NEUROLOGICAL MANAGEMENT

Clinical genetics in neurological disease

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Inherited neurological disorders have traditionally engendered feelings of therapeutic nihilism in the medical community: not only are these conditions often difficult to diagnose with any certainty, they are also perceived as "untreatable" whether or not a firm diagnosis has been reached. This negative response is due in part to a lack of knowledge concerning the underlying pathological basis for these disorders.

The 1980s and early 1990s have seen a rapid increase in our understanding of the molecular mechanisms behind many of these debilitating conditions, largely due to advances in recombinant DNA technology. This article aims to summarise the role of clinical genetic and molecular genetic practice in the management of the major inherited neurological diseases. We consider diagnostic issues as they pertain to the individual case—that is, the specific disorder affecting the patient and its likely prognosis—and counselling issues for the family, such as the identification of individuals who may develop symptoms in later life (presymptomatic testing) or risks for the inheritance of the disorder by a future family member (prenatal testing and carrier detection). To date there have not been equivalent advances in our therapeutic options in the management of these conditions although experimental protocols have been initiated with this in mind, some of which show promising results in animal models.

The disorders and the relevant molecular genetic analyses under consideration are discussed in two groups: those for which one or more genetic loci have been identified but the gene(s) not yet cloned; and those for which the genes have been isolated and the relevant product characterised. We begin by reviewing the available data from epidemiological and family studies on the frequency of these disorders.

Epidemiology

Individual genetic neurological diseases are not common but as a group they account for a significant burden of disability in a generally younger age group than affected by other neurological diseases. In a review of reports published throughout the world, Emery¹ estimated a population prevalence for the inherited neuromuscular diseases of over 1 in 3000. The authors' study in southeast Wales² suggests a minimum prevalence for the major inherited neurological disorders of over 58 per 100 000 population (approximately 1 in 1700 population). Table 1 shows the disorders and the prevalence of symptomatic individuals in the southeast Wales population.

These figures do not take into account asymptomatic gene carriers who may develop clinical disease in the future; nor do they allow for the unaffected but "at-risk" family members who are also likely to request information on the disease (this latter group are more likely to seek guidance from a genetics unit but a not insignificant number may be seen initially by the neurologist dealing with the index case).

Linkage analysis**DISORDERS LOCALISED TO SPECIFIC****CHROMOSOMAL SITES BUT NOT YET CLONED**

The number of disorders in this group decreases monthly as a result of the continual isolation of new genes for specific diseases. The technology used in "linkage analysis" merits a brief description as it needs to be appreciated that there are limitations to its usefulness in many clinical situations.

Linkage analysis has been the first stage in the localisation of a gene responsible for many genetic disorders. It makes use of restriction fragment length polymorphisms (RFLPs). These are lengths of DNA that have been cut at each end at a specific site

Table 1 Prevalence of major, single-gene neurological disorders in southeast Wales

Disorder	Prevalence (/100 000)	95% confidence interval
Hereditary motor and sensory neuropathy types I,II,III and V	12.9	10.7-15.4
Myotonic dystrophy	7.1	5.5-9.1
Duchenne muscular dystrophy	9.6*	7.0-12.9
Becker muscular dystrophy	5.0*	3.2-7.6
Facioscapulohumeral muscular dystrophy	2.9	1.9-4.1
Spinal muscular atrophy (all types)	1.3	0.7-2.2
Huntington's disease	8.4	6.6-10.5
Tuberous sclerosis	1.6	0.9-2.6
Von Hippel Lindau disease	0.6	0.2-1.4
Hereditary spastic paraplegia	3.4	2.3-4.8
Neurofibromatosis type 1	13.3	11.1-15.8

*Per 100 000 males.

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recognised by a bacterial restriction endonuclease enzyme. Different enzymes recognise and cleave at different DNA sequence motifs of variable numbers of base pairs. The great variability in human DNA results in differences in the length of sequence between cleavage sites in unrelated individuals. Within a family, however, these anonymous DNA segments (RFLPs) are inherited in a mendelian fashion and can be tracked through a family pedigree using standard laboratory techniques. Botstein *et al*³ first suggested the use of these in the construction of genetic linkage maps for the localisation of disease genes to particular segments of chromosomes and they have superseded the blood group polymorphisms used in early studies.

