NEUROLOGICAL INVESTIGATIONS

Diagnosis of inherited metabolic disorders affecting the nervous system

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The number of metabolic disorders that can produce neurological symptoms is daunting. The clinician cannot simply send off to the laboratory a blood sample and ask for a metabolic "screen" that will detect a hypothetical metabolic abnormality. The range of possible conditions must be narrowed to the most likely before deciding which investigative approach should be taken. Most biochemical disorders encountered by neurologists are genetically determined. Thus one of the most important elements of the history is the family history. It is not sufficient to ask "has anyone in your family had a neurological problem?". The clinician must be aware of the different forms of inheritance patterns (autosomal dominant, autosomal recessive, X linked, mitochondrial) and must obtain enough information to construct a meaningful family tree. Directed questions must be asked about siblings as well as parents, grandparents, uncles, aunts, cousins, and children. Table 1 lists features that characterise single gene inheritance patterns.¹⁻³

Most of the clear cut genetic diseases are due to single gene abnormalities. There are several different mechanisms, however, that produce abnormal genes. A *point mutation* of a single DNA nucleotide can result in a different amino acid being coded for in the resulting polypeptide or protein. There can be *deletion* of a segment of a gene or *insertion* of one or more nucleotides. *Duplication* of a gene has been reported in Charcot-Marie-Tooth disease type 1A. Unstable expansions of portions of a gene (trinucleotide repeats) are being increasingly found in autosomal

dominant neurodegenerative diseases. The pace of new discoveries in medical genetics is incredibly rapid. Many of the new discoveries have led to diagnostic methods that were unanticipated only a few years ago.

Certain terms used by medical geneticists should be familiar to neurological practitioners. *Anticipation* refers to a disease beginning earlier and often being more severe in succeeding generations. In some autosomal dominant disorders, such as myotonic dystrophy and spinocerebellar ataxia 1, this phenomenon seems to be related to the length of an expanded trinucleotide repeat in the abnormal gene.⁴ *Penetrance* refers to the proportion of subjects with the abnormal gene who will develop symptoms if they live long enough. The degree of *expression* of a genetic disease refers to the variation of severity of the phenotype that is seen in a patient population. *Mosaicism* refers to variation among different cells and tissues in the chromosome complement. This occurs normally in women due to *lyonisation*, in which one of each cell's two X chromosomes is randomly inactivated. Mitochondrial disorders (see later) are associated with *heteroplasmy*, a term that refers to variation in the proportion of normal or genetically abnormal mitochondria in different tissues.

In *autosomal dominant* disorders, multiple generations are usually affected, although this might not have occurred if the affected patient represents a new mutation. Male to male transmission only occurs with autosomal dominant transmission. Each child of an affected parent will have a 50% chance of having or not having the abnormal gene.

Autosomal recessive disorders occur when expression of the disease requires the abnormal gene to be inherited from both parents, so that the affected person's cells have two abnormal alleles. Many autosomal recessive disorders are associated with defective enzymes. The low level of enzyme activity often leads to accumulation of the enzyme substrate with resultant toxicity to susceptible cells. The carrier parents seldom manifest symptoms because the normal gene codes for normal enzyme that is active enough to prevent substrate accumulation. Consanguineous marriages between cousins are more common in families with autosomal recessive diseases.

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Table 1 Some characteristics of single gene inheritance

Type of inheritance	Characteristics
Autosomal dominant	Multiple generations affected Father to son transmission (rules out X linked and mitochondrial inheritance patterns) Males and females equally affected
Autosomal recessive	Only siblings affected Parental consanguinity common Males and females equally affected
X Linked	Never father to son transmission Transmission through unaffected mothers Only males affected (rare exceptions) 50% risk to sons of carrier women All daughters of affected men are carriers
Mitochondrial	Maternally inherited Never father to child transmission Both sons and daughters can be affected

Adapted from table 9-1.³

X Linked disorders are due to abnormal genes located on the X chromosome. Clinical disease characteristically occurs in males who have inherited the abnormal gene from a carrier mother. Occasionally the mother or daughter with one normal and one abnormal gene will manifest symptoms, which almost always are milder than in the affected son or father, who has only one X chromosome. Male to male transmission cannot occur because a son receives the X chromosome from his mother.

Mitochondrial disorders are due to abnormalities in genes (deletions, point mutations) located in mitochondrial DNA. Both male and female mitochondria are derived from the ovum rather than the sperm. Both males and females can be affected by mitochondrial disorders, but father to child transmission does not occur. Because tissues and cells vary in the proportion of normal and abnormal mitochondria they carry (*heteroplasmy*), the expression of the disorder in different tissues and in different subjects can be extremely variable.

This contribution will not include much discussion of diseases that primarily affect muscle or nerve, as these have been the subjects of previous articles in this series. Some metabolic conditions, however, including metachromatic leukodystrophy and certain mitochondrial disorders that affect both central and peripheral structures, will be included. Non-genetic metabolic conditions such as hypoglycaemia, hepatic encephalopathy, deficiency diseases, and electrolyte disorders will not be discussed. Emphasis will be on conditions that are seen in adults by neurologists but many of these disorders will be variants of diseases that usually have their first manifestations in infancy or childhood. The first section contains brief discussions of categories of metabolic disease likely to be

encountered by neurologists. The second section contains a discussion of differential diagnoses of metabolic conditions that might produce particular complexes of neurological symptoms, including mental retardation, dementia, ataxias, motor neuron disease, movement disorders, and stroke.

Categories of metabolic disorders

AMINOACIDURIAS AND ORGANIC ACIDAEMIAS

These are the classic disorders of infancy and childhood associated with mental retardation and seizures. The *aminoacidurias* include phenylketonuria, maple syrup urine disease, homocystinuria, and other disorders listed in table 2.⁵ Screening the urine for amino acids is routinely done in clinical chemistry laboratories. Although it would be very unusual for first symptoms to occur in adult life, patients with treated phenylketonuria eventually will be seen as adults by neurologists.

Organic acidaemias, including methylmalonic acidaemia and propionic acidaemia, usually have their onset in infancy. Symptoms of dehydration are associated with ketoacidosis, hypoglycaemia, and hyperammonaemia. Diagnosis is made by urinalysis for organic acids, and can be confirmed by measuring activity of the abnormal enzyme in cultured fibroblasts.

These disorders are becoming better understood since the advent of modern molecular investigative techniques. For example, it is now known that there are three genes located on three different chromosomes (1, 6, 19) that code for the structural proteins unique to the deficient enzyme (branched chain ketoacid dehydrogenase complex [BCKAD]) in maple syrup urine disease. Mutations in different components of the complex can lead to variable clinical manifestations.⁶

Table 2 Aminoacidurias and organic acidaemias

Disease	Clinical features	Increased substances	Enzyme defect
Phenylketonuria	Mental retardation	Phenylalanine Phenylpyruvic acid	Phenylalanine hydroxylase
Maple syrup urine disease	Ketoacidosis Infantile seizures Characteristic odour Progressive encephalopathy Milder variants	Branched chain aminoacids (leucine, isoleucine, valine) (urine: branched chain ketoacids)	Branched chain ketoacid dehydrogenase complex
Homocystinuria	Ectopia lentis, stroke	Homocystine	Cystathionine β -Synthase
Non-ketotic hyperglycinaemia	Coma, progressive encephalopathy	Glycine	Glycine clearance system (4 proteins)
Methylmalonic acidaemia	Ketoacidosis Hyperammonaemia	Methylmalonic acid	Methyl-malonyl CoA mutase
Propionic acidaemia	Ketoacidosis Hyperammonaemia	3-hydroxypropionic acid	Propionyl CoA carboxylase
Isovaleric acidaemia	Ketoacidosis Hyperammonaemia	Isovaleric acid	Isovaleryl CoA dehydrogenase
Glutaric aciduria Type 1 Type 2	Progressive encephalopathy Spasticity, choreoathetosis Three groups—renal cystic dysplasia Hypoglycaemia, acidosis Muscle weakness	Glutaric, 3-hydroxyglutaric acids Glutaconic acid	Glutaric CoA dehydrogenase Electron transfer flavoprotein (ETF) and ETF ubiquinone oxidoreductase
γ -Hydroxybutyric aciduria	Mental retardation Seizures Developmental delay and motor abnormalities	γ -Hydroxybutyric acid	Succinic semialdehyde dehydrogenase

LYSOSOMAL DISORDERS

The term lysosome was proposed by DeDuve *et al* in 1955 for intracellular granules that were rich in hydrolytic enzymes.⁷ A lysosomal disease is associated with an abnormal enzyme that results in defective breakdown of the enzyme substrate.⁸ The product accumulates and eventually alters cell function. Because hydrolytic enzymes are present in many tissues, the diagnosis of the accumulated product or of the enzyme deficiency usually can be made with readily accessible tissues such as peripheral white blood cells or skin fibroblasts.⁹⁻¹¹ Although most of these disorders produce symptoms at a young age, some mutations, as in adult onset GM₁ gangliosidosis, lead to onset of symptoms later in life.¹²

Lysosomal disorders can be subcategorised according to the type of accumulated storage product. The two principal groups are lipid storage diseases and mucopolysaccharidoses. As these conditions have been more fully characterised, it has become clear that a great deal of heterogeneity exists among them, in many cases due to different point mutations in the gene. Thus these disorders should be considered in the differential diagnosis of atypical degenerative disorders.

