European Journal of Neurology 2001, **8:** 407–424

SPECIAL ARTICLE

EFNS task force on molecular diagnosis of neurologic disorders Guidelines for the molecular diagnosis of inherited neurologic diseases Second of two parts

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Keywords:
DNA-testing,
genetics, inherited
neurologic disorders,
molecular genetic
diagnosis, mutation

Received 10 January 2001
Accepted 1 March 2001

Introduction

With the Human Genome Project nearing completion, the molecular basis of an increasing number of inherited neurologic diseases has been elucidated. Molecular diagnosis of these disorders is becoming a matter of clinical practice. These *guidelines*, established by the task force on molecular diagnosis of the EFNS, are designed to provide practical help for the clinical neurologist to make appropriate use of the possibilities of molecular diagnosis of neurologic disorders in Europe.

In the first part, which appeared in the last issue of the European Journal of Neurology, general principles of molecular diagnosis, including genetic counselling, have been described. A second section contains a summary of the possibilities and limitations of molecular genetic diagnosis of some important inherited neurologic diseases. Practical issues, such as diagnostic criteria that help to decide whether a molecular diagnostic should be ordered, are emphasized, and basic aspects are covered only as needed.

A table at the end of section II will offer a more comprehensive listing of neurogenetic disorders, which are dealt within the respective issue, with information on the types of mutation and the availability of molecular diagnosis. This listing will not be complete, but attempts to be helpful in most clinical cases. Original papers on gene mapping and cloning are referenced here.

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Section II (second of two parts): Molecular Diagnosis of Neurologic Disorders

Because of the large number of disorders covered, this section of the *guidelines* is published in this and the previous issue of the *European Journal of Neurology*. In the current issue, skeletal muscle channelopathies, neuropathies, epilepsies, neurovascular disorders, neurocutaneous syndromes and mitochondrial diseases are covered. For non-degenerative movement disorders, inherited ataxias, neurodegenerative disorders, dementias and myopathies and muscular atrophies the reader is asked to refer to the last issue of the Journal.

Molecular diagnosis of skeletal muscle channelopathies
Frank Lehmann-Horn and Karin Jurkat-Rott

The skeletal muscle channelopathies, non-dystrophic myotonias and periodic paralyses, are the first disorders for which an ion channel defect was identified to cause the pathology (Lehmann-Horn and Jurkat-Rott, 1999). Predominant symptoms of these diseases are transiently occurring muscle stiffness and episodes of muscle weakness with next to no symptoms in the interval. Whilst myotonia is caused by increased excitability of the sarcolemma, paralysis is associated with reduced excitability. Both symptoms are mediated by long-lasting spells of muscle fiber depolarization provoked by internal or environmental triggers. The cause for this diversity of symptoms is the varying degree of

Table 1 Skeletal muscle voltage-gated channelopathies

Disorder	Symptoms	Clinical diagnosis
Myotonia congenita Becker and Thomsen	ar (Becker), ad (Thomsen); generalized myotonia, warm-up phenomenon, muscle hypertrophy, transient weakness (Becker)	Family history, staircase test, EMG exclusion in case of cataracts
Potassium-sensitive myotonia	ad; Generalized myotonia of variable severity, aggravation by potassium administration, no weakness	Family history, EMG, potassium administration in milder cases
Paramyotonia congenita	ad; Paradoxical myotonia, cold-induced muscle stiffness followed by weakness/paralysis	Family history, lid paramyotonia test, EMG, provocation by cold
Hyperkalemic periodic paralysis	ad; Episodic attacks of mainly generalized weakness, hyperkalemia during episode, triggering by rest after body exertion or potassium intake	Family history, trigger history, potassium administration, EMG, ictal serum potassium level
Hypokalemic periodic paralysis	ad; Episodic attacks of mainly generalized weakness, hypokalemia during episode, triggering by carbohydrate-rich food or exercise	Family history, trigger history, glucose-and- insulin administration, ictal serum potassium level exclusion if myotonia present

Abbreviations: ad = autosomal dominant; ar = autosomal recessive.

depolarization: slight depolarization induces hyperexcitability and strong depolarization underexcitability. The transition from one condition to the other is so narrow that some patients present with alternating myotonia and peridoic paralysis (Terwindt *et al.*, 1998) (for symptom overview and clinical diagnostic testing see Table 1).

The group of disorders is caused by mutations in genes encoding voltage-gated chloride, sodium, and calcium channels. Generally, reliable information for determining the range of diagnoses may be gained by examination of additional relatives because of the intra-ad interpersonal variability. Next to clinical features, also electromyography and provocative testing are helpful for diagnosing. Genetic studies focus on confirming clinical diagnosis but may be quite useful for exclusion of dystrophy-related myopathies (i.e. testing of CTG repeats in the myotonin protein kinase gene on chromosome 19q, see section on myotonic dystrophy), an important factor for prognosis. Additionally, valuable information on epidemiology and indications for heterogeneity may be obtained by the genetic studies, so that they are highly recommended.