In a family linkage analysis, each member of the family is assessed for the presence of the disease of interest and their status (RFLP) for the marker locus is ascertained. If the disease locus and the marker locus are close together on the same chromosome (linked) then independent assortment at meiosis will be rare and both traits will be inherited in the offspring. The closer they are to each other the less likely it is that they will separate during the pairing of homologous chromosomes at meiosis—in other words, that a recombination will occur. The recombination fraction, known as theta (θ) gives an indication of the genetic distance between the two loci. Genetic distance is expressed in centiMorgans where 1 cM is defined as a segment of chromosome with a 1% chance of recombination per meiosis⁴ and this is equivalent to a θ of 0.01. A recombination fraction of 0.5 means either that the two loci are on different chromosomes or that they are far apart on the same chromosome.

The successful application (accuracy) of linkage analysis in the clinical situation is dependent on several factors: it is crucial that the diagnosis is correct; and the disease must have been localised (mapped to a segment of chromosome) with close polymorphic markers available. Affected individuals should be heterozygous for the marker polymorphisms ("informative"), there should not (ideally) be genetic heterogeneity (more than one disease locus for the clinical phenotype), and the family relationships, especially paternity, must be clearly established. The disease status for each individual typed must be accurate (especially where the disease is of late onset and "unaffected" individuals are crucial to the analysis). The figure shows a family typed for a hypothetical two-allele polymorphism known to be linked to the disease of interest. Individual I₂ is affected by the disorder and is heterozygous for the polymorphism (she types 2-1); her unaffected spouse is homozygous (2-2) as is her unaffected son II₁. Individual II₂ is clinically affected and has inherited allele 1 from his mother, and has subsequently transmitted this allele to individual III₁. This three-generation situation is ideally suited to linkage analysis as phase is known—that is, the chromosome carrying the mutant gene can be identified—and affected

Figure The lanes in the autoradiograph contain DNA from the individuals indicated in the pedigree. The DNA has been digested using a restriction endonuclease enzyme, subjected to electrophoresis on an agarose gel and transferred to a nylon membrane. The DNA probe of interest has been labelled with radioisotope and incubated overnight with the membrane. The membrane has been stored at -20°C adjacent to radiographic film for seven days. Bands are assigned numbers with the largest band designated 1.

individuals are heterozygous for the polymorphisms of interest. It is possible to predict for individual III₂, who may be too young for the disease to have manifested, the likelihood that they will develop the disease from a knowledge of their genotype. In this situation the presence of allele 1 indicates a high risk, the actual value of which would depend on the recombination fraction between the marker and the disease. In general it is unlikely that a marker would be used whose rate of recombination with the disease was greater than 3%. In the example shown the actual value would be 97%, using a marker with 3% recombination, as it is a fully informative situation. This approach can also be used to increase the reproductive options for couples at risk by permitting prenatal diagnosis through linkage analysis and, where affected, termination of an affected pregnancy. Individual III₁ in the figure could represent a conceptus from which chorionic villus sampling would provide appropriate tissue for analysis in early pregnancy.

Specific disorders

Table 2 lists those diseases linked to specific chromosomes, but with the gene not yet isolated, for which family linkage studies can play a useful role in patient and family management. It is not intended to be a comprehensive list of all mapped neurogenetic diseases; the interested reader should consult sources such as the bimonthly listings in *Neuromuscular disorders* (Pergamon Press), the summaries of the Human Genome Mapping Project annual meetings,

Table 2 Chromosomal localisations for major genetic neurological disorders for which the gene has not yet been cloned

Disorder	Localisation(s)
Emery-Dreifuss muscular dystrophy	Xq27·3–28
Facioscapulohumeral* muscular dystrophy	4q35
Familial spastic paraplegia (one dominant type)	14q
Friedreich's ataxia	9q13–21
Limb-girdle muscular dystrophy (dominant)	5q22·3–31·3
Spinal muscular atrophy recessive types I, II and III	5q11·2–13·3
Tuberous sclerosis (see also table 3)	9q34

Gene locations are identified by the chromosome number followed by an indication of which arm (p = short, q = long) and the sub-band.

*But see below for comments on the molecular rearrangement reported in this disorder.

