NEUROLOGICAL INVESTIGATIONS

Investigation of muscle disease

F L Mastaglia, N G Laing

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.1-2 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 glycyrrhizinic 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, hypothroid, 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

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Inducing drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myalgia</td>
<td>Suxamethonium, danaanol, colchiate, salbutamol, lithium, captorpril, colchicine, procainamide, metoclopride, cyclosporin, cyclosporin, zidovudine, ifosfamide, levetiracetam, pindolol, ziconotide, penicillin, gold, enalapril, ampicillin, tizanidine, nitidilpine</td>
</tr>
<tr>
<td>Myotonia</td>
<td>Diazacolesterol, β-blockers, β-fonato (fenoterol, ritodrine), clofibrate, diuretics (frumside, ethacrynic acid, mesalazine), acetazolamide</td>
</tr>
<tr>
<td>Necrotising myopathy</td>
<td>Alcohol, glibenclamide, sirolastatin, colchiate, e-aminoacaproic acid, cyclosporin, zidovudine, cocaine, etamine</td>
</tr>
<tr>
<td>Mitochondrial myopathy</td>
<td>Zidovudine</td>
</tr>
<tr>
<td>Inflammatory myopathy</td>
<td>D-penicillamine, t-cryptophan, others rarely</td>
</tr>
<tr>
<td>Autoimmune myopathy</td>
<td>Chloroquine, vincristine, colchicine, amiodarone, perchlorine</td>
</tr>
<tr>
<td>Type 2 atrophy</td>
<td>Corticosteroids</td>
</tr>
<tr>
<td>Localised myopathy</td>
<td>Intramuscular antibiotics, narcotics</td>
</tr>
</tbody>
</table>

*May exacerbate myotonia.
†May cause myotonia in animals.

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

<table>
<thead>
<tr>
<th>Inflammatory</th>
<th>Hereditary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral myositis</td>
<td>Disorders of glycogen metabolism</td>
</tr>
<tr>
<td>Pyomyositis</td>
<td>Carbohydrate intolerance deficiency</td>
</tr>
<tr>
<td>Parasitic myositis</td>
<td>Myoadenylate deaminase deficiency</td>
</tr>
<tr>
<td>Polymyositis/dermatomyositis</td>
<td>Mitochondrial myopathy</td>
</tr>
<tr>
<td>Granulomatous myositis</td>
<td>Dystrophinopathy</td>
</tr>
<tr>
<td>Interstitial myositis</td>
<td>Sodium channel myositis</td>
</tr>
<tr>
<td>Localised nodular myositis</td>
<td>Malignant hyperthermia</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>Others</td>
</tr>
<tr>
<td>Eosinophilic fasciitis</td>
<td>Fibromyalgia</td>
</tr>
<tr>
<td>Toxic</td>
<td>Polymyalgia rheumatica</td>
</tr>
<tr>
<td>Acute alcoholic myopathy</td>
<td>Postviral myalgia/fatigue</td>
</tr>
<tr>
<td>Acute/subacute drug induced myopathies</td>
<td>Muscle overuse syndromes</td>
</tr>
<tr>
<td>Myopathies due to envenomation</td>
<td>Myopathy with tubular aggregates</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Endocrine</th>
<th>Hypothyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteomalacia</td>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Hyperparathyroidism</td>
<td>Hyperthyroidism</td>
</tr>
</tbody>
</table>
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 extracocular 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 extracocular muscles, and often of the bulbar and limb muscles; fatigability is a prominent feature. In myotonic dystrophy there is often less 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” 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 scapulopereonal syndrome.

The limb-girdle syndrome, in which there is involvement of the girdle and proximal limb muscles, is relatively non-selective 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 hypertropy is common in mytonia congenita. A hypertrophic myopathy may occasionally occur in patients with amyloidosis, sarcoidosis, or cysticercosis. Muscle contractures occur relatively in idiopathic 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 muscular dystrophy in the 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.11 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

<table>
<thead>
<tr>
<th>Physical exercise</th>
<th>Muscle trauma</th>
<th>Pressure</th>
<th>Falls</th>
<th>Intravenous injection</th>
<th>Acute psychosis/delirium</th>
<th>Dystrophias</th>
<th>Drugs</th>
<th>Alcohol</th>
<th>Others*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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

Myoglobinamaemia and myoglobinuria may be due to various causes 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 inflammatory 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. 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.