Myotonia congenita Thomsen and Becker (MIM 160 100)

The classical non-dystrophic myotonic disorders are caused by dominant point mutations (Thomsen myotonia) or recessive mis-sense and non-sense mutations (Becker myotonia) in the skeletal muscle voltage-gated chloride channel. Over 40 mutations are known, distributed all over the gene, which is encoded by 23 exons. These account for approximately 30% of the cases making genetic studies quite arduous. Again, the important issue genetically and prognostically (and in some cases diagnostically) is to exclude the myotonic dystrophy CTG repeats. If genetic screening does not yield a result, linkage analysis including additional family of defined clinical status is a very useful tool to confirm clinical diagnosis.

Potassium-aggravated myotonia (MIM 603 967)

Genetic studies are very important for the discrimination from myotonia congenita. There are seven mutations known in five of the 24 exons of the α-subunit of the voltage-gated sodium channel and account for approximately 20% of the cases if only a single individual of a kinship is studied. A much larger percentage can be diagnosed by testing additional affected and unaffected family members for linkage to the gene located on chromosome 17q.

Paramyotonia congenita and hyperkalemic periodic paralysis (MIM 168 300)

Recent results have demonstrated these disorders to be heterogenous, whereby the most frequent paramyotonia form is linked to 12 mutations in four of the 24 exons of the α-subunit of the voltage-gated sodium channel. For hyperkalemic periodic paralysis, there are five mutations known. Both disorders can be genetically diagnosed with a success rate of almost 50% by allele-specific tests. In case of a negative result, linkage analysis of further family members is recommended.

Hypokalemic periodic paralysis (MIM 600 304)

This disorder is usually caused by mutations in the gene for a calcium channel on chromosome 1, but one family has been identified, in whom a sodium-channel mutation is responsible (see Table 2). If a positive family history can be obtained, one of the three most common

Table 2 Neurogenetic disorders

Disease	Symbol	Inheritance	Position	Gene product	Mutation	Molecular diagnosis	Reference	Remarks	MIM-number
Potassium sensitive myotonia	SCN4A	AD	17q23	Sodium channel α-subunit	Pm	B	Lerche *et al.*, 1993		603 967
Hyperkalemic periodic paralysis	SCN4A	AD	17q23	Sodium channel α-subunit	Pm	B	Fontaine *et al.*, 1990		168 300
Paramyotonia congenita	SCN4A	AD	17q23	Sodium channel α-subunit	Pm	B	McClatchey *et al.*, 1992		168 300
Potassium-aggravated myotonia	SCN4A	AD	17q23	Sodium channel α subunit	Pm	B	Lerche *et al.*, 1993		168 300
Hypokalemic periodic paralysis 1	CACN1AS	AD	1q31-32	Calcium channel	Pm	B	Jurkat-Rott *et al.*, 1994		600 304
Hypokalemic periodic paralysis 2	SNCA4	AD	17q23	Sodium channel α-subunit	Pm	B	Jurkat-Rott *et al.*, 2000		600 304
Hypokalemic periodic paralysis 3	KCNE3	AD	11q13-14	Potassium channel β-subunit	Pm	B	Abbott *et al.*, 2001		600 304
Myotonia congenita Thomsen	CLCN1	AD	7q35	Chloride channel	Pm	B	Koch *et al.*, 1992		160 800
Myotonia congenita Becker	CLCN1	AR	7q35	Chloride channel	Pm, Del, Ins	B	Koch *et al.*, 1992		255 700
Neuropathies									
Charcot-Marie-Tooth Type Ia	CMT1a	AD	17p11.2	PMP-22	Dupl/Pm	A	Lupski *et al.*, 1991	In 70% duplication of a 1.5-Mb-fragment	118 220
Charcot-Marie-Tooth Type Ib	CMT1b	AD	1q22-23	P_o	Pm	B	Hayasaka *et al.*, 1993		118 200
Type II (neuronal)	CMT 2a 118 210	AD	1p36	Unknown	Unknown	D	Ben Othmane *et al.*, 1993a		118 210
	CMT2b	AD	3q13-q22	Unknown	Unknown	D	Kwon *et al.*, 1995		600 882
	CMT2d	AD	7p14	Unknown	Unknown	D	Ionasescu *et al.*, 1996		601 472
Charcot-Marie-Tooth Type IVa	CMT4a 214 400	AR	8q	Unknown	Unknown	D	Ben Othmane *et al.*, 1993b		214 400
	CMT4b	AR	8q	Unknown	Unknown	D	Bolino *et al.*, 1996	One family	
Charcot-Marie-Tooth, X-chromosomal	CMTX	XL	Xq13.1	Connexin-32	Pm	B	Bergoffen *et al.*, 1993		302 800
Hereditary sensory neuropathy	HSN I	AD	9q22.1-22.3	Unknown	Unknown	D	Nicholson *et al.*, 1996		162 400
Hereditary motor neuropathy	HMN II	AD	12q24	Unknown	Unknown	D	Timmerman *et al.*, 1996		158 590
	HMN V	AD	7p	Unknown	Unknown	D	Christodoulou *et al.*, 1995		
Hereditary neuralgic amyotrophy	HNA	AD	17q24-25	Unknown	Unknown	D	Pellegrino *et al.*, 1997		
Tomaculous Neuropathy (liability to pressure palsies)	HNPP	AD	17p11.2	PMP-22	Del/Pm	A	Chance *et al.*, 1993	Mostly deletion of a 1.5-Mb-fragment (complementary to CMT1)	162 500
Inherited tumor syndromes									
Neurofibromatosis 1 (v. Recklinghausen)	NF1	AD	17q11.2	Neurofibromin	Del/Pm	B	Wallace *et al.*, 1991		162 200
Neurofibromatosis 2	NF2	AD	22q12.2	Merlin	Del/Pm	B	Trofatter *et al.*, 1993		101 100
von Hippel-Lindau disease	VHL	AD	3p25	VHL-gene	Pm/Del	B	Richards *et al.*, 1993		193 300
Tuberous sclerosis	TSC1	AD	9q34	Hamartin	Pm/Del/Ins	B	van Slegtenhorst *et al.*, 1997		191 100
	TSC2	AD	16p13	Tuberin	Del	B	European Chromosome 16 Tuberous Sclerosis Consortium 1993		191 092