McKusick's *Mendelian inheritance in man* (published and "online" versions) and periodic reviews.⁵

It may be possible to provide presymptomatic testing for these disorders for relatives at risk if the family structure is suitable but the presence of genetic heterogeneity, as in tuberous sclerosis, can make even this very difficult in all but the largest families. Various Bayesian approaches have been proposed to take such additional factors into account when counselling individuals in these situations.⁶

Although the gene for facioscapulohumeral (FSH) muscular dystrophy has not yet been cloned, it is possible to make diagnostic use of molecular DNA analysis where it is thought that an affected individual represents a new dominant mutation for the disorder. This paradoxical situation arises as the result of the presence of detectable rearrangements associated with a series of 3·2 kb tandem repeat elements in the subtelomeric region of the long arm of chromosome 4.⁷ (1 kb = 1000 base pairs.) The DNA probe p13E-11 (locus D4F104S1) detects bands over 50 kb in size after *EcoRI* digestion in normal individuals and a band less than 35 kb in length in individuals with FSH dystrophy. In most cases thought to represent new mutations, it is possible to show that both parents possess two normal-sized alleles whereas the affected individual has one normal-sized and one rearranged fragment.

Some disorders, mostly rare, have not yet been mapped to a specific chromosome(s) and genetic management is confined to counselling families on the inheritance and nature of the disease.

Mutation analysis

Once a gene associated with a particular disease has been isolated it becomes possible to test DNA samples from affected individuals for mutations within that gene. These may take the form of point mutations that substitute alternative nucleic acid bases and result in changes in the amino-acid sequence (missense mutations) or introduce stop codons with premature truncation of the translation product (nonsense mutations). Other mutations may involve the insertion or deletion of a number of base pairs (from one to many thousands) resulting in disruption of the normal function of the gene. Occasionally dele-

tions or duplications of whole chromosome segments are found in association with specific disorders. In addition to these "classical" types of mutation, we have seen in the past two years, the identification of a new class of mutation that is often unstable in its transmission between generations. This is the trinucleotide repeat, the principal characteristic of which is instability, giving varying molecular and clinical changes in the different affected members of a single kindred, as discussed more fully below.

Mutation analysis has the advantage (over linkage analysis) that it is not dependent on family structure and can therefore be applied to an isolated case of a disorder suspected to be genetic. A variety of laboratory techniques are used in the detection of mutations⁸ and it may take several weeks for a specific defect to be identified, depending on whether the method is in regular service use or still at the research stage.

DISORDERS FOR WHICH THE RESPONSIBLE GENE HAS BEEN CHARACTERISED

Table 3 lists some of those disorders for which the gene responsible has been identified and mutation analysis is feasible (the special situation for facioscapulohumeral muscular dystrophy has been mentioned above). The number of disorders in this category is increasing rapidly and individual genetic units cannot be expected to provide every analysis. In the United Kingdom, the clinician primarily responsible for the individual patient's care should consult their local genetics unit for information on which molecular services are available locally and those which can be obtained elsewhere (usually from other genetic laboratories in the National Health Service). It should be stressed that a comprehensive genetic counselling service should be available to all individuals on whom a molecular genetic analysis is undertaken. This is particularly relevant where specific mutation analysis is requested but the individual has not been aware that the disorder may be hereditary. The analyses may have significant implications for siblings or offspring. Examples of these difficulties are given below in respect of specific disorders.

In several disorders listed, a single, or very small number, of distinct mutations in the same gene are responsible for the same phenotype: thus in Leber's hereditary optic neuropathy (LHON) a point mutation at base pair 11 778 (in the gene for subunit 4 of the reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase (ND4) complex in the respiratory chain) of the mitochondrial genome accounts for 50% of cases, whereas other causative mutations giving an identical clinical picture occur at base pairs 3460 (in ND1), 4160 (ND1) and 15 257 (cytochrome b).⁹ Mutation analysis in this situation therefore requires careful planning and interpretation. Recent work has suggested that some mitochondrial mutations may be synergistic—for example, mutations at base pairs 13 708, 15 257, 15 812 and 5244—and the accumu-

