Genet* 1995;56:374-80.
 - 143 Jurkat-Rott K, Lehmann-Horn F, Elbaz A, et al. A calcium channel mutation causing hypokalaemic periodic paralysis. *Human Molecular Genetics* 1994;3:1415-9.
 - 144 Ptacek LJ, Trimmer JS, Agnew WS, Roberts JW, Petajan JH, Leppert M. Paramyotonia congenita and hyperkalaemic periodic paralysis map to the same sodium channel gene locus. *Am J Hum Genet* 1991;49:851-4.
 - 145 Rojas CV, Wang J, Schwartz LS, Hoffman EP, Powell BR, Brown RHJ. A MET to VAL mutation in the skeletal muscle Na⁺ channel α -subunit in hyperkalaemic periodic paralysis. *Nature* 1991;354:387-9.
 - 146 Baquero JL, Ayala RA, Wang J, et al. Hyperkalaemic periodic paralysis with cardiac dysrhythmia: a novel sodium channel mutation? *Ann Neurol* 1995;37:408-11.
 - 147 Tawil R, Ptacek LJ, Pavlakis SG, et al. Andersen's syndrome: potassium-sensitive periodic paralysis, ventricular ectopy, and dysmorphic features. *Ann Neurol* 1994;35:326-30.
 - 148 Fardeau M. Congenital myopathies. In: Mastaglia FL, Walton JN, eds. *Skeletal muscle pathology*. 2nd ed. Edinburgh: Churchill-Livingstone, 1992:237-81.
 - 149 Laing NG, Majda BT, Akkari PA, et al. Assignment of a gene (NEM1) for autosomal dominant nemaline myopathy to chromosome 1. *Am J Hum Genet* 1992;50:576-83.
 - 150 Laing NG, Wilton SD, Akkari PA, et al. A mutation in the α -tropomyosin gene TPM3 associated with autosomal dominant nemaline myopathy. *Nature Genet* 1995;9:75-9.

- 151 Beggs AH, Byers TJ, Knoll JHM, Boyce FM, Bruns GAP, Kunkel L. Cloning and characterization of two human skeletal muscle alpha-actinin genes on chromosome one and eleven. *J Biol Chem* 1992;267:9281-8.
- 152 Haan EA, Freemantle CJ, McCure JA, Friend KL, Mulley JC. Assignment of the gene for central core disease to chromosome 19. *Hum Genet* 1990;86:187-90.
- 153 Kausch K, Lehmann-Horn F, Janka M, Wieringa B, Grimm T, Muller CR. Evidence for linkage of the central core disease locus to the proximal long arm of human chromosome 19. *Genomics* 1991;10:765-9.
- 154 Quane KA, Healy JMS, Keating KE, et al. Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia. *Nature Genet* 1983;5:51-5.
- 155 Quane KA, Keating KE, Healy JMS, et al. Mutation screening of the RYR1 gene in malignant hyperthermia: detection of a novel tyr-ser mutation in a pedigree with associated central cores. *Genomics* 1994;23:236-9.
- 156 Zhang Y, Chen HS, Khanna VK, et al. A mutation in the human ryanodine receptor gene associated with central core disease. *Nature Genet* 1993;5:46-50.
- 157 Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 1988;331:717-9.
- 158 Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutations associated with Leber's hereditary optic neuropathy. *Science* 1988;242:1427-30.
- 159 Jackson MJ, Schaefer JA, Johnson MA, Morris AAM, Turnbull DM, Bindoff LA. Presentation and clinical investigation of mitochondrial respiratory chain disease. *Brain* 1995;118:339-57.
- 160 Johns DR. Mitochondrial DNA and disease. *New Engl J Med* 1995;333:638-44.
- 161 Morgan-Hughes JA. Mitochondrial diseases. In: Mastaglia FL, Walton JN, eds. *Skeletal muscle pathology*. 2nd ed. Edinburgh: Churchill-Livingstone, 1992: 367-424.
- 162 DiMauro S, Moraes CT. Mitochondrial encephalomyopathies. *Arch Neurol* 1993;50:1197-208.