Table 2 (Continued)

Disease	Symbol	Inheritance	Position	Gene product	Mutation	Molecular diagnosis	Reference	Remarks	MIM-number
Dementias									
Familial Alzheimer's disease	AD1	AD	21q21	Amyloid precursor protein	Pm	C	Goate *et al.*, 1991		104 760
	AD2	AD	19q13.2	ApoE	Pm	C	Pericak-Vance *et al.*, 1991		104 310
	AD3	AD	14q24.3	Presenilin 1	Pm	C	Sherrington *et al.*, 1995		104 311
	AD4	AD	1q31-q42	Presenilin 2	Pm	C	Rogaev *et al.*, 1995		600 759
Frontotemporal dementia with parkinsonism	FTDP-17	AD	17q21	MAPTAU	Pm	C	Hutton *et al.*, 1998		601 630
Fam. Creutzfeld-Jakob disease	PRNP	AD	20pter-p12	Prion-Protein	Pm/ins	B	Owen *et al.*, 1989	5–10% of CJD cases	123 400
Gerstmann–Sträussler-Syndrome	PRNP	AD	20pter-p12	Prion-Protein	Ins/Pm	B	Hsiao *et al.*, 1989	Part of the CJD spectrum	137 440
Fatal familial insomnia	PRNP	AD	20pter-p12	Prion-Protein	Pm	B	Medori *et al.*, 1992	Part of the CJD spectrum	600 072
Familial amyotrophic lateral sclerosis	SOD1	AD	21q22	Superoxide dismutase 1	Pm	C	Rosen *et al.*, 1993	20% of hereditary ALS	105 400
Other neurodegenerative disorders	SOD1	AD	21q22	Superoxide dismutase 1	Pm	C	Rosen *et al.*, 1993	20% of hereditary ALS	105 400
Fam. spastic paraplegia	SPG1	X	Xq28	L1CAM	Pm	C	Jouet *et al.*, 1994	'Complicated form'	312 900
	SPG2	X	Xq21	Proteolipid-protein	Pm. Dupl, Del	B	Saugier-Veber *et al.*, 1994	Allelic to Pelizeus-Merzbacher-disease	312 920
	SPG3	AD	14q11.2-24.3	Unknown	Unknown	D	Hazan *et al.*, 1993		182 600
	SPG4	AD	2p24-p21	Spastin	Pm	C	Hazan *et al.*, 1993		182 601
	SPG5	AR	8p12-q13	Unknown	Unknown	D	Hentati *et al.*, 1994		270 800
	SPG6	AD	15q11.1	Unknown	Unknown	D	Fink *et al.*, 1995		600 363
	SPG7	AR	16q24.3	Paraplegin	Del/ins	D	Casari *et al.*, 1998		
	SPG8	AD	8q23-24	Unknown	Unknown	D	Hedera *et al.*, 1999	One family	603 563
Epilepsies									
Benign familial neonatal convulsions	EBN1	AD	20q13.3	KCNQ2	Pm, Del, ins	C	Biervert *et al.*, 1998;		121 200
	EBN2	AD	8q24	KCNQ3	Pm	C	Singh *et al.*, 1998 Charlier *et al.*, 1998		121 201
Familial nocturnal frontal lobe epilepsy	ADNFLE	AD	20q13	CHRNA4	Pm, ins	C	Steinlein *et al.*, 1995		600 513
	ADNFLE	AD	8q24	CHRNB2	Pm	C	Phillips *et al.*, 2001		
Febrile seizures	GEFS+	AD?	19q13.1	SCN1B	Pm	C	Wallace *et al.*, 1998		604 236
Febrile seizures	GEFS2+	AD?	2q24-q33	SCN1A	Pm	C	Escayg *et al.*, 2000		604 233
Febrile seizures	FEB1	AD	8q13	Unknown	Unknown	D	Wallace *et al.*, 1996		602 476
Febrile seizures	FEB2	AD	19p13.3	Unknown	Unknown	D	Johnson *et al.*, 1998		602 477