Lipid storage diseases

Table 3 lists the lipid storage diseases that cause neurological dysfunction. These disorders are diagnosed either by finding high concentrations of substrate in tissues or by showing pronounced reduction in concentrations of the lysosomal enzyme responsible for degrading the accumulated storage substance.

In each disorder, subtypes have been delineated. In Gaucher's disease, associated with accumulation of glucocerebroside, and in Niemann-Pick disease, with sphingomyelin accumulation, hepatosplenomegaly is prominent in all types; however, only types 2 and 3 Gaucher's disease and types A and C Niemann-Pick disease are associated with neurological deterioration. Globoid leukodystrophy (Krabbe's disease), metachromatic leukodystrophy (MLD) and Tay-Sachs disease are inherited as autosomal recessive disorders and can be diagnosed by measurement of enzyme concentrations in peripheral

white blood cells or cultured skin fibroblasts. Clinical variants are found in each disorder. In MLD some mutations in the arylsulphatase A gene on chromosome 22 have been correlated with different phenotypes.¹³ Similarly, in Tay-Sachs disease over 20 mutations including nucleotide insertions, deletions, and substitutions on the α subunit (chromosome 15) and the β subunit (chromosome 5) of the hexosaminidase enzyme have been described.¹⁴ Late onset cases may develop weakness, fasciculations, ataxia, and psychiatric symptoms.¹⁵

GM₁ gangliosidosis, due to deficiency of the lysosomal enzyme β -galactosidase, can produce symptoms in infancy, childhood, or adult life. At least 16 mutations have been identified in the β -galactosidase gene.^{12 16 17} Severity of the diseases can be correlated with the amount of residual enzyme activity, the infantile form having no demonstrable activity and the adult forms having 4–8% of normal activity. Leinekugel *et al* found that 10–15% of β -hexosaminidase A and arylsulphatase A activities were sufficient to degrade substrate.¹⁸

The adult form of the disorder is slowly progressive and may produce gait disorders, involuntary choreoathetoid movements, bradykinesia, or dementia. Storage of GM₁ ganglioside can be much more pronounced in the striatum than in other parts of the brain by contrast with younger onset patients, in whom storage is more widespread.^{12 19}

Diagnosis is confirmed by finding much reduced lysosomal acid β -galactosidase activity in leucocytes. Single base mutations can be found on the β -galactosidase gene. The ⁵¹isoleucine (ATC) → threonine (ACC) mutation in the gene is common in Japanese adult onset GM₁ gangliosidosis.¹²

Mucopolysaccharidoses

These lysosomal disorders are associated with accumulation of complex glycosaminoglycans (mucopolysaccharides), due to genetic defects resulting in deficiencies of degradative enzymes.^{20 21} The stored substances include dermatan sulphate, heparan sulphate, keratan sulphate, and chondroitin 4/6 sulphates, which are detectable in the urine. Of the 12 described disorders all are autosomal recessive

Table 3 Common lipid storage diseases

Disease	Neurological symptom	Major lipid accumulated	Enzyme defect
Gaucher's	Hepatosplenomegaly, neurological deterioration in type 2 Dementia, seizures in type 3	Glucocerebroside	Glucocerebroside- β -glucosidase
Niemann-Pick	Hepatosplenomegaly, psychomotor deterioration in types A and C	Sphingomyelin Cholesterol (type C)	Sphingomyelinase (type A)
Globoid leukodystrophy (Krabbe's)	Progressive encephalopathy, seizures, spasticity, blindness	Galactocerebroside	Galactocerebroside β -Galactosidase
Metachromatic leukodystrophy	Progressive encephalopathy, neuropathy, ataxia, spasticity	Sulphatide	Arylsulphatase A
Fabry	Pain, rare strokes, X linked, renal insufficiency, skin lesions	Ceramide trihexoside	Ceramide trihexoside α -Galactosidase
Tay-Sachs	Progressive encephalopathy	GM ₂ ganglioside	Hexosaminidase A
Tay-Sachs variant	More rapid progression	Globoside and GM ₂	Hexosaminidase A and B
GM ₁ gangliosidosis	Dementia, progressive ataxia, choreoathetosis	GM ₁ ganglioside	β -Galactosidase 16 point mutations

except for Hunter syndrome which is X linked recessive. Among these conditions are Hurler's, Scheie's, Sanfilippo, Morquio's, and Maroteaux-Lamy diseases, and β -glucuronidase deficiency. Clinical signs such as coarse facial features, corneal clouding, hearing difficulties, hepatosplenomegaly, or joint abnormalities are usually detected during the first year of life. Later, developmental delay or mental regression may become apparent.

PEROXISOMAL DISORDERS

The peroxisome is an organelle that is found in most tissues. It contains over 40 enzymes including oxidases and catalase. Moser *et al* list 11 disorders attributable to defects in peroxisomal enzymes.²²⁻²³ These include disorders of peroxisome biogenesis (Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum's syndrome, and hyperpipecolic acidemia). The first two of these disorders can be associated with neonatal seizures, hypotonia, and developmental delay.

Of the disorders associated with peroxisomal enzyme abnormalities, X linked adrenoleukodystrophy is the most likely to be seen by neurologists.

Adrenoleukodystrophy and adrenomyeloneuropathy are X linked disorders and are associated with raised blood concentrations of very long chain fatty acids (VLCFAs) due to impaired peroxisomal β oxidation. The genetic defect seems to result from deletions in the peroxisomal membrane protein gene.²⁴ Some different phenotypes occur.²²⁻²⁶ Neurologists are most likely to encounter an adult patient with adrenomyeloneuropathy, with slowly progressive paraparesis as the main neurological manifestation and adrenocortical failure as a common occurrence. Adult onset cerebral adrenoleukodystrophy is manifested by dementia, confusional states, and sometimes progressive ataxia or psychiatric disturbances.²⁷ Symptom progression is usually slower in patients with adult onset.

Assays for VLCFAs (C24:0, C26:0) are carried out in specialised lipid laboratories using gas liquid chromatography or mass spectrometry. Although the vast majority of patients have raised plasma concentrations of these fatty acids, an occasional family will

have VLCFA concentrations within the usual "normal" range.²⁸ Molecular genetic analysis now makes it possible to detect point mutations within the adrenoleukodystrophy gene.²⁹

MITOCHONDRIAL ENCEPHALOPATHIES

A high index of suspicion is aroused for the presence of a disorder involving an abnormal mitochondrial gene if there are clinical features of Leigh's disease (subacute necrotising encephalomyelopathy), Kearns-Sayre syndrome (progressive external ophthalmoplegia, retinal pigmentary degeneration, and other symptoms), MELAS (mitochondrial encephalomyelopathy with lactic acidosis and stroke-like episodes), MERRF (myoclonic epilepsy with ragged red fibres), Leber's hereditary optic neuroretinopathy, or NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa).³⁰⁻³⁶ Various other features have been seen including short stature, deafness, diabetes mellitus, peptic ulceration, severe constipation, and migraine.