Table 3 Cloned genes, their products, functions and mutations

Disease	Chromosomal localisation	Gene product	Function	Mutation	Mutations identified (%)
DMD/BMD	Xp21	Dystrophin	Cytoskeletal protein	Deletions, duplications Point mutations	50
Neurofibromatosis type 1	17q11-2	Neurofibromin	Tumour suppressor	Deletions Point mutations	20
Neurofibromatosis type 2	22q11	Merlin	Tumour suppressor	Deletions Point mutations	50
Tuberous sclerosis (one type)	16p13	Tuberlin	Tumour suppressor	Deletions	Uncertain
Myotonic dystrophy	19q13-2	Myotonin	Protein kinase	Expanded trinucleotide repeat sequence	100
Huntington's disease	4p16-3	Huntingtin	Unknown	Expanded trinucleotide repeat sequence	99
Charcot-Marie-Tooth disease type 1A	17p11-2	PMP-22	Myelin protein	Duplication Point mutations	<100 Few
Charcot-Marie-Tooth disease type 1B	1q22-23	Po	Myelin protein	Point mutations	100
Charcot-Marie-Tooth disease X-linked	Xq13	Connexin	Cell contact	Point mutations, deletions	80
Spinal and bulbar muscular atrophy (Kennedy's disease)	Xq13	Androgen receptor	Unknown on anterior horn cells	Expanded trinucleotide repeat sequence Point mutations	100 Unknown
Prion diseases	20pter-p12	Prion protein	Unknown	Point mutations	14
Familial Alzheimer's disease	21pter-q21	Amyloid precursor protein	Unknown	Point mutations	Unknown
Von Hippel Lindau disease	3p25-26	Unknown	Tumour suppressor	Deletions, insertions Point mutations	Unknown
Familial ALS (motor neuron disease)	21q22-1	Cu, Zn-superoxide dismutase (SOD 1)	Free-radical removal	Point mutations	Unknown
Spinocerebellar ataxia	6p24-p23	Unknown	Unknown	Expanded trinucleotide repeat sequence	Unknown
Fragile X syndrome A	Xq27	Unknown	RNA binding protein	Expanded trinucleotide repeat sequence Point mutations	<100 Few
Fragile X syndrome E	Xq29	Unknown	Unknown	Expanded trinucleotide repeat sequence	Unknown

DMD = Duchenne muscular dystrophy; BMD = Becker muscular dystrophy; ALS = amyotrophic lateral sclerosis.

lation of these in an individual increases the likelihood that clinical disease will result.¹⁰ Even in Duchenne and Becker muscular dystrophies (DMD and BMD) clustering of mutations (in exons 3, 8, 13, 43, 44, 47, 50, 51, and 52) has allowed the development of practical screening strategies (multiplex polymerase chain reaction) that optimise detection (up to 90% of all deletions) within ever-present financial constraints.¹¹

In a number of disorders the situation is less satisfactory: table 3 gives, for each disorder, an indication of the proportion of affected individuals in which a mutation in the relevant gene can be identified. It is important to realise, therefore, that failure to identify a mutation does not necessarily mean that the individual does not have the disease in question. It may be possible to use an alternative strategy to confirm the diagnosis and this is the case with DMD and BMD. The characterisation of dystrophin at the DNA level allows the prediction of structure and function as well as the synthesis of antibodies to several domains of the protein. These can be used histochemically to indicate whether a normal distribution of protein is present in association with the muscle cell membrane—that is, not DMD/BMD—or the amount is reduced or distribution discontinuous (BMD) or the protein is absent (DMD). This is important in giving a prognosis to the parents of boys with an Xp21 dystrophy identified in screening programmes of neonates¹² if the type of molecular change has not itself suggested the likely phenotype.¹¹ In cases where no mutation is found in dystrophin, and antibody studies show a normal amount and distribution of protein in muscle, alterna-

tive diagnoses need to be considered. Recent work has clarified the situation in the autosomal recessive "Duchenne-like" dystrophy and Fukuyama-type dystrophy, both of which arise as the result of abnormalities of proteins found in association with dystrophin.^{13,14} The genes for autosomal recessive and autosomal dominant limb-girdle dystrophy have been localised to specific chromosomes (5 and 15) but not yet identified and a molecular test is not therefore available for these.

Molecular testing may not be of significant clinical help in some situations, as in neurofibromatosis type 1, where only a small proportion of cases (20%) have an identifiable mutation in the neurofibromin gene.¹⁵ Fortunately the disease is clearly identifiable (fully penetrant) by the age of five years and an individual clinically normal after this can be reassured. Because of the low rate of mutation identification, prenatal testing for this disorder is still largely dependent on linkage analysis although its accuracy has improved with the availability of intragenic markers.