Progressive myoclonic–epilepsy of Unverricht–Lundborg type	EPM1	AR	21q22.3	Cystatin B, CSTB	12 bp repeat expansion, pm, del	A	Pennacchio *et al*., 1996 Lalioti *et al*., 1997	The dodecamer repeat expansion for accounts appr. 90% of disease alleles world-wide	254 800
Lafora's disease	MELF	AR	6q23-q25	Laforin	Microdel, pm, ins	B	Minassian *et al*., 1998 Serratosa *et al*., 1999	Not all families are linked to chromosome 6q	254 780
Neuronal ceroid lipofuscinosis, infantile, variant late infantile, variant juvenile	CLN1	AR	1p32	Palmitoyl-protein thioesterase (PPT)	Pm, ins, del	C	Vesa *et al*., 1995		256 730
Neuronal ceroid lipofuscinosis, classical late infantile	CLN2	AR	11p13	Pepstatin-insensitive protease	Pm, ins, del	C	Sleat *et al*., 1997		204 500
Neuronal ceroid lipofuscinosis, juvenile	CLN3	AR	16p12	Novel membrane protein	Del, pm, ins	C	The International Batten Disease Consortium, 1995		204 200
Neuronal ceroid lipofuscinosis, Finnish late-infantile	CLN5	AR	13q22	Novel membrane protein	Pm, del	C	Savukoski *et al*., 1998		256 731
Neuronal ceroid lipofuscinosis, variant late infantile	CLN6	AR	15q21	Unknown	Unknown	C	Sharp *et al*., 1999		601 780
Neuronal ceroid lipofuscinosis, progressive epilepsy with mental retardation	CLN8	AR	8p23	Novel membrane protein	Pm	C	Ranta *et al*., 1999	So far described only in Finland	600 143
Neurovascular disorders									
CADASIL	CADASIL	AD	19p13.1	Notch3	Pm	A	Joutel *et al*., 1996		125 310
Hereditary cerebral hemorrhage with amyloidosis	HCHWA-D	AD	21q21	Amyloid precursor protein	Pm	C	Levy *et al*., 1990		104 760
	HCHWA-I	AD	20p11.2	Cystatin C	Pm	C	Abrahamson *et al*., 1992		105 150
Cavernous malformations	CCM1	AD	7q21-22	KRIT1	Pm, del, ins	C	Laberge-le *et al*., 1999		116 860
	CCM2	AD	7p13-15	Unknown	Unknown	D	Craig *et al*., 1998		603 284
	CCM3	AD	3q25.2-27	Unknown	Unknown	D	Craig *et al*., 1998		603 285
Migraine									
Familial hemiplegic migraine	FHM1	AD	19p13	Calcium channel	Pm	C	Ophoff *et al*., 1996	Allelic to SCA6 and EA2, see, Channelopathies	141 500
	FHM2	AD	1q21-31	Unknown	Unknown	D	Gardner *et al*., 1997 Ducros *et al*., 1997		602 481
Mitochondrial diseases									
Chronic progressive external ophthalmoplegia	CPEO	spor		mtDNA	Del, Dupl, Pm	A	Holt *et al*., 1988	Molecular genetic analysis should be performed in muscle	258 450
Autosomal dominant PEO	adCPEO	ad	10q23-24 3p14-21	Unknown	Unknown	D	Suomalainen *et al*., 1995	Multiple deletions of mtDNA result from a nuclear defect	550 000
Autosomal recessive PEO	arPEO	ar	Unknown	Unknown	Unknown	D	Bohlega *et al*., 1996	Multiple deletions of mtDNA result from a nuclear defect	–

Table 2 (Continued)