Mutations in mitochondrial DNA have been discovered in patients with several clinical presentations. Deletions of mitochondrial DNA are common in Kearns-Sayre syndrome.² In MELAS, point mutations include an A $\rightarrow$ G mutation at nt3243 in 80% of cases, as well as T $\rightarrow$ C mutations at nt3271 and at nt9957, and an A $\rightarrow$ G mutation at nt11084. An A $\rightarrow$ G nt8344 mutation has been found in MERRF, and a T $\rightarrow$ G or T $\rightarrow$ C mutation at nt8993 in Leigh's syndrome.³⁷⁻³⁸

Diagnostic laboratory tests include: (a) serum pyruvate and lactate concentrations; (b) muscle biopsy to assess for the presence of ragged red fibres, and as a source of mitochondria for DNA analysis; and (c) molecular genetic studies on blood or muscle in a specialised laboratory to assess for known mutations.

DISORDERS ASSOCIATED WITH EXPANDED TRINUCLEOTIDE REPEATS

One of the most exciting developments in neurology has been the discovery of neuro-genetic diseases in which the abnormal gene mutation results in expansion of a repeated sequence of trinucleotides. Table 4 lists many

Table 4 Disorders associated with unstable expanded trinucleotide repeats

Disorder	Chromosome	Number of repeats		Disease	Translation of repeat
		Extended trinucleotide	Normal		
Fragile X syndrome (FRXA)	X	CGG	6-50	Premutation: 52-200 Disease: 200 to >1000	No
Fragile X E (FRXE)	X	GCC	6-25	>200	No
Myotonic dystrophy	19q	CTG	<30	Premutation: 42-180 Disease: 200->1000	No
Bulbar spinomuscular atrophy (Kennedy's syndrome)	Xq11-12	CAG	17-26	40-52	Yes
Huntington's disease	4p16.3	CAG	11-34	37-121	Yes
Autosomal dominant spinocerebellar ataxias					
Spinocerebellar ataxia 1	6p22-23	CAG	19-36	42-81	Yes
Machado-Joseph disease	14q32.1	CAG	13-36	68-79	Yes
Dentatorubral-pallidolulysian atrophy (DRPLA)	12p12-ter	CAG	7-23	49-75	Yes

of the presently known disorders of this type.³⁹⁻⁵⁰ They are either X linked or autosomal dominant disorders and include the dominant spinocerebellar ataxias SCA1 and Machado-Joseph disease, Huntington's disease, fragile X syndrome, myotonic dystrophy, and spinal bulbar muscular atrophy (Kennedy's syndrome). In many of these disorders, the length of the expanded trinucleotide repeat is unstable. Succeeding generations have expansions of greater length which may be associated with earlier onset and more severe disease manifestations. It is likely that additional neurodegenerative disorders will be added to this list.⁴³

Genetics laboratories have the capability of definitively diagnosing the presence or absence of some of these disorders on a single sample of blood, using molecular genetic techniques. Commercial availability is limited at this time.

DISORDERS OF COPPER METABOLISM

Two diagnosable genetic disorders are associated with defects in copper metabolism: Menkes' disease and Wilson's disease.

Menkes' "kinky hair" (steely hair) disease is an X linked disorder with manifestations in early infancy.⁵¹⁻⁵² Infants feed poorly, become hypothermic, gain weight slowly, develop seizures, and show progressive neurological deterioration. They have colourless, friable hair which has a characteristic microscopic appearance. Danks *et al* suggested that the disease was due to a disorder of copper metabolism.⁵³ The gene has been isolated and is a copper transporting ATPase. Until recently, diagnosis if suspected clinically was established by demonstrating low ceruloplasmin and serum copper concentrations and abnormalities in fibroblast copper uptake.⁵⁴⁻⁵⁵ In the newborn, however, copper and ceruloplasmin are normally low, so reliable detection of abnormally low concentrations cannot be made until the third or fourth week of life. The diagnosis can be made by DNA analysis.⁵⁶

Wilson's disease is an autosomal recessive disorder due to an abnormal gene at q14.3 on chromosome 13. This gene codes for a copper transporting P-type ATPase that is presumably important for hepatic incorporation of copper into ceruloplasmin and for excretion of copper into bile.⁵⁷⁻⁵⁹ The enzyme is also expressed in the kidney. Twenty five mutations have been identified in the Wilson's disease gene, accounting for the great variability in clinical symptomatology.⁶⁰ The pathogenetic role of reduced synthesis or impaired function of the copper transporting protein ceruloplasmin is not clear.⁶¹ In the course of Wilson's disease, increased storage of copper occurs in liver, brain, cornea (Kayser-Fleischer ring in Descemet's membrane), and kidneys. Neurological and psychiatric symptoms can occur secondary to deposition of copper in the brain or as a result of hepatic encephalopathy due to copper induced liver damage. The diagnosis can and should be made before the onset of

symptoms in close relatives of affected patients.⁶²⁻⁶⁴ The diagnosis can be made with the assistance of the following laboratory findings.⁶³ (a) increased excretion of copper into the urine (normal <30 µg/24 h; Wilson's disease 100-1000 µg/24 h); (b) decreased serum concentration of total copper (normal 85-145 µg/dl); (c) decreased serum concentration of ceruloplasmin (normal range 25-45 mg/dl); about 5% of cases will have normal ceruloplasmin concentrations.⁶⁵

Symptomatic approach to the diagnosis of inherited metabolic disorders

MENTAL RETARDATION OR DETERIORATION

Fragile X syndrome

Over 100 X linked mental retardation syndromes are known at the present time.⁶⁶ Of these, fragile X syndrome is the leading genetic cause of mental retardation.⁶⁷ The syndrome is so named because of the instability of the X chromosome when incubated in folic acid deficient media.⁶⁸⁻⁶⁹ This was the first neurological disorder to be associated with an unstable trinucleotide repeat sequence.⁷⁰⁻⁷¹ Clinically, patients may have prominent ears, large testicles, high arched palates, and behavioural deficits. Definitive diagnosis can be made by the demonstration of an expanded trinucleotide CGG repeat sequence. Abnormality in the FMR1 gene results in lack of production of the FMR1 protein.

Diagnosis of mental deterioration or progressive encephalopathy in infants may require various metabolic tests if the suspected diagnosis is not already obvious.⁷² A metabolic screen of the urine carried out by a hospital laboratory usually includes a nitroprusside-cyanide test, a ferric chloride test, tests for ketoacids and mucopolysaccharides, and two dimensional amino acid chromatography. Serum analyses for amino acids and organic acids will usually be diagnostic for aminoacidurias and organic acidaemias. Studies to search for other disorders mentioned (Menkes' disease, lipid storage diseases) will require special testing by genetics laboratories.

JUVENILE OR ADULT PATIENTS PRESENTING WITH DEMENTIA

Laboratory investigations are of limited usefulness in dementia diagnosis. The most common dementing illnesses such as Alzheimer's disease are not yet diagnosable biochemically on a routine basis. In a few families with familial Alzheimer's disease, point mutations have been found in the gene on chromosome 21 that codes for the amyloid precursor protein (APP) gene.⁷³⁻⁷⁴ Most families, however, do not have these mutations. In young onset families, linkage analysis has located the gene defect to a locus on chromosome 14.⁷⁵ Ultimately it may be possible to diagnose the disorder by molecular genetic techniques. At present, diagnosis of the rare APP mutation can be carried out only by specialised research laboratories studying this disorder.

In some patients with progressive cognitive impairment, a high degree of suspicion based on clinical or radiological clues may justify carrying out further metabolic studies to confirm a suspected diagnosis (table 5). Vitamin B-12 deficiency rarely produces dementia alone but should be excluded.