DISEASES ASSOCIATED WITH EXPANSIONS IN TRINUCLEOTIDE REPEAT SEQUENCES

The molecular abnormalities in six disorders in table 3 have been identified as arising from the expansion of a trinucleotide repeat sequence in association with the identified gene. The term *association* is chosen deliberately as the locations of these pathological sequences differs; in Huntington's disease and X-linked spinal and bulbar muscular atrophy, it lies within the coding sequence, whereas in myotonic dystrophy it is 3' (downstream) to the coding sequence. In the fragile

X mental retardation syndrome associated with a cytogenetically detectable fragile site at Xq27.3 (FRAXA), the expanded sequence lies 5' (upstream) to the coding sequence. It appears that in this disorder there is a critical degree of expansion beyond which there is silencing of gene expression¹⁶ possibly due to hypermethylation within the gene¹⁷ and it is this deficiency that is responsible for the disease phenotype. In the fragile X syndrome with a fragile site at Xq28 (FRAXE), there is amplification of a GCC repeat with associated hypermethylation of the neighbouring CpG island (a region associated with an active gene) implying a similar disease mechanism to that seen in FRAXA.¹⁸ In myotonic dystrophy it has been shown that the level of myotonin protein kinase messenger mRNA is reduced when the mutant allele has less than 200 repeats and very low when there are 800 repeats.¹⁹ In congenital cases it has been reported that not only is no mutant mRNA detectable but also that there is a reduced level of the normal paternal mRNA.²⁰ Another group has, however, reported an increase in the level of mutant mRNA in congenital cases.²¹ This conflict needs to be resolved.

A specific characteristic of this type of mutation is its instability; the length of the repeat sequence tends to increase as it undergoes successive meiotic passages (as it passes from one generation to the next). In myotonic dystrophy this successive expansion accounts for the long debated phenomenon of anticipation whereby the clinical manifestations (phenotype) become more severe in subsequent generations. It does not, however, account for the observation that congenital cases of myotonic dystrophy are always born of affected mothers.

These trinucleotide repeat amplifications are quantitative mutations and have been the subject of several studies examining correlations between degree of amplification and the age at onset of symptoms.²² The question arises as to whether it is possible to predict the age at disease onset and severity of the disease phenotype knowing the degree of expansion in the DNA sequence.²³ We would caution against attempting this in a specific clinical situation. Although there is a clear distinction between the degree of expansion in congenital cases of myotonic dystrophy and late onset/minimal manifesting individuals, such as cataract only, there is considerable overlap in other situations, even within the same family.

Phenotype prediction on the basis of degree of sequence expansion in Huntington's disease is fraught with even more difficulties: we have shown that the individual variation in age at onset for a given repeat number can range over a span of 20–30 years²⁴ and, in addition, that the size of the repeat sequence on the normal paternal allele has an effect in maternally transmitted disease.²⁵ This may account for the larger variability in age at onset in sibships with disease of maternal compared with paternal ori-

gin. As with any inherited disorder, the disease-specific mutation is acting within the individual's unique (with the exception of identical twins) genetic background which may contain several modifying loci. It could well be argued that the lack of a clinically useful correlation has potential benefits to an individual requesting presymptomatic testing: the psychological well being of an individual and his or her family would be unlikely to be helped by foreknowledge of a precise age at onset of this devastating disorder.

HEREDITARY MOTOR AND SENSORY NEUROPATHY TYPE I

In considering the applicability of molecular genetic testing, the case of type I hereditary motor and sensory neuropathy (HMSN I) illustrates some of both the benefits and limitations of using direct mutational analysis in the clinical situation. HMSN type I (also known as Charcot-Marie-Tooth disease type 1) is an autosomal dominant condition with extreme variability in phenotype. The manifestations range from significantly reduced peripheral nerve conduction velocity (median motor conduction velocity <38 m/s) with no clinical signs or symptoms in some gene carriers, to wheelchair dependence in later life in others. The disorder shows genetic heterogeneity with genes on chromosomes 17, 1 and another, as yet unidentified, locus. In an individual male case it is not possible clinically to distinguish CMT 1 from the X-linked form (CMTX).