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). 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.

Pharmacological testing

Although in some families with malignant hyperthermia mutations have been found in the rydoline 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. Ab normal responses to caffeine and calcium ions have also been described using the skinned muscle fibre technique. 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-mytopathic 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,19 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.20

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-waves 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 technitium 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 calcification 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 neuromuscular 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. 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 sncroglycanopathies. 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. 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, Bonnemann et al 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 sncroglycanopathy. Immunohistochemistry or immunoblotting can also predict the severity of disease—for example, in both the dystrophinopathies and the sncroglycanopathies. On the other hand, molecular analysis cannot always predict severity of disease, as people with apparently the same mutation can have different severities of disease perhaps due to alternate splicing around the mutation.

**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. 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 sncroglycanopathies, 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.

**ACCURATE, SIMPLPE PREGNATAL 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.

**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. Presymptomatic diagnosis is also possible by molecular techniques in any of the later onset diseases, such as hypokalaemic periodic paralysis.

The polymerase chain reaction (PCR), 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 messages or the creation of restriction enzyme sites to discriminate between normal and mutant alleles.

**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, BgII, or BamHI digestion.
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.
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.

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

5. Mitochondrial myopathies, in which the common point mutations can be tested for using PCR followed by enzyme digestion or SSCP analysis.

**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, 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 review summarised the techniques then current such as the simple single strand conformation analysis (SSCA), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis (HA), RNAsese A cleavage, chemical mismatch cleavage (CMC), and direct sequencing. Techniques which should perhaps be added to the list include restriction endonuclease fingerprinting (REF), dideoxyfingerprinting, and enzymatic mismatch cleavage (EMC).

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 SSRPs. The short cut alternative is to examine the cDNA for mutations. 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. 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 messages 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 message—is fraught with difficulties.61 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 dominant limb-girdle muscular dystrophy,65 66 X linked centronuclear (myotubular) myopathy,67 oculopharyngeal muscular dystrophy,68 and recessive nemaline myopathy69 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 not72 73 and virtually only single families have shown linkage to other regions of the genome.74 The limb-girdle muscular 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 coli77 and Duchenne muscular dystrophy,78 (fig 1), but it has also been applied to neurofibromatosis.79 80 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 26 31 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.60 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 al51 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). 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
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. 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),
which gives a diagnosis in hours rather than the previous days or weeks.\textsuperscript{25-27} Prenatal diagnosis is also achieved with multiplex PCR when a family deletion has been identified, and by linkage using microsatellite\textsuperscript{28} 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.\textsuperscript{29,30,31} Identification of carriers of dystrophinopathies has been achieved with dosage Southern blotting,\textsuperscript{32-34} presence of junction fragments,\textsuperscript{35} and non-inheritance of alleles.\textsuperscript{36} 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\textsuperscript{37-39} as has RT-PCR from illegitimate transcription of the dystrophin message in circulating lymphocytes.\textsuperscript{40}

The increasing diversity of dystrophinopathy phenotypes has been reviewed by Beggs \textit{et al}.\textsuperscript{41} and includes quadriiceps myopathy,\textsuperscript{42} cramps and myalgia,\textsuperscript{43,44} hyperCKaemia,\textsuperscript{45,46} and X linked cardiomyopathy.\textsuperscript{47,48}

\textbf{Limb-girdle muscular dystrophies}

The first recessive limb-girdle muscular dystrophy gene to be localised (LGMD2A) was on chromosome 15\textsuperscript{49-51} 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.\textsuperscript{52} (Two different autosomal recessive limb-girdle muscular dystrophies segregate in the Amish community—the other is LGMD2E.\textsuperscript{53}) The identification of calpain as the mutant gene in LGMD2A was the first implication of a non-structural protein in a muscular dystrophy.\textsuperscript{54} No abnormalities in the dystrophin associated glycoproteins were detected in patients with LGMD2A.\textsuperscript{55-57}