Huntington's disease

The diagnosis of Huntington's disease can now be confirmed by commercially available DNA testing. The presence of an extended CAG repeat in the Huntington's disease gene on chromosome 4 establishes the diagnosis. A normal allele has less than 35 CAG repeats. The Huntington's disease gene has more than 38 repeats in 98–99% of cases.⁷⁶

Leukodystrophies

In addition to multiple sclerosis and progressive multifocal leukoencephalopathy, cerebral white matter can be affected by several metabolic disorders, with dementia as a major symptom. These include adult onset metachromatic leukodystrophy (MLD), adrenoleukodystrophy, Pelizaeus-Merzbacher disease, Krabbe's disease, and cerebrotendinous xanthomatosis (CTX). In each disorder, symptoms are progressive, usually over months to years. Inherited in an autosomal recessive manner, MLD would likely be associated with clinical or EMG/nerve conduction velocity evidence of a peripheral neuropathy. Adrenoleukodystrophy might be associated with adrenal insufficiency and, as it is an X linked disorder, would occur predominantly in males. Patients with cerebrotendinous xanthomatosis usually develop prominent xanthomas in large tendons such as the Achilles tendon.

Adrenoleukodystrophy—This disorder is characterised by varying modes of onset at different ages. Most affected people are male,

as the disorder is X linked. Some heterozygous women may, however, develop spastic paraparesis.²² The phenotypes delineated by Moser *et al* are: childhood cerebral (48%), adolescent cerebral (5%), adult cerebral (3%), adrenomyeloneuropathy (25%), Addisonian only (10%), asymptomatic (8%).²⁶ The disorder is suspected in children with learning disorders and dementia. Adrenomyeloneuropathy usually begins with progressive paraparesis.

Pelizaeus-Merzbacher disease—This disorder is X linked. Symptoms usually begin in infancy or childhood, but onset in early adulthood has been reported.⁷⁷ Symptoms include psychomotor delay and later, dementia, nystagmus, ataxia, spasticity, and involuntary movements. Mutations in the gene coding for proteolipid protein result in defective myelin. Mutations in the proteolipid protein gene can now be determined in genetics laboratories.^{78 79} Prenatal diagnosis is also possible.⁸⁰

Canavan's disease—Another rare leukodystrophy is Canavan's disease, characterised by infantile and juvenile forms with severe progressive neurological deterioration. Raised urinary *N*-acetylaspartic acid and deficiency of the enzyme aspartoacylase in skin fibroblasts confirm the diagnosis.⁸¹

Lipid storage diseases

Metachromatic leukodystrophy (MLD) and Krabbe's disease (globoid leukodystrophy), as well as other lipid storage diseases such as GM₁ gangliosidosis and type 3 Gaucher's disease can produce dementia as part of more generalised neurological deterioration. Urinary sulphatides will be abnormally increased in MLD. In the other conditions, confirmation of clinical suspicion will require white blood cell or fibroblast enzyme determinations carried out by a specialised laboratory.

Neuronal ceroid lipofuscinosis (Batten's disease and variants)—This is a disorder associated with storage of a complex lipopigment. The disease has not yet been characterised enzymatically, although linkage analysis has located the gene for the infantile form (CLN1) to chromosome 1p32, and that for the juvenile form (CLN3) to chromosome 16p12.^{82 83} Patients usually develop retinal degeneration, myoclonus, seizures, and dementia. Diagnosis is made by demonstrating accumulated storage product in buffy coat or in skin biopsies, which show curvilinear bodies or a "fingerprint" pattern on electron microscopy. The early onset forms are autosomal recessive. Both autosomal dominant and autosomal recessive inheritance has been reported in the adult form (Kufs' disease) which is not associated with pigmentary retinal degeneration.^{84 85}

ATAXIAS

Progressive ataxia can result from several conditions that have metabolic causes. The MRI will have assisted in diagnosing multiple sclerosis, cerebellar neoplasms, and the diagnoses of alcoholic cerebellar degeneration and

Table 5 Disorders associated with dementia

Disorder	Diagnostic test
Alzheimer's disease	In known families, genetics laboratory for DNA point mutation in the APP gene
B-12 deficiency	Serum B-12 level Schilling test
Huntington's disease	Genetics laboratory for DNA screen (CAG > 37 repeats)
Adrenoleukodystrophy	Lipid laboratory for plasma VLCFA
Pelizaeus-Merzbacher disease	Genetics laboratory for PLP gene
Canavan's disease	Urine for <i>N</i> -acetylaspartic acid Fibroblasts for aspartoacylase
Metachromatic leukodystrophy	Arylsulphatase A
GM ₁ gangliosidosis	β -galactosidase
Globoid leukodystrophy (Krabbe's)	Galactocerebrosidase
Cerebrotendinous xanthomatosis	Lipid laboratory for cholestanol
Ceroid-lipofuscinosis	EM of buffy coat for curvilinear bodies

VLCFA = very long chain fatty acids; PLP = proteolipid protein; APP = amyloid precursor protein; EM = electron microscopy.

paraneoplastic syndromes will have been considered.

Genetic causes are of special importance in this group of disorders. As yet the diagnosis of Friedreich's ataxia is based on clinical, not biochemical findings. The genetics laboratory can, however, assist in diagnosing several of these disorders, including spinocerebellar ataxia type I (SCA-1) and Machado-Joseph's disease.⁴⁴⁻⁸⁶

Ataxia-telangiectasia, an autosomal recessive disorder, is the most common cause of ataxia in children under the age of 10.⁷² Usually the diagnosis is evident clinically, with ataxia, nystagmus, choreoathetosis, and characteristic auricular and conjunctival telangiectases being evident. The abnormal gene on chromosome 11q22-23, which is important for DNA repair, has recently been identified.⁸⁷ The gene product is likely to be a phosphatidylinositol-3' kinase. Presumably more definitive DNA diagnostic testing will become available. Symptoms of ataxia-telangiectasia can begin in early adult life.⁸⁸ Some serum abnormalities are found in patients with this disorder, including raised α -fetoprotein in 95% of cases, alterations in serum immunoglobulins, and raised carcinoembryonic antigen concentrations.⁸⁹

Autosomal dominant cerebellar ataxias are being reclassified as their genetic defects are discovered. Abnormal genes have been found on chromosomes 6, 11, 12, 14, and 16.^{42-46, 86, 90-92} Dubourg *et al* sampled DNA from 88 families with inherited ataxias and from 16 patients with sporadic ataxia to determine the frequency of the SCA1 mutation on chromosome 6.⁴ Twelve of the families and none of the sporadic cases carried the SCA1 mutation (unstable expanded CAG repeat).

Clinical characteristics do not readily distinguish the subtypes of cerebellar ataxias.⁹³ Many patients will have additional signs such as extensor plantar responses, decreased vibration sense, ophthalmoplegias, and increased or decreased tendon reflexes. In SCA1, instability of the mutation is more common with male transmission and the age of onset of symptoms is lower in patients with a higher number of CAG repeats (anticipation).

Dentatorubral-pallidoluysian atrophy (DRPLA) is a rare autosomal dominant neurodegenerative disorder, usually classified under the ataxias. The disorder can be associated with myoclonus, chorea, dementia, and seizures. Juvenile and adult onsets are reported.⁹⁴ The disorder may be misdiagnosed as Huntington's disease.⁴⁸ The molecular defect is an expanded CAG repeat on the gene located on chromosome 12p and can be diagnosed by molecular analysis.⁴⁹⁻⁵⁰

Another disorder that may present with ataxia is *Cerebrotendinous xanthomatosis*. This condition is suspected in a patient who has tendon xanthomas, often in the Achilles tendons.⁹⁵ The diagnosis is confirmed by showing raised concentrations of cholestanol (dihydrocholesterol) in blood or tissue.⁹⁶ This

test will involve contacting a specialised lipid laboratory, but it is important to do, as the disease can be treated by giving chenodeoxycholic acid.⁹⁷ Several mutations of the gene on chromosome 2 that codes for the enzyme sterol 27-hydroxylase have been found in families with this disorder.⁹⁸⁻¹⁰⁰ Presymptomatic cases and heterozygotes can now be detected by molecular diagnostic techniques.¹⁰¹

Ataxia with isolated vitamin E deficiency (AVED)—Patients have been described with progressive ataxia and other features of Friedreich's disease, in which vitamin E concentrations were reduced in the absence of malabsorption.¹⁰² In the patient described by Stumpf *et al*, serum vitamin E concentrations were below 1.0 (normal 5-20) $\mu\text{g/ml}$ and could be restored to normal with an oral dose of 800 mg/day of DL- α -tocopherol.¹⁰³ This disorder is autosomal recessive. The gene maps to chromosome 8q13.^{104, 105} Affected patients have mutations in the α -tocopherol transfer protein resulting in impaired ability to incorporate α -tocopherol into lipoproteins.¹⁰⁶