In over 90% of cases of HMSN I the disorder arises as a result of the duplication of 1.1 million base pairs (1.1 Mb) of DNA on the proximal short arm of chromosome 17 (CMT at this locus is identified as CMT1A).²⁶ Within this region is a gene coding for peripheral myelin protein-22 (PMP-22), an integral membrane protein that plays an important role in arresting Schwann cell division. Mutations in this gene cause a peripheral neuropathy in the *trembler* mouse model.²⁷ Individuals with this duplication have three copies of the normal gene and the phenotype probably results from over-expression of the gene. In addition, at least two families have now been identified in which the CMT 1/HMSN I phenotype is associated with a point mutation in the DNA sequence of the PMP-22 gene.²⁸

Although this is of great interest to the developmental neurobiologist, it poses the problem of how much molecular analysis should we undertake if the first mutation screen (in this case analysis for the 1.1 Mb duplication) is negative? This may be of crucial importance to patients and their families who will often be concerned about the risk of the same condition arising in either siblings or offspring and to the clinician (geneticist or neurologist) counselling them on those risks.

We are of the opinion that screening for point mutations in duplication-negative HMSN I cases is not practicable as a clinical service (although it may be possible to find a research unit willing to undertake the analy-

sis). One situation that has been clarified with mutation analysis for CMT1/HMSN I is the case of the isolated, affected individual who may be considered to have the autosomal recessive form of HMSN I. Hoogendijk *et al*²⁹ have shown that in nine out of 10 isolated cases of HMSN I (in whom both parents were clinically and electrophysiologically normal), the disorder had arisen from a *de novo* duplication occurring in one of the parental germ cells. The risk to subsequent offspring of such an affected individual is clearly 50% (and not "minimal" as may previously have been counselled) whereas the risk to siblings of such patients would be minimal (this may be modified in some cases by gonadal mosaicism in the parent) rather than the previously counselled 25%. Counselling of a duplication-negative isolated male case remains difficult in view of the possibility that he has the X-linked form of CMT. Hayasaka *et al*³⁰ have recently reported the identification of point mutations in the gene for Po, another integral membrane protein of peripheral nervous system myelin, in individuals with CMT1b. Where this complexity of situation occurs there is much to be said for the continued application of standard clinical screening protocols (measurement of motor nerve conduction velocities and sensory nerve action potentials) for individuals at risk in addition to direct molecular analysis.

Considerations when requesting mutation analysis

In myotonic dystrophy, Huntington's disease and Charcot-Marie-Tooth type 1 (HMSN I) the success of mutation analysis makes it tempting to expand the range of the clinical picture that prompts the dispatch of a blood sample for "DNA analysis". We have already stated our view that, before any sample is taken, the patient should be made aware of the potential genetic implications of a "positive" result. This may not cause undue anxiety if the individual believes other family members show similar signs already, or he or she perceives the disorder not to be unduly serious.

Three disorders merit closer examination in considering the clinical spectrum that should prompt a "DNA analysis": Huntington's disease; Alzheimer's disease; and prion dementia. All may manifest with progressive dementia with or without additional features. Should we therefore screen all cases of dementia for the specific mutations associated with these disorders? Several problems arise in considering this.

Firstly, there is the question of consent. Can the patients be made aware of the implications of a positive result or should their families make the decision on their behalf? After all it is they (the family) who will be most affected by such a result.

Secondly, the sensitivity of the analysis should be considered. In those situations where we believe this to be approaching

100% (Huntington's disease) the family can be reassured by a negative result. In familial Alzheimer's disease, however, there is genetic heterogeneity, and the available molecular analysis (looking for point mutations in the APP gene) would screen only one gene accounting for a small proportion of cases.³¹ A negative result would not therefore exclude a diagnosis of familial Alzheimer's disease. Screening DNA from patients with dementia for mutations in the prion protein gene associated with Gerstmann-Sträussler syndrome and familial Creutzfeldt-Jakob disease would, superficially, seem reasonable in view of the reported cases where dementia is the only manifestation.³²

The implications for the siblings and offspring of a patient with an identifiable prion mutation are, however, less clear. Whereas an asymptomatic gene carrier for Huntington's disease has approaching a 100% likelihood of developing clinical manifestations (barring other causes of death), there are currently only limited data with which to counsel an asymptomatic carrier of a prion gene mutation; Collinge *et al* give an empiric risk of 95%.³³ Any reservations on the application of these mutation analyses in a demented patient would be removed should an effective treatment become available.