Disease	Symbol	Inheritance	Position	Gene product	Mutation	Molecular diagnosis	Reference	Remarks	MIM-number
Kearns–Sayre syndrome	KSS	spor		mtDNA	Del, Dupl, Pm	A	Zeviani *et al.*, 1988	Molecular genetic analysis should be performed in muscle	530 000
Pearson's bone marrow–pancreas syndrome		spor		mtDNA	Del, Dupl	A	Rotig *et al.*, 1989	Surviving infants may later develop KSS	557 000
Mitochondrial encephalomyopathy with lactacidosis and 'stroke-like episodes'	MELAS	Mat	np 3243 (np 3271)	^{mt}-tRNA^{Leu}	Pm	A	Goto *et al.*, 1990	Other point mutations have been described in rare cases	540 000
Myoclonus epilepsy with ragged red fibres	MERRF	mat	np 8344	^{mt}-tRNA^{Lys}	Pm	A	Shoffner *et al.*, 1990	Other point mutations have been described in rare cases	545 000
Leber's hereditary optic neuropathy	LHON	mat	np 11778 np 3460 np 14484	Complex I of the respiratory chain	Pm	A	Wallace *et al.*, 1988	Other point mutations have been described in rare cases	535 000
Neurogenic weakness, ataxia and retinitis pigmentosa	NARP	mat	np 8993	ATPase 6 of the respiratory chain	Pm	A	Holt *et al.*, 1990	High percentage of thi mutation may lead to MILS	551 500
Maternally inherited Leigh syndrome	MILS	mat	np 8993	ATPase 6 of the respiratory chain	Pm	A	Tatuch *et al.*, 1992	Lower percentage of this mutation may lead to NARP	516 060
Leigh syndrome	LS	ar	9q34	SURF-1	Pm	C	Zhu *et al.*, 1998	Genetically very heterogeneous	185 620
Myo-neuro-gastro-intestinal encephalomyopathy	MNGIE	ar	22q13-qter	Thymidine phosphorylase	Pm	C	Nishino *et al.*, 1999	The nuclear defect leads to multiple deletions or depletion of mtDNA	550 900

Note: This compilation of inherited neurologic disorders is not complete. The selection of the listed diseases was chosen relatively at random, its purpose is to give the reader an orientation point. The speed in which progress is made in molecular genetic research, however, causes such tables to become outdated very quickly. In case of doubt it is recommended that current publications or special centres be consulted. For many if not for most of the diseases listed here, mutations in other currently unknown genes may also be responsible.

Abbreviations: AD = Autosomal dominant; AR = autosomal recessive; X = X-chromosomal; mat = maternal (mitochondrial) transmission; spor = sporodic Pm = point mutation; Del = deletion; Ins = insertion; Trinuc = trinucleotid-repeat expansion.

Availability of molecular diagnosis: A: Routine procedure, commercially available, results usually within 4 weeks, B: Routine procedure, but may be time-consuming and expensive, usually due to occurrence of multiple mutations; results may take several months, C: Usually available only within research setting, D: Not yet available.

known mutations can be detected in 40% of the cases. Differentiating this disorder from the hyperkalemic variant is of prognostic and therapeutic relevance. Because of the difficulties in diagnosis and genetic heterogeneity, testing of additional family members for linkage to the known loci is recommended in case of negative results.

Molecular diagnosis of inherited neuropathies

Eric LeGuern and Thomas Gasser

The molecular basis of many inherited forms of peripheral neuropathies with or without recognized metabolic defect has now been delineated (Pareyson, 1999; Schenone and Mancardi, 1999) In addition to the *Charcot-Marie-Tooth* (CMT) syndrome which is the most frequent inherited neuropathy, other genetic neuropathies were identified: *hereditary neuropathy with liability to pressure palsies* (HNPP, MIM 162 500), *familial brachial plexus* neuropathy (hereditary neuralgic amyotrophy (MIM 162 100), the *hereditary sensory and autonomic neuropathies*, and some forms of amyloid neuropathy. Peripheral neuropathy can be also part of more complex neurologic syndromes, including many autosomal recessive inborn errors of metabolism. Neuropathy, usually of the axonal type (neuroneopathy) also occurs, to a varying extent, in inherited neurologic disorders discussed elsewhere in these guidelines, e.g. almost invariably in Friedreich's ataxia, frequently in the dominant spinocerebellar ataxias (SCAs) and occasionally in spastic paraplegia or even in complicated forms of frontotemporal dementia (in the form of amyotrophy). In addition, numerous familial syndromes have been described in which neuropathy is accompanied by features such as blindness, seizures, dementia, or mental retardation.

For practical purposes, the CMT syndrome is the most important hereditary neuropathy. The CMT-syndrome is clinically and genetically heterogeneous. The classification currently used is based on the type of the neuropathy, which may be axonal or demyelinating, and on the mode of inheritance.

Two major categories were defined, based on nerve conduction velocities (NCV), especially on motor nerve conduction velocity of median nerve (MNCV): the demyelinating CMT with slow MNCV (<35 m/s in the upper extremity) and the neuronal CMT with normal or near-normal MNCV (>40 m/s MNCV). For each type, different modes of inheritance are observed: autosomal dominant (AD), X-linked dominant, or autosomal recessive (AR).

Whilst the classification into CMT1 (AD demyelinating CMT) and CMT2 (AD axonal CMT) follows electrophysiological and neuropathological criteria as well as modes of inheritance, their subdivision (for example CMT1A, 1B, 1C for AD demyelinating CMT) is based on the underlying genetic defects.

The autosomal recessive forms are referred to as CMT4, CMTX is X-linked dominant (Pareyson, 1999; Schenone and Mancardi, 1999).