A- β -lipoproteinaemia—Patients with this disorder usually develop steatorrhea in infancy followed in the second decade by progressive ataxia and peripheral neuropathy.⁷² The patients have absence of serum β -lipoproteins and very low concentrations of α -tocopherol. Dietary supplementation with high doses of vitamin E (100 mg/kg/day) may arrest the neurological manifestations.¹⁰⁷ This condition is associated with defective genes coding for the larger subunit of the microsomal triglyceride transfer protein (MTP), resulting in abnormal VLDL secretion and impairing delivery of vitamin E and other fat soluble substrates.¹⁰⁸

The autosomal recessive disorder *Friedreich's ataxia* is associated with an as yet unidentified genetic defect on chromosome 9. Because of clinical similarities to AVED, it has been suggested that the Friedreich's ataxia gene may also be involved in α -tocopherol metabolism.¹⁰⁹

Ataxia associated with mitochondrial disorders—Ataxia can be a feature of several of the mitochondrial disorders, especially the MERRF and MELAS syndromes, and the rare syndrome termed NARP.³⁴ The presence of retinitis pigmentosa should raise the index of suspicion for NARP, which is sometimes due to a point mutation at bp8993 resulting in substitution of arginine for leucine in subsequence 6 of the mitochondrial H⁺ATPase. Patients with this disorder may have seizures, muscle weakness, mental retardation and long tract signs with symptoms beginning from infancy to late adulthood.

MOTOR NEURON DISEASE

Most patients with suspected amyotrophic lateral sclerosis (ALS) have no known biochemical defect. Kennedy's syndrome (spinal bulbar muscular atrophy) may clinically resemble the bulbar form of ALS, though progression is usually slow. A genetics

laboratory can confirm this diagnosis by determining the presence of an expanded CAG repeat on the X chromosome (table 4).

About 20% of cases of familial ALS have been discovered to have one of several mutations of the gene on chromosome 21 that codes for the Cu/Zn binding superoxide dismutase enzyme (SOD).¹¹⁰⁻¹¹²

In patients with mutations in this gene, the concentration and specific activity of Cu/Zn SOD are reduced in erythrocytes by about 50%¹¹³; however, these changes do not correlate with disease severity. Detection of the Cu/Zn SOD mutation requires DNA analysis of a blood sample by a genetics laboratory studying this disorder.

MOVEMENT DISORDERS (TABLE 6)

Wilson's disease

Patients with Wilson's disease can develop symptoms of hepatic or neurological dysfunction. Symptoms that may bring a patient to a neurologist include involuntary movements (tremor, dystonia, chorea, spasms), and behavioural and personality changes. Psychiatric symptoms account for a high proportion of presenting complaints.⁶⁴ A Kayser-Fleischer ring may be seen on examination of the cornea.

Wilson's disease is commonly but not invariably associated with reduction in serum ceruloplasmin concentrations (normal range 25-45 mg/dl). Total serum copper concentrations may not be raised. "Free" serum copper can be calculated by subtracting from the total copper ($\mu\text{g/dl}$), that amount bound to ceruloplasmin (multiply by 3 the ceruloplasmin concentration (in mg/dl) to obtain the bound copper in $\mu\text{g/dl}$). The free copper concentration should be $<10-15 \mu\text{g/dl}$. Laboratory standards are variable, however, so it is recommended that a 24 hour urinary copper be obtained. In Wilson's disease copper excretion is $>100 \mu\text{g/24 h}$ (normal $<30 \mu\text{g/24 h}$).

In an asymptomatic patient at risk for Wilson's disease, liver biopsy may be necessary to detect increased copper content in the liver (patients with Wilson's disease have $>200 \mu\text{g/g}$ wet weight).

Other metabolic causes of dystonia

Adult onset of dystonia can occur in GM₁ gangliosidosis.¹² The disorder is autosomal recessive and is diagnosed by showing pronounced reduction of activity of the enzyme β -galactosidase which can be measured in leucocytes. Progressive *dopa responsive dystonia* has been recently reported to be associated with mutations in a gene on chromosome 14q22.1-22.2 which codes for the enzyme GTP cyclohydrolase 1.¹¹⁴

Other movement disorders

Choreic movements are characteristic of Huntington's disease, which is discussed in the section on dementia. Another disorder in which chorea can occur is ataxia-telangiectasia, discussed in the section on ataxias.

STROKE

Metabolic causes of stroke are rare, but must be considered in children and young adults presenting with an acute ischaemic event. Disorders to consider include MELAS syndrome, homocystinuria, sulphite oxidase deficiency, and Fabry's disease. MELAS syndrome can be associated with stroke-like episodes in young adults.¹¹⁵ Diagnosis may be difficult (see section on mitochondrial disorders) and requires strong suspicion and communication with a genetics laboratory specialising in mitochondrial disorders.

Homocystinuria is much more common than *sulphite oxidase deficiency*. Both disorders may be suspected by the presence of ectopia lentis. Chronic homocysteine infusions in baboons produce sustained endothelial cell loss and result in accelerated atherosclerosis.¹¹⁶ It has been suggested that moderate homocysteinaemia in otherwise normal subjects may be a risk factor for atherosclerotic stroke.¹¹⁷ The disorder is due to an inborn error of cystathionine synthase. Urinary amino acid screen will detect raised concentrations of homocystine. *Sulphite oxidase* deficiency can be associated with stroke-like episodes in infancy and childhood.¹¹⁸ Amino acid screening of the urine must be done carefully and on fresh urine, and should show the presence of S-sulphocysteine.¹¹⁹ S-sulphocysteine and sulphite are also detectable in plasma.¹²⁰ Basal ganglia infarction in children, and adult onset chorea and dementia have been reported in propionic acidemia.^{121 122}

Fabry's disease

This X linked lysosomal storage disease is associated with accumulation of trihexose ceramide due to deficiency in the enzyme ceramide trihexoside α -galactosidase (table 3). At an early age, patients develop pinpoint skin lesions, especially on the abdomen. Trihexose ceramide accumulates in blood vessel walls, in kidney, and in myocardium. Death is usually due to renal or heart failure. Patients have been reported to develop episodic stroke-like symptoms and headaches. Small infarcts have been found in cerebral hemispheres, associated with proliferative

Table 6 Metabolic disorders associated with involuntary movements

Disease	Type of movement	Associated features	Diagnostic test
Huntington's disease	Chorea Dystonia (juvenile)	Dementia	Molecular analysis for CAG repeat expansion
Wilson's disease	Tremor, dystonia chorea	Hepatic insufficiency Kayser-Fleischer ring	Ceruloplasmin, serum and urine copper
Dentatorubral pallidolusian atrophy (DRPLA)	Chorea	Ataxia, myoclonus, dementia	Molecular analysis for CAG repeat expansion
GM ₁ gangliosidosis	Dystonia, Choreoathetosis	Gait disturbance	Leucocyte lysosomal acid β -galactosidase activity
Dopa responsive dystonia	Dystonia	Levodopa responsiveness	Research laboratory for GTP cyclohydrolase mutation
Ataxia-telangiectasia	Choreoathetosis	Ataxia, auricular, and conjunctival telangiectases	Decreased α -fetoprotein, immunoglobulin abnormalities

changes in vessel intima and media, presumably due to glycolipid deposition.¹²³

Summary

Knowledge of the molecular causes for genetic diseases that affect the nervous system is rapidly expanding. Especially striking has been the finding in several autosomal dominant neurodegenerative disorders that unstable expansions of trinucleotide repeats are responsible for the genetic disorder and that the length of the repeat can be correlated with the age of onset and the severity of symptoms. Phenotypic heterogeneity in many disorders associated with enzyme deficiencies can often be linked to the amount of residual enzyme activity occurring with different gene mutations.