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,\textsuperscript{58} 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,\textsuperscript{59,60} The other (LGMD1B) is not yet localised.\textsuperscript{61}

\textbf{Sarcoglycanopathies}

Adhalinopathy (\(\alpha\)-sarcoglycanopathy) was the first sarcoglycanopathy to be identified, initially by deficiency in immunohistochemical staining\textsuperscript{62} and later by mutations in the adhalin gene.\textsuperscript{63,64,65} 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 \(\beta\)-sarcoglycan gene on chromosome 4 are associated with LGMD2E, the second limb-girdle muscular dystrophy found among the Amish\textsuperscript{66} and also other communities.\textsuperscript{67} Mutations in the \(\gamma\)-sarcoglycan gene on chromosome 13 are associated with severe congenital autosomal recessive muscular dystrophy (SCARMD, LGMD2C).\textsuperscript{68}

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 \textit{et al}\textsuperscript{69} state in the discussion of their paper, only molecular investigation of all the candidate genes can provide a definitive diagnosis.

\textbf{Congenital muscular dystrophy}

Congenital muscular dystrophies are charac-
terised by severe dystrophic changes of muscle from birth. Tomé et al. 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. Subsequently mutations of the LAMA2 gene were identified in patients with congenital muscular dystrophy.

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, and thus a different as yet unidentified gene must be involved. Fukuyama muscular dystrophy and Walker-Wallberg syndrome may be genetically identical.

**Facioscapulohumeral muscular dystrophy**

A gene for facioscapulohumeral muscular dystrophy (4q35) was first mapped in 1990 to chromosome 4 near the telomere. Subsequently, altered sized fragments involving a repeated sequence with homeodomain homology were identified in familial and sporadic cases of facioscapulohumeral muscular dystrophy. 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 documented and not all facioscapulohumeral muscular dystrophy families show linkage to 4q35. 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-myosin and in the same region as a gene for dominant distal myopathy.

**Emery-Dreifuss muscular dystrophy**

The Emery-Dreifuss muscular dystrophy gene has been identified and its protein product named emerin. 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 Gowers and the other for the recessive Miyoshi myopathy. It is interesting that the MPD1 gene maps to a similar region of chromosome 14 as a gene for oculopharyngeal muscular dystrophy 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), raising the possibility that the two conditions may be caused by mutations in the same gene.

### 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. 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. 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. 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. The incidence of mitral valve prolapse is also increased in some families. Investigation of cardiac function, including ECG and echocardiography, should therefore be performed routinely and, when indicated, ECG monitoring and radionuclide

<table>
<thead>
<tr>
<th>Table 5 Classification of hereditary myotonias</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myotonia</strong></td>
</tr>
<tr>
<td>- Myotonic dystrophy</td>
</tr>
<tr>
<td>- Proximal myotonic dystrophy</td>
</tr>
<tr>
<td><strong>Gene</strong></td>
</tr>
<tr>
<td>- Chloride channel myotonias:</td>
</tr>
<tr>
<td>- Autosomal dominant (Thomsen)</td>
</tr>
<tr>
<td>- Autosomal recessive (Becker)</td>
</tr>
<tr>
<td>- Sodium channel myotonias:</td>
</tr>
<tr>
<td>- Paralympotonia congenita</td>
</tr>
<tr>
<td>- Myotonia floridans</td>
</tr>
<tr>
<td>- Myotonia permanens</td>
</tr>
<tr>
<td>- Dystrophic myotonias:</td>
</tr>
<tr>
<td>- Myotonic dystrophy</td>
</tr>
<tr>
<td><strong>Myotin protein kinase</strong></td>
</tr>
</tbody>
</table>
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 myotonic 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 BglII or BamHI.44 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).132,133 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.135

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 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.139 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 a 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 periodic 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, mexiltiline.138 A fourth variety of sodium channel myotonia with continuous severe myotonia, muscle stiffness, and hypertrophy has also been described and designated myotonia perennans.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
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. 15 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. 8 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. 6

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. 10 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 exercise test described by McManis et al. 16 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. 142 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 g/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 hypokalaemia, intravenous potassium (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

In the potassium sensitive forms of periodic paralysis attacks of weakness may be induced by oral potassium loading. 9, 11 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.