There have been no reservations, however, in applying molecular analyses of the dystrophin gene and its product, despite the knowledge that a positive result identifies an untreatable disorder. Hoffman *et al*³⁴ have shown that at least 10% of women with a myopathy and raised plasma creatine kinase concentration have an identifiable abnormality in the dystrophin gene or the dystrophin protein, or both. The perceived benefit that these women and their families can now be counselled on the appropriate risks of recurrences would appear to have been justification enough for testing.

The availability of analyses for the trinucleotide repeat amplification in the androgen receptor gene in bulbospinal neuronopathy³⁵ and mutations in the copper/zinc superoxide dismutase gene (SOD1)³⁶ in some families with autosomal dominant motor neuron disease, makes feasible the screening of isolated individuals (especially males) with motor neuron disease. We are of the opinion that, whereas screening for the androgen receptor gene trinucleotide repeat expansion is valuable (a normal result excludes Kennedy's disease), SOD1 analysis in the absence of a significant family history is not an appropriate use of laboratory services.

Table 4 shows the mutation analyses that we consider should be available to neurologists as part of a regional molecular genetic service and the clinical situations in which we feel the request is appropriate. Fragile X (type A) mutation analysis is usually requested by paediatricians when faced with a child with developmental delay but could be added to this list. It would however, add considerably to the workload and would require appropriate additional staff.

Table 4 Clinical indications for specified mutation analyses

Mutation analysis	Clinical indication
Dystrophin gene	"Limb girdle" dystrophy with raised CK *Raised neonatal creatine kinase
CMT1A duplication	Demyelinating neuropathy
Myotonin-kinase CTG repeat	Muscular dystrophy with myotonia
IT15 CAG repeat	Adult onset chorea Juvenile rigid syndrome
LHON mutations	Acute bilateral optic atrophy
MELAS/MERRF mutations†	Myoclonic epilepsy, proximal myopathy with appropriate histology, young stroke victims

*Ethically approved research protocol.

†On DNA extracted from blood and muscle.

LHON = Leber's hereditary optic neuropathy; MELAS = mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes; MERRF = myoclonus epilepsy with ragged-red fibres.

The issue of which cases should be referred to a clinical geneticist in conjunction with the request for a specific mutation analysis should be addressed locally, taking into consideration the available staffing levels (see below) and the specific interests of the clinicians. Access to diagnostic molecular services should not be limited by the availability of a clinical geneticist, although it should be a standard of acceptable practice that the appropriateness of the request for mutation analysis should be discussed with a genetics colleague whenever possible.

Once a positive result has been generated the clinician is duty bound to transmit this information to the patient and, with his or her consent, to the family. Clinical neurologists will often find this a most useful time to engage the help of their clinical genetics colleagues, if they were not approached initially to advise on what molecular test may have been applicable. Extended family counselling on the nature and implications of a specific diagnosis is often time consuming and may involve other health professionals such as specialist genetics nurses and clinical psychologists if presymptomatic testing is requested by siblings or offspring.

SITUATIONS WHERE MUTATION ANALYSIS IS INAPPROPRIATE

We are also aware of the situations where a molecular DNA test should not be undertaken. The most obvious situation is where the patient does not want it. This is no different from an individual declining a test for HIV carrier status. Most other situations occur where presymptomatic testing is requested and these will usually be directed towards the geneticist. Practising neurologists should be aware of these situations, however, as family members may approach them initially, following the diagnosis of a heritable disorder in the patient. Occasionally, an individual at risk for an inherited disorder may approach a neurologist with symptoms unrelated to the genetic disease. In this situation it would not be appropriate to offer specific mutation analysis. What is less clear is the merit of mutation analysis in the presence of vague symptoms which may be disease related, such as clumsiness or forgetfulness, where there is a family history of Huntington's disease. The identification of a disease-specific mutation in this situation may

not offer a definitive explanation for the individual's symptoms.

Many inherited neurological disorders have onset in mid to later life and presymptomatic testing of children for these is not appropriate.³⁷ This would change should effective therapy to prevent clinical disease become available. Individuals should not be under pressure from third parties, such as insurance companies, to test and those requesting testing should be made aware of the possible interests of such parties in the outcome. It may be useful to consider what impact a positive test would have on one's own family and social situation to gain an appreciation of the possible consequences for the individual in question.