Making a specific diagnosis of a neurological disorder associated with genetically determined metabolic defects requires access to a laboratory that can assist in arranging for appropriate testing to be carried out. In some disorders such as the aminoacidurias diagnostic metabolic studies can be performed in hospital clinical chemistry laboratories. In others, such as the lysosomal storage diseases, a laboratory that carries out special lipid analyses and white blood cell enzyme assays will be necessary. DNA mutational analyses are becoming commercially available for diagnosing many disorders such as mitochondrial diseases and those conditions associated with expanded trinucleotide repeats. It may be necessary to contact individual research laboratories when confronted with a disorder that has been newly discovered or that is very rare. A computerised directory of specialised laboratories that perform disease specific testing for genetic disorders should be useful in choosing the appropriate diagnostic or research laboratory.

- 1 Beaudet AL, Scriver CR, Sly WS, *et al.* Genetics and biochemistry of variant human phenotypes. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic basis of inherited disease*. 6th ed. New York: McGraw-Hill, 1989:3-53.
- 2 Harding AE. The DNA laboratory and neurological practice. *J Neurol Neurosurg Psychiatry* 1993;56:229-33.
- 3 Bird TD, Farrell DF. Genetics and neurology. In: Swanson PD, ed. *Signs and symptoms in neurology*, Philadelphia: JB Lippincott, 1984:345-77.
- 4 Dubourg O, Durr A, Cancel G, *et al.* Analysis of the SCA1 CAG repeat in a large number of families with dominant ataxia: clinical and molecular correlations. *Ann Neurol* 1995;37:176-80.
- 5 Nyhan WL, Haas R. Inborn errors of amino acid metabolism and transport. In: Rosenberg RN, Prusiner SB, DiMauro S, *et al.*, eds. *The molecular and genetic basis of neurological disease*, Boston: Butterworth-Heinemann, 1993:145-65.
- 6 Cox RP, Chuang DT. Maple syrup urine disease: clinical and molecular genetic considerations. In: Rosenberg RN, Prusiner SB, DiMauro S *et al.*, eds. *The molecular and genetic basis of neurological disease*. Boston: Butterworth-Heinemann, 1993:189-207.
- 7 de Duve C, Pressman BC, Gianetto R, *et al.* Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem J* 1955;60:604-17.
- 8 Hers HG. Inborn lysosomal diseases. *Gastroenterology* 1965;48:625-33.
- 9 Brady RO. Biochemical genetics in neurology. *Arch Neurol* 1976;33:145-51.
- 10 Kolodny EH. Current concepts in genetics. Lysosomal storage diseases. *New Engl J Med* 1976;294:1217-20.
- 11 Suzuki K. Enzymatic diagnosis of sphingolipidoses. *Methods in enzymology* 1987;138:727-62.
- 12 Yoshida K, Ikeda S, Kawaguchi K, Yanagisawa N. Adult GM₁ gangliosidosis: immunohistochemical and ultrastructural findings in an autopsy case. *Neurology* 1994;44:2376-82.
- 13 Kolodny EH. Metachromatic leukodystrophy and multiple sulfatase deficiency: sulfatide lipidosis. In: Rosenberg RN, Prusiner SB, DiMauro S *et al.*, eds. *The molecular and genetic basis of neurological disease*. Boston: Butterworth-Heinemann, 1993:497-503.
- 14 Kolodny EH. The GM2 Gangliosidosis. In: Rosenberg RN, Prusiner SB, DiMauro S *et al.*, eds. *The molecular and genetic basis of neurological disease*. Boston: Butterworth-Heinemann, 1993:531-40.
- 15 Navon R, Argov Z, Frisch A. Hexosaminidase A deficiency in adults. *Am J Hum Genet* 1986;24:179-96.
- 16 Yoshida IC, Oshima A, Sakuraba H, *et al.* GM₁ gangliosidosis in adults: clinical and molecular analysis of 16 Japanese patients. *Ann Neurol* 1992;31:328-32.
- 17 Nishimoto J, Nanba E, Inui K, *et al.* GM1-gangliosidosis (genetic β -galactosidase deficiency): identification of four mutations in different clinical phenotypes among Japanese patients. *Am J Hum Genet* 1991;49:566-72.
- 18 Leinekugel P, Michel S, Conzelmann E, Sandhoff C. Quantitative correlation between the residual activity of β -hexosaminidase A and arylsulfatase A and the severity of the resulting lysosomal storage disease. *Hum Genet* 1992;88:513-23.
- 19 Goldman JE, Katz D, Rapin I, *et al.* Chronic GM₁-gangliosidosis presenting as dystonia: I. clinical and pathological features. *Ann Neurol* 1981;9:465-75.
- 20 Neufeld EF, Lim TW, Shapiro LJ. Inherited disorders of lysosomal metabolism. *Ann Rev Biochem* 1975;44:357-76.
- 21 Matalon R, Kaul R, Michals K. The mucopolysaccharidoses and the mucopolipidoses. In: Rosenberg RN, Prusiner SB, DiMauro S *et al.*, eds. *The molecular and genetic basis of neurological disease*, Boston: Butterworth-Heinemann, 1993:401-9.
- 22 Moser HW, Moser AE, Singh I, O'Neill BP. Adrenoleukodystrophy: survey of 303 cases: biochemistry, diagnosis and therapy. *Ann Neurol* 1984;16:628-41.
- 23 Moser HW. Peroxisomal disorders. In: Rosenberg RN, Prusiner SB, DiMauro S, *et al.*, eds. *The molecular and genetic basis of neurological disease*. Boston: Butterworth-Heinemann, 1993:351-87.
- 24 Mosser J, Douar AM, Sarde CO, *et al.* Putative X-linked adrenoleukodystrophy gene shows unexpected homology with ABC transporter. *Nature* 1993;361:726-30.
- 25 van Geel BM, Assies J, Weverling GJ, Barth PG. Predominance of the adrenomyeloneuropathy phenotype of X-linked adrenoleukodystrophy in the Netherlands: A survey of 30 kindreds. *Neurology* 1994;44:2343-6.
- 26 Moser HW, Moser AB, Smith KD, *et al.* Adrenoleukodystrophy: phenotypic variability and implications for therapy. *J Inher Metab Dis* 1992;15:645-64.
- 27 Farrell DF, Hamilton SR, Knauss TA, *et al.* X-linked adrenoleukodystrophy: adult cerebral variant. *Neurology* 1993;43:1518-22.
- 28 Kennedy CR, Allen JT, Fensom AH, Steinberg SJ, Wilson R. X-linked adrenoleukodystrophy with non-diagnostic plasma very long chain fatty acids. *J Neurol Neurosurg Psychiatry* 1994;57:759-61.
- 29 Vorgerd M, Fuchs S, Tegenthoff M, Malin J-P. A missense point mutation (Ser515Phe) in the adrenoleukodystrophy gene in a family with adrenomyeloneuropathy: a clinical, biochemical, and genetic study. *J Neurol Neurosurg Psychiatry* 1995;58:229-31.
- 30 Crimmins D, Morris JGL, Walker GL, *et al.* Mitochondrial encephalomyopathy: variable clinical expression within a single kindred. *J Neurol Neurosurg Psychiatry* 1993;56:900-5.
- 31 DeVivo DC. The expanding clinical spectrum of mitochondrial diseases. *Brain Dev* 1993;15:1-22.
- 32 Fryer A, Appleton R, Sweeney MG, *et al.* Mitochondrial DNA 8993 (NARP) mutation presenting within a heterogeneous phenotype including "cerebral palsy". *Arch Dis Child* 1994;71:419-22.
- 33 Lestienne P, Bataille N. Mitochondrial DNA alterations and genetic diseases: a review. *Biomed Pharmacother* 1994;48:199-214.
- 34 Holt IJ, Harding AE, Petty RKH, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 1990;46:428-33.
- 35 Lodi R, Montagna P, Iotti S, *et al.* Brain and muscle energy metabolism studied in vivo by 31P magnetic resonance imaging in NARP syndrome. *J Neurol Neurosurg Psychiatry* 1994;57:1492-6.
- 36 Zeviani M, Taroni F. Mitochondrial diseases. *Bailliere's clinical neurology* 1994;3:315-34.
- 37 Santorelli FM, Shanske S, Jain K, *et al.* A T $\rightarrow$ C mutation at nt8993 of mitochondrial DNA in a child with Leigh syndrome. *Neurology* 1994;44:972-4.
- 38 Tatuch Y, Christodoulou J, Feigenbaum A, *et al.* Heteroplasmic mtDNA mutation (T $\rightarrow$ G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. *Am J Hum Genet* 1992;50:852-8.
- 39 La Spada AR, Paulson HL, Fishbeck KH. Trinucleotide repeat expansion in neurological diseases. *Ann Neurol* 1994;36:814-22.
- 40 La Spada, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 1991;352:77-9.