- 163 Mechler F, Johnson MA, Mastaglia FL, et al. Mitochondrial myopathies. A clinico-pathological study of cases with and without extra-ocular muscle involvement. *Aust NZ J Med* 1986;16:185-92.
- 164 Zeviani M, Moraes CT, DiMauro S, et al. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 1988;38:1339-46.
- 165 Zeviani M, Servidei S, Gellera C, Bertini E, DiMauro S, DiDonato S. An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. *Nature* 1989;339:309-11.
- 166 Mechler F, Fawcett PRW, Mastaglia FL, Hudson P. Mitochondrial myopathy: a study of clinically affected and asymptomatic members of a six-generation family. *J Neurol Sci* 1981;50:191-200.
- 167 Ozawa M, Goto Y-I, Sakuta R, Tanno Y, Tsuji S, Nonaka I. The 8,344 mutation in mitochondrial DNA: a comparison between the proportion of mutant DNA and clinicopathological findings. *Neuromusc Disord* 1995;5:483-8.
- 168 Suomalainen A, Ciafaloni E, Koga Y, Peltonen L, DiMauro S, Schon EA. Use of single strand conformation polymorphism analysis to detect point mutations in human mitochondrial DNA. *J Neurol Sci* 1992;111:222-6.
- 169 Kawai H, Akaike M, Yokoi K, et al. Mitochondrial encephalomyopathy with autosomal dominant inheritance: a clinical and genetic entity of mitochondrial diseases. *Muscle Nerve* 1995;18:753-60.
- 170 Cheng S, Higuchi R, Stoneking M. Complete mitochondrial genome amplification. *Nature Genet* 1994;7:350-1.
- 171 Howell N, Kubacka I, Halvorson S, Howell B, McCullough DA, Mackey D. Phylogenetic analysis of the mitochondrial genomes from Leber hereditary optic neuropathy pedigrees. *Genetics* 1995;140:285-302.
- 172 Lertrit P, Kapsa RMI, Jean-Francois MJB, et al. Mitochondrial DNA polymorphism in disease: a possible contributor to respiratory dysfunction. *Human Molecular Genetics* 1994;3:1973-81.
- 173 DiMauro S, Tonin P, Servidei S. Metabolic myopathies. In: Rowland LP, DiMauro S, eds. *Handbook of clinical neurology*. Amsterdam: Elsevier, 1992:479-526.
- 174 MacLennan DH, Duff C, Zorzato F, et al. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature* 1990;343:559-61.
- 175 Zorzato F, Fujii J, Otsu K, et al. Molecular cloning of cDNA encoding human and rabbit forms of the Ca²⁺ release channel (ryanodine receptor) of skeletal muscle. *J Biol Chem* 1990;265:2244-56.
- 176 Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. I. Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. *Ann Neurol* 1984;16:193-208.
- 177 Karpati G, Carpenter S. Pathology of the inflammatory myopathies. In: Mastaglia FL, ed. *Inflammatory myopathies*. Bailliere's Clinical Neurology 1993;2/3: 527-56.
- 178 Bernstein RM. Autoantibodies in myositis. In: Mastaglia FL, ed. *Inflammatory myopathies*. Bailliere's Clinical Neurology 1993;2/3:599-615.
- 179 Dalakas MC, Pezeshpour GH. Neuromuscular diseases associated with human immunodeficiency virus infection. *Ann Neurol* 1988;23(suppl):S38-S48.
- 180 Mastaglia FL, Ojeda VJ. Inflammatory myopathies: Part I. *Ann Neurol* 1985;17:215-27.
- 181 Mastaglia FL, Laing BA, Zilko P. Treatment of inflammatory myopathies. In: Mastaglia FL, ed. *Inflammatory myopathies*. Bailliere's Clinical Neurology 1993;2/3: 717-40.
- 182 Woolf RE, Baethge BA. Interleukin-1 α , interleukin-2, and soluble interleukin-2 receptors in polymyositis. *Arth Rheum* 1990;33:1007-14.