Susceptibility genes

This article has focused on those neurological disorders whose aetiology is primarily "single gene" and where advances in our knowledge have followed the isolation of that gene. In many of the most prevalent neurological disorders (multiple sclerosis, idiopathic epilepsy, stroke) there may be a significant genetic component, as recognised from familial clustering of cases or from twin studies, but where true mendelian inheritance is not seen. Attention is now focusing on the identification of "susceptibility genes" which may predispose an individual to the development of these conditions. Harding *et al*³⁸ have proposed that mitochondrial loci may be implicated in the susceptibility to develop multiple sclerosis. It has long been recognised that there is a higher frequency of specific HLA haplotypes in individuals with multiple sclerosis, suggesting additional immunological factors (reviewed in reference 39). It is not known how relevant any single susceptibility locus will turn out to be, nor how amenable to modification this predisposition will be in an individual.

We are in no doubt, however, that individuals with these disorders and their families will in future expect to receive counselling on the genetic contribution to disease susceptibility and advice on strategies for modifying their risks. We are equally convinced that society will require considerable education on the relevance of these factors, if individuals who have been identified to be at increased risk are not to be discriminated against in education, employment, and insurance. Legislative intervention may be necessary to avoid the creation of a genetically stigmatised subpopulation.

Applying molecular analysis

SAMPLES

Most analyses are carried out on DNA isolated from peripheral blood lymphocytes collected in containers with EDTA as an anticoagulant. A sample of 20 ml of blood provides sufficient DNA for all analyses. Where polymerase chain reaction technology is used, considerably less is required, so that

the dried blood spot on a Guthrie card may be adequate. Some mutations may be tissue specific (such as some mitochondrial gene mutations) and the appropriate sample will be required if a false-negative result is to be avoided. Transportation of blood using the fastest available postal service (approximately two days delay) is adequate provided that arrangements have been made with the recipient laboratory to expect the sample. Tissue such as muscle biopsy specimens may require snap freezing in liquid nitrogen, especially if RNA analysis is required, and again this should be discussed with the unit undertaking the analysis.

SERVICES

The availability of a comprehensive molecular genetic laboratory service is essential if one is to make optimal clinical use of the advances outlined in this review. The pace at which mutation analysis is becoming feasible for genetic disorders in general, precludes any single diagnostic unit from providing such a service and many laboratories have implemented consortium arrangements to enable the widest possible range of services to be available. Single gene neurological disorders do, however, account for 54% of all clinical referrals to regional genetic units⁴⁰ and it would seem appropriate that a core molecular neurogenetic diagnostic service should be available at a regional level.

We feel that the minimal provision should include molecular analyses for the trinucleotide repeat expansions associated with myotonic dystrophy and Huntington's disease, the CMT1A duplication, a deletion screening protocol for Duchenne and Becker muscular dystrophies, a core mitochondrial mutation screen (see table 4) and linkage analysis for the autosomal recessive spinal muscular atrophies of childhood. The workload generated at a regional level should be able to be handled by two trained laboratory scientific officers working alongside colleagues carrying out other analyses. Additional analyses will generally be available by negotiation with other units. The Laboratory and National Consortium Directory, compiled by the Clinical Molecular Genetics Society, lists by disease and centre the services available throughout the United Kingdom. This publication also indicates the target times by which a result should be available (these vary by disease and centre).

The provision of a clinical service to complement the molecular analyses is essential. As we discussed above, it will often be necessary to undertake extended family counselling when a genetic diagnosis is made. Most districts will have access to the services of a clinical geneticist and a specialist genetic nurse, who will be able to provide such a service. We feel that, in addition, there should be, at a regional level, the provision of a clinical specialist with training in both clinical neurology and clinical and molecular genetics. This individual would be able to offer an optimal

clinical service for patients and colleagues and provide informed interpretation of relevant molecular DNA analyses.

Conclusions

Molecular genetic studies have begun to elucidate the pathobiological mechanisms underlying many of the inherited neurological disorders. Improved diagnostic accuracy enables a more accurate prognosis to be given to the patient and facilitates appropriate genetic counselling for the family unit. It is likely that more effective therapy will follow for some of these disorders but the prospect of a "cure", as perceived by the patient, remains a matter of speculation. This should not prevent individuals and their families benefiting from the advances made to date.

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