- 41 Plassart E, Fontaine B. Genes with triplet repeats: a new class of mutations causing neurological diseases. *Biomed Pharmacother* 1994;48:191-7.
- 42 Stevanin G, Le Guern E, Ravise N, et al. A third locus for autosomal cerebellar ataxia (ADCA) type 1 maps to chromosome 14q26.3qter: evidence for the existence of a fourth locus. *Am J Hum Genet* 1994;52:11-20.
- 43 Willems PJ. Dynamic mutations hit double figures. *Nature Genetics* 1994;8:213-5.
- 44 Kawaguchi Y, Okamoto T, Taniwaki M, et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nature Genetics* 1994;8:221-7.
- 45 Ranum LPW, Chung M, Banfi S, et al. Molecular and clinical correlations in spinocerebellar ataxia 1 (SCA1): evidence for familial effects on the age of onset. *Am J Hum Genet* 1994;55:244-52.
- 46 Giunti P, Sweeney MG, Spadaro M, et al. The trinucleotide repeat expansion on chromosome 6p (SCA 1) in autosomal dominant cerebellar ataxias. *Brain* 1994; 227:645-9.
- 47 Duryao M, Ambrose C, Myers R et al. Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nature Genetics* 1993;4:387-92.
- 48 Potter NT, Meyer MA, Zimmerman AW. Molecular and clinical findings in a family with dentatorubral-pallidoluysian atrophy. *Ann Neurol* 1995;37: 273-7.
- 49 Nagafuchi S, Yanagisawa H, Sato K, et al. Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. *Nature Genetics* 1994;6:14-8.
- 50 Nagafuchi S, Yanagisawa H, Ohsaki E, et al. Structure and expression of the gene responsible for the triplet repeat disorder, dentatorubral and pallidoluysian atrophy (DRPLA). *Nature Genetics* 1994;8:177-82.
- 51 Menkes JH, Alter M, Steigleder GK et al. A sex-linked recessive disorder with growth retardation, peculiar hair, and focal cerebral and cerebellar degeneration. *Pediatrics* 1962;29:764-79.
- 52 Menkes JH. Kinky hair disease: twenty-five years later. *Brain Dev* 1988;10:77-9.
- 53 Danks DM, Campbell PE, Stevens BJ et al. Menkes' kinky hair syndrome: an inherited defect in copper absorption with widespread effects. *Pediatrics* 1972;50: 188-201.
- 54 Gunn TR, Macfarlane S, Phillips LI. Difficulties in the neonatal diagnosis of Menkes' kinky hair syndrome-trichopoliodystrophy. *Clinical Pediatr* 1984;23:514-6.
- 55 Tonnesen T, Horn N. Prenatal and postnatal diagnosis of Menkes disease, an inherited disorder of copper metabolism. *J Inherit Metab Dis* 1989;12 (suppl1): 207-14.
- 56 Turner Z, Tonnesen T, Bohmann J, et al. First trimester prenatal diagnosis of Menkes disease by DNA analysis. *J Med Genet* 1994;31:615-7.
- 57 Bull PC, Cox DW. Wilson disease and Menkes disease: new handles on heavy-metal transport. *Trends Genet* 1994;10:246-52.
- 58 Tanzi RE, Petrukhin K, Chernov I et al. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nature Genetics* 1993;5:344-50.
- 59 Yamaguchi Y, Heiny ME, Gitlin JD. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. *Biochem Biophys Res Commun* 1993;197:271-7.
- 60 Thomas GR, Forbes JR, Roberts EA. The Wilson disease gene: spectrum of mutations and their consequences. *Nature Genetics* 1995;9:210-7.
- 61 Czaja MJ, Weiner FR, Schwarzenberg FJ, et al. Molecular studies of ceruloplasmin deficiency in Wilson's disease. *J Clin Invest* 1987;80:1200-4.
- 62 Walshe JM. Diagnosis and treatment of presymptomatic Wilson's disease. *Lancet* 1988;ii:435-7.
- 63 Walshe JM. Wilson's disease: yesterday, today, and tomorrow. *Mov Dis* 1988;3:10-29.
- 64 Walshe JM, Yealland M. Wilson's disease: the problem of delayed diagnosis. *J Neurol Neurosurg Psychiatry* 1992;55:692-6.
- 65 Gibbs K, Walshe JM. A study of the ceruloplasmin concentrations found in 75 patients with Wilson's disease, their kinships and various control groups. *Quart J Med* 1979;48:447-63.
- 66 Neri G, Chiurazzi P, Arena JF, Lubs HA. XLMR genes: update 1994. *Am J Med Genet* 1994;51:542-9.
- 67 Jacobs PA, Glover TW, Mayer M, et al. X-linked mental retardation: a study of 7 families. *Am J Med Genet* 1980;7:471-89.
- 68 Turner G, Brookwell R, Daniel A, et al. Heterozygous expression of X-linked mental retardation and X-chromosome marker fra(X) (q27). *N Engl J Med* 1980; 303:662-4.
- 69 Nielson KB. Diagnosis of the fragile X syndrome: clinical findings in 27 males with the fragile site at Xq28. *Journal of Mental Deficiency Research* 1983;27: 211-26.
- 70 Verkerk AJMH, Pieretti M, Sutcliffe JS, et al. Identification of a gene (FMR-1) containing CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991;65:905-14.
- 71 Fu Y-H, Kuhl DPA, Pizzuti A. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell* 1992;67: 1047-58.
- 72 Menkes JH. *Textbook of child neurology*, 5th ed. Baltimore: Williams and Wilkins, 1995.
- 73 Goate A, Chartier Harlin MC, Mullan M, et al. Segregation of missense mutations in amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 1991;349:704-6.
- 74 Hardy J. Alzheimer's disease. Molecular genetics. *J Fla Med Assoc* 1994;91:759-61.
- 75 Schellenberg GD, Bird T, Wijsman E et al. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science* 1992;258:668-71.
- 76 Kremer B, Goldberg P, Andrew SE et al. A worldwide study of the Huntington's disease mutation. The sensitivity and specificity of measuring CAG repeats. *N Engl J Med* 1994;330:1401-6.
- 77 Saito Y, Ando T, Doyu M, et al. An adult case of classical Pelizaeus-Merzbacher disease-magnetic resonance images and neuropathological findings. *Rinsho Shinkeigaku. Clinical Neurology* 1993;33:187-93.
- 78 Pham-Dinh D, Boespflug-Tanguy O, Mimault C, et al. Pelizaeus-Merzbacher disease: a frameshift deletion/insertion event in the myelin proteolipid gene. *Human Molecular Genetics* 1993;2:465-7.
- 79 Boespflug-Tanguy O, Mimault C, Melki J, et al. Genetic homogeneity of Pelizaeus-Merzbacher disease: tight linkage to the proteolipoprotein locus in 16 affected families. *Am J Hum Genet* 1994;55:461-7.
- 80 Strautnieks S, Rutland P, Winter RM, et al. Pelizaeus-Merzbacher disease: detection of mutations THR¹⁸¹ → Pro and Leu²²³ → Pro in the proteolipid protein gene, and prenatal diagnosis. *Am J Hum Genet* 1992;51: 871-8.
- 81 Matalon R, Michals K, Sebastia D, et al. Aspartoacylase deficiency and N-acetylaspatic aciduria in patients with Canavan disease. *Am J Med Genet* 1988;29: 463-71.
- 82 Gardiner RM. Genetic analysis of Batten disease. *J Inherit Metab Dis* 1993;16:787-90.
- 83 Mitchison HM, Taschner PE, O'Rawe AM et al. Genetic mapping of the Batten disease locus (CLN3) to the interval D16S288-D16S383 by analysis of haplotypes and allelic association. *Genomics* 1994;22:465-8.
- 84 Ferrer I, Arbizu T, Pena J, Serra JP. A Golgi and ultrastructural study of a dominant form of Kufs' disease. *J Neurol* 1980;222:183-90.
- 85 Martin J-J. Adult type of neuronal ceroid-lipofuscinosis. *J Inherit Metab Dis* 1993;16:237-40.
- 86 Rosenberg RN. Autosomal dominant cerebellar phenotypes: the genotype has settled the issue. *Neurology* 1995;45:1-5.
- 87 Savitsky K, Bar-Shira A, Gilad S, et al. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 1995;268:1749-53.
- 88 Serizawa M, Sakamoto M, Hirabayashi K, et al. Histological and radiobiological study of adult cases with ataxia telangiectasia. *Rinsho Shinkeigaku. Clin Neurol* 1994;34:38-42.
- 89 Boder E. Ataxia-telangiectasia. In: Gomez MR, ed. *Neurocutaneous diseases. A practical approach*, Boston: Butterworths, 1987:95-117.
- 90 Gardner K, Alderson K, Galster B, et al. Autosomal dominant spinocerebellar ataxia: clinical description of a distinct hereditary ataxia and genetic localization to chromosome 16 (SCA4) in a Utah kindred. *Neurology* 1994;92:1S.
- 91 Benomar A, Le Guern E, Dure A. Autosomal dominant cerebellar ataxia with retinal degeneration (ADCA type II) is genetically different from ADCA type I. *Ann Neurol* 1994;35:439-44.
- 92 Ranum LPW, Schut LJ, Lundgren JK, et al. Spinocerebellar ataxia type 5 in a family descended from the grandparents of President Lincoln maps to chromosome 11. *Nature Genetics* 1994;8:280-4.
- 93 Harding AE. The clinical features and classification of the late onset autosomal dominant cerebellar ataxias. *Brain* 1982;105:1-28.
- 94 Smith JK, Gonda VE, Malamud N. Unusual form of cerebellar ataxia. Combined dentato-rubral and pallido-luysian degeneration. *Neurology* 1958;8:205-9.
- 95 Schimschock JR, Alvord EC, Swanson PD. Cerebro-tendinous xanthomatosis: clinical and pathological studies. *Arch Neurol* 1968;18:688-98.
- 96 Menkes JH, Schimschock JR, Swanson PD. Cerebro-tendinous xanthomatosis: the storage of cholestanol within the nervous system. *Arch Neurol* 1968;19:47-53.
- 97 Berginer VM, Salen G, Shefer S. Long-term treatment of cerebrotendinous xanthomatosis with chenodeoxycholic acid. *New Engl J Med* 1984;311:1649-52.
- 98 Leitersdorf E, Reshef A, Meiner V, et al. Frameshift and splice-junction mutations in the sterol 27-hydroxylase gene cause cerebrotendinous xanthomatosis in Jews of Moroccan origin. *J Clin Invest* 1993;91:2488-96.
- 99 Kim K-S, Kubota S, Kuriyama M, et al. Identification of new mutations in sterol 27-hydroxylase gene in Japanese patients with cerebrotendinous xanthomatosis (CTX). *J Lipid Research* 1994;35: 1031-9.
- 100 Nakashima N, Sakai Y, Sakai H, et al. A point mutation in the bile acid biosynthetic enzyme sterol 27-hydroxylase in a family with cerebrotendinous xanthomatosis. *J Lipid Research* 1994;35:663-8.
- 101 Meiner V, Meiner Z, Reshef A, et al. Cerebrotendinous xanthomatosis: molecular diagnosis enables presymp-