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 (CACNL1A3) on chromosome 1q31–32 affecting two amino acid residues. 98, 102, 142, 143 The mutations can be detected by PCR followed by enzyme digestion as they either create or destroy restriction enzyme sites or by SSCP. 98 Mutations in the gene encoding the α subunit of the skeletal muscle sodium channel (SCN4A) causing hyperkalaemic periodic paralysis were first described in 1991. 104, 105 A recent review by Lehmann-Horn and Rüdel 160 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

Table 6 Classification of periodic paralyses

<table>
<thead>
<tr>
<th>Hereditary:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypokalaemic (DHP receptor; 1q31–32)</td>
</tr>
<tr>
<td>Potassium sensitive (SCN4A; 17q23–25)</td>
</tr>
<tr>
<td>Hyperkalaemic</td>
</tr>
<tr>
<td>Paramyotonia congenita</td>
</tr>
<tr>
<td>Periodic paralytic paralysis</td>
</tr>
<tr>
<td>Andersen’s syndrome</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acquired:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypokalaemic</td>
</tr>
<tr>
<td>Hyperkalaemic</td>
</tr>
<tr>
<td>Thyrotoxic</td>
</tr>
</tbody>
</table>
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 congenital 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 2.76 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 hyperthermia152 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 1988157 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 extracocular 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 MERRF syndromes) (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 8 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 8 Mitochondrial myopathies and encephalomyopathies**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive external ophthalmoplegia</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Familial</td>
<td></td>
</tr>
<tr>
<td>Kearns-Sayre syndrome</td>
<td>Ophthalmoplegia</td>
</tr>
<tr>
<td>Pigmented retinopathy</td>
<td>Cardiac conduction defects</td>
</tr>
<tr>
<td>Cerebellar ataxia</td>
<td>Neurosensorineal deafness</td>
</tr>
<tr>
<td>LHG myopathy</td>
<td>Infantile myopathy</td>
</tr>
<tr>
<td>Benign reversible</td>
<td>Severe fatal</td>
</tr>
<tr>
<td>MERRF syndrome</td>
<td>Myoclonus</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>MELAS syndrome</td>
</tr>
<tr>
<td>Myopathy</td>
<td>Lactic acidosis</td>
</tr>
<tr>
<td>Stroke-like episodes</td>
<td></td>
</tr>
</tbody>
</table>

*Predisposition to malignant hyperthermia.
Table 9 Clinical manifestations of mitochondrial disease

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

Heterogeneity is characteristic of this group of disorders 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 a single major deletion, but point mutations have been found in some patients. Similarly, those patients with the Kearns-Sayre syndrome have single large mtDNA deletions which occur sporadically. 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. Such a gene has recently been linked to chromosome 10q23–24.3–24.3 in a Finnish family with autosomal dominant CPEO. Cases presenting purely with a proximal myopathy may also be dominantly inherited 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 syndrome and tRNA-leu (nucleotides 3243, 3251, 3252, 3271 or 3291) in the MELAS syndrome.

**Acute rhabdomyolysis and myoglobinuria**

Various hereditary and acquired disorders may lead to episodes of severe widespread muscle fibre destruction and myoglobinuria. 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, hyperventilation 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 hyperthermia 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 19171 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.24 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.12 24 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 palmitoyl 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 palmitoyl transferase activity as well as a muscle biopsy for biochemical 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 these 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
Investigation of muscle disease


33. Noguchi S, McNally EM, Ohtsane KB, et al. Mutations in...


The 8,344 mutation in the cytochrome c oxidase subunit 6A gene (MT-CO6A) is associated with mitochondrial encephalomyopathy with autosomal dominant inheritance. A clinical and genetic entity of mitochondrial diseases. 