The genotype–phenotype relationship in the CMT-syndrome is complex. Different mutations in the same gene may give rise to different neuropathies, whereas the same phenotype may be associated with mutations of different genes. Because of this complexity, the role of molecular diagnosis is still limited in many cases, with the notable exception of the most frequent form of CMT1, which is caused by a duplication of the chromosomal segment bearing the gene for peripheral myelin protein-22 (PMP22).

Charcot-Marie-Tooth disease, type 1 (CMT1, previously called HMSN I) is inherited with an autosomal dominant transmission and represents the most common form of the familial neuropathies in Europe. The clinical phenotype is variable, ranging from asymptomatic carriers with only diminished MNCVs to chair bound patients. The diagnosis must be suspected when a typical clinical picture (progressing, predominantly distal neuropathy and pes cavus deformity) is found in association with marked slowing of nerve conduction (median nerve conduction velocity of less than 35 m/s).

The most common subtype, CMT1A (MIM 118 220) (75–80%) is linked to chromosome 17p11.2, and mutations affect the gene for peripheral myelin protein 22 (PMP-22). CMT1A is most commonly caused by a duplication of a 1.5 megabase chromosomal region bearing the PMP-22 gene, resulting in the presence of three copies of the PMP-22 gene in patients. DNA-based testing for the PMP-22 duplication (CMT1A) is performed by gene dosage experiments on southern blotting or by fluorescent *in situ* hybridization on metaphase chromosomes (FISH), or by genotyping microsatellites or by detection of junction fragments in CMT1A-REP, the two very homologous regions flanking the duplication. These molecular diagnoses are widely available and detect $>98\%$ of patients with CMT1A. Point mutations in the PMP-22 gene are very rare, and cause $<2\%$ of cases of CMT1A without duplication. Point mutations will not be identified by this routine screening technique.

About 5–10% of CMT1 is designated CMT1B (MIM 118 200) and caused by point mutations in the myelin P0 protein (MPZ) gene (locus on 1q22). The remaining $\sim 15\%$ of patients with CMT1 are designated as having CMT1C (MIM 601 098), but the causative gene(s) and

chromosomal assignments are not known in most cases. Recently, however, mutations in the gene for epidermal growth response 2 (EGR2), a transcription factor involved in the regulation of gene expression and possibly cell proliferation, have been identified in some families with a CMT1-phenotype, but the quantitative importance of this locus is still unknown.

If no 17p11.2 duplication is found, point mutations in the PMP-22, MPZ or EGR2 gene may be responsible for the disease. Clinical and electrophysiological features cannot reliably predict which of the genes is involved, whilst the phenotypical spectrum for a mutation is very large and overlaps with those caused by other mutations. Because of this genetic heterogeneity, sequence analysis is time-consuming and expensive, and will be restricted to a research setting in most cases, but is also available from some commercial laboratories.

Other forms of Charcot-Marie-Tooth disease

CMT2 (HMSN II) is diagnosed in patients with a progressive peripheral motor and sensory neuropathy with normal or near-normal NCV that is inherited in an autosomal dominant manner. The relative proportions of CMT2A (MIM 118 210), 2B (MIM 600 882), 2C (MIM 158 580) and 2D (MIM 601 472) are yet to be determined. The chromosomal loci for CMT2A, CMT2B, and CMT2D have been mapped, but the genes have not been identified (see Table 2). DNA-based testing is not available clinically. Interestingly, in some cases point mutations in the MPZ gene (usually associated with the CMT1-phenotype) have been identified in patients with CMT2, but associated with a late age at onset (third to fourth decade), an Argyll–Robertson sign and a progressive decrease of MNCV.

CMT type 4 (MIM 214 400) refers to CMT with autosomal recessive inheritance. The affected persons have the typical CMT phenotype of distal muscle weakness and atrophy associated with sensory loss and, frequently, pes cavus foot deformity. None of the genes for the CMT4 subtypes have been identified. The diagnosis is based on clinical, pathological, and genetic linkage criteria.

CMTX (MIM 302 800) is a CMT that is inherited with an X-linked dominant pattern and associated with mutations in the connexin 32 (Cx32) gene. Clinical and electrophysiologic features are often in between CMT1 and CMT2. Affected males have a moderate to severe peripheral neuropathy, whereas female carriers of the mutation have a mild neuropathy or are asymptomatic.

Dejerine-Sottas disease (DSD, also called HMSN III, MIM145 900) has been regarded as a distinct disease entity before it became evident from molecular studies that mutations in all four genes associated with demyelinating CMT (PMP-22, MPZ, Cx32, and EGR2) have been found in series of patients with DSD.

The DSD is only a severe form of CMT1: by definition, onset is by age 2, and very low conduction velocities (usually < 12 m/s) indicate severe hypo-and demyelination of peripheral nerves.

Hereditary neuropathy with liability to pressure palsies (HNPP, MIM 162 500)

Hereditary neuropathy with liability to pressure palsies (HNPP) is a disorder of peripheral nerves in which individuals are predisposed to repeated pressure palsies, such as carpal tunnel syndrome and peroneal palsy with foot drop. Recovery from acute neuropathy is often complete; when recovery is not complete, the resulting disability is usually mild. Some affected persons also have signs of a mild to moderate peripheral neuropathy. The diagnosis is established in an adult with recurrent focal compression neuropathies who has a family history consistent with autosomal dominant inheritance. The disease is most commonly caused by a deletion of the same 1.5 Mb region of chromosome 17 that is duplicated in CMT1A. DNA testing for this deletion that includes the PMP-22 gene detects about 70–80% of cases, the remainder having a PMP-22 frame shift mutation.

The proportion of patients with a single episode of compression neuropathy who have a 17p11 deletion is not clear. However, with the exception of the situation that a molecular diagnosis is wanted for example for career counselling purposes, testing should be offered only to those patients with (1) more than one episode of compression neuropathy, or (2) one episode of compression neuropathy and an unexplained polyneuropathy, or (3) one episode of compression neuropathy and a family history of neuropathy (Dubourg *et al.*, 2000).

Other inherited neuropathies

Many gene loci have been mapped in families with other rare forms of hereditary neuropathies including genetically distinct forms of *hereditary motor neuropathies* (HMNs), *hereditary sensory and autonomic neuropathies* (HSANs) and *hereditary neuralgic amyotrophy* (HNA, also called familial brachial plexus palsy) (see table). Except for HSAN type IV (congenital insensitivity to pain with anhidrosis), which has been associated with mutations in the gene encoding TRKA/

NGF, a receptor tyrosine kinase for nerve growth factor, no genes have been identified in any of these disorders yet.

Molecular diagnosis of epileptic disorders
Ortrud K. Steinlein and Anna-Elina Lehesjoki

Although idiopathic epilepsies are thought to be mainly caused by genetic factors, not much is known today about the specific genetic mechanisms. Most forms of idiopathic epilepsy are probably caused by complex inheritance with several different genes contributing to the phenotype (Gardiner, 1999; Leppert and Singh, 1999). So far, only genes for some rare monogenic forms of idiopathic epilepsy have been described (Steinlein, 1998).

Familial nocturnal frontal lobe epilepsy (ADNFLE, MIM 600 513)

The syndrome is genetically heterogeneous. Two loci have been identified so far, the α4-subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4) on chromosome 20q13.3 and the β2 subunit (CHRN82 on chromosome 8q24). Mutations are clustered in the second transmembrane region, which can be tested by direct sequencing (Steirtein *et al*.., 1995; Phillips *et al*., 2001).

Benign familial neonatal convulsions (BFNC, MIM 121201, MIM 121200)

Again, mutations in different genes can cause this common epileptic syndrome (genetic heterogeneity). The major locus is a gene coding for a voltage gated potassium channel, KCNQ2 located on chromosome 20q13.3, accounting for more than 90% of cases with identified genetic defects. Molecular diagnosis is possible, but time-consuming because of the large size of the KCNQ-genes. At present, the probability to detect a mutation in families that show linkage to chromosome 20q is about 60%, but only 5% in sporadic cases or nuclear families (Biervert *et al*., 1998; Singh *et al*., 1998).

A minor locus has been mapped to chromosome 8q24 in one single family, the causative gene coding for another potassium channel, KCNQ3 (Charlier *et al*., 1998).

Generalized epilepsy with febrile seizures plus (GEFS+, MIM 604 236, MIM 604 233)

Febrile seizures are a clinically and genetically heterogeneous syndrome. Point mutations in two genes encoding voltage gated sodium channels have been identified (SCN1A and SCN1B).

Symptomatic epilepsies

There are over 150 rare Mendelian disorders, in which epilepsy occurs as part of a more complex phenotype. These include for example several neurodegenerative, neurodevelopmental and metabolic disorders. Significant advances have recently been made in elucidating the molecular genetic basis of several symptomatic epilepsies. In this context only disorders, in which epileptic seizures constitute a prominent component of the phenotype are summarized.

Progressive myoclonus epilepsy of Unverricht-Lundborg type (EPM1; chromosome 21q22.3, MIM 254 800) is caused by mutations in the gene encoding cystatin B (CSTB), a cysteine protease inhibitor. The most common underlying mutation world-wide is an unstable expansion of a dodecamer minisatellite repeat unit in the promoter region of CSTB. It accounts for approximately 90% of disease alleles. In addition, five point mutations have been described. Analysis for the dodecamer expansion can be used to confirm the clinical diagnosis of EPM1 (Lehesjoki and Koskiniemi, 1998).

Lafora's disease (EPM2A, MIM 254 780) is another form of progressive myoclonus epilepsy. Mutations in the gene encoding a novel protein tyrosine phosphatase ('laforin') on chromosome 6q24 are responsible for the primary defect in Lafora's disease. Two microdeletions and several DNA sequence variations segregating with the disease phenotypes have been identified. No single common mutation, like in EPM1, is responsible for Lafora's disease. The disorder is heterogeneous as some 20% of Lafora's disease families do not map to the EPM2A region on chromosome 6q24.

The *neuronal ceroid lipofuscinoses (NCLs)* are a genetically heterogeneous group of neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigment in many cell types. At least eight genes (CLN1–CLN8) are known to underlie NCLs. Five of these have been isolated and mutations characterized. The known NCL mutations are summarized at: http://http://www.ucl.ac.uk/ncl. The *CLN1* gene on chromosome 1p32 encodes for a lysosomal enzyme, palmitoyl-protein thioesterase (PPT). Mutations in PPT have been found to cause the *infantile NCL* (INCL; *MIM 256 730*) as well as a variant form of late infantile *NCL (*vLINCL) and a variant form of juvenile NCL (vJNCL). Altogether 37 mutations have been identified, three of which predominate. The CLN1 associated phenotypes all share a similar

ultrastructure of granular osmiophilic deposits. The *CLN2* gene on chromosome 11p15 also encodes a lysosomal enzyme, tripeptidyl peptidase (TPP1). The CLN2 gene underlies the *classical late infantile form of NCL* (cLINCL, *MIM 204 500*). Altogether 41 mutations have been described. Of these, two are predominant. The *CLN3* gene for juvenile NCL (JNCL, *MIM 204 200*) on chromosome 16p12 is a novel gene encoding a predicted membrane protein. Twenty-five mutations have been characterized. A 1.0-kb deletion removing exons 7 and 8 is the most common mutation in Europe and USA, whereas the other mutations have mostly been described from single families. The *CLN5* gene on chromosome 13q22 underlies the Finnish variant form of LINCL (MIM 256 731). Like *CLN3*, it encodes a novel predicted transmembrane protein. Four mutations have been described. *Progressive epilepsy with mental retardation* (EPMR, also called *Northern epilepsy* (*MIM 600 143*) was recently recognized as a new NCL subtype *CLN8*. It has so far been described only from Finland, where all patients are distant relatives. The underlying gene on chromosome 8p23 is the third NCL gene encoding a putative novel transmembrane protein. A single founder mis-sense mutation has been detected in the Finnish patients. The locus for *CLN6*, another variant LINCL (*MIM 601 780*) is located on chromosome 15q21-23, but the underlying gene has not yet been identified. A group of families previously designated CLN7 show evidence for linkage to CLN8 and may thus be allelic variants of this gene (Mitchel *et al.*, 2001). The locus for *CLN4* (adult form of NCL, MIM 204 300) remains to be mapped.

Molecular diagnosis of inherited cerebrovascular disorders
M. Dichgans

CADASIL

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by mutations in the gene for *Notch3*. Mutations are located within exons coding for epidermal growth factor (EGF)-like repeat domains. Mutations involve highly conserved cysteine residues. All mutations result in an uneven number of cysteines within EGF-like repeat domains. In the majority of cases point mutations are found. However, splice site mutations and small in-frame deletions have been reported. Mutations are strongly clustered with exons 3 and 4 which contain 70% of all mutations. Direct sequencing of these two exons is suggested as a first step if clinical suspicion is high (recurrent transient and lasting ischemic deficits starting in early to mid-adulthood, prominent white matter abnormalities and lacunar infarcts on MRI, and a family history compatible with dominant inheritance; no vascular risk factors). Screening of the 32 other exons encoding EGF-like repeat domains may be pursued as a second step. In about 10% of the cases no mutations are found (Joutel *et al.*, 1997).

The disease may also be diagnosed by (skin) biopsy. Ultrastructural examination of small arterioles reveals characteristic osmiophilic deposits within the vascular basal membrane.

Amyloid angiopathies

The majority of central amyloid angiopathies (CAA) occur sporadically and are not associated with any known mutations. Two Mendelian variants of CAA have been described: *cerebral amyloid angiopathy, Dutch type* (*MIM 104 760*), caused by a point mutation within the Amyloid Precursor Protein gene (guanine-to-cytosine at nucleotide 1852), resulting in a substitution of glutamine for glutamic acid at position 693. In *cerebral amyloid angiopathy, Icelandic type* (*MIM 105 150*), a point mutation within the Cystatin C gene results in a change of leucine in position 68 to glutamine. In both cases, mutations can be detected by PCR-amplification of the respective exon followed by restriction digest. The possibility of a molecular diagnosis should therefore be entertained in patients with early onset of recurrent cerebral hemorrhages in association with prominent white matter changes.

Cavernous malformations

Cerebral cavernous malformations (CCM) are vascular malformations causing hemorrhagic strokes and seizures. In up to 50% of cases the condition is inherited as an autosomal dominant trait with incomplete penetrance. Amongst Mexican-Americans, most CCM cases are the result of a founder mutation localized to chromosome 7q21–22 (CCM1, MIM 116 860). This locus also accounts for 40–65% of the non-Hispanic cases. The *CCM1* gene has recently been identified. Point mutations and frameshift deletions as well as frame shift