- omatic detection of a treatable disease. *Neurology* 1994;44:288-90.
- 102 Harding AE, Matthews S, Jones S, *et al.* Spinocerebellar degeneration associated with a selective defect of vitamin E absorption. *N Engl J Med* 1985;313:32-5.
 - 103 Stumpf DA, Sokol R, Bettis D, *et al.* Friedreich's disease: V. Variant form with vitamin E deficiency and normal fat absorption. *Neurology* 1987;37:68-74.
 - 104 Ben Hamida M, Belal S, Sirugo G, *et al.* Friedreich's ataxia phenotype not linked to chromosome 9 and associated with selective autosomal recessive vitamin E deficiency in two inbred Tunisian families. *Neurology* 1993;43:2179-83.
 - 105 Ben Hamida C, Doerflinger N, Belal S, *et al.* Localization of Friedreich ataxia phenotype with selective vitamin E deficiency to chromosome 8q by homozygosity mapping. *Nature Genetics* 1993;5:195-200.
 - 106 Ouahchi K, Arita M, Kayden H, *et al.* Ataxia with isolated vitamin E deficiency is caused by mutations in the α -tocopherol transfer protein. *Nature Genetics* 1995;9:141-5.
 - 107 Kayden HJ. The neurologic syndrome of vitamin E deficiency: a significant cause of ataxia. *Neurology* 1993;43:2167-9.
 - 108 Sharp D, Blinderman L, Combs KA *et al.* Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinemia. *Nature* 1993;365:65-9.
 - 109 DiDonato S. Can we avoid AVED? *Nature Genetics* 1995;9:106-7.
 - 110 Rosen DR, Siddique T, Patterson D, *et al.* Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;362:59-62.
 - 111 Deng H-X, Hentati A, Tainer JA. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. *Science* 1993;261:1047-51.
 - 112 Aoki M, Ogasawara M, Matsubara Y, *et al.* Familial amyotrophic lateral sclerosis (ALS) in Japan associated with H46R mutation in Cu/Zn superoxide dismutase gene: a possible new subtype of familial ALS. *J Neurological Sciences* 1994;126:77-83.
 - 113 Bowling AC, Barkowski EE, McKenna YD, *et al.* Superoxide dismutase concentration and activity in familial amyotrophic lateral sclerosis. *J Neurochem* 1995;64:2366-9.
 - 114 Ichinose H, Ohye T, Takahashi E, *et al.* Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase 1 gene. *Nature Genetics* 1994;8:236-41.
 - 115 Lertrit P, Noer AS, Jean-Francis MJB, *et al.* A new disease related mutation for mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes syndrome affecting the ND4 subunit of complex I. *Am J Hum Genet* 1992;52:457-68.
 - 116 Harker LA, Ross R, Slichter SJ, Scott CR. Homocystine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. *J Clin Invest* 1976;58:731-41.
 - 117 Brattstrom LE, Hardebo JE, Hultberg BC. Moderate homocysteinemia—a possible risk factor for arteriosclerotic cerebrovascular disease. *Stroke* 1984;15:1012-6.
 - 118 Riela AR, Roach S. Etiology of stroke in children. *J Child Neurol* 1993;8:201-20.
 - 119 Mudd SH, Irreverre F, Laster L. Sulfite oxidase deficiency in man: demonstration of the enzymatic defect. *Science* 1967;156:1599-602.
 - 120 Shih VE, Abroms IF, Johnson JL, *et al.* Sulfite oxidase deficiency. Biochemical and clinical investigations of a hereditary metabolic disorder in sulfur metabolism. *New Engl J Med* 1977;297:1022-8.
 - 121 Haas RH, Marsden DL, Capistrano-Estrada S, Hamilton R *et al.* Acute basal ganglia infarction in propionic acidemia. *J Child Neurol* 1995;10:18-22.
 - 122 Sesthi KD, Ray R, Roesel RA. Adult-onset chorea and dementia with propionic acidemia. *Neurology* 1989;39:1343-5.
 - 123 Christensen Lou HO, Reske-Nielsen E. The central nervous system in Fabry's disease. *Arch Neurol* 1971;25:351-9.

NEUROLOGICAL STAMP

Rosa canina (dog rose)

The Roman naturalist Pliny attributed the name dog rose to a belief that the plant's root could cure the bite of a mad dog. It is not clear if the dogs were rabid. The use of the dog rose in prescriptions dates back to Hippocrates but precisely what for is unclear. As a medicinal plant it came into full bloom in the second world war. When Britain was unable to import fresh citrus fruits the government organised the gathering of dog rose fruits, or hips, which were known to be a rich source of vitamin C. Processed into syrup the rose hips helped to prevent scurvy and, today, the plant is valued as a rich source of vitamin C.

The dog rose is illustrated as part of a commemorative set issued for the International Congress of Pharmacology held in Prague in 1971 (Stanley Gibbons 1980, Scott 1773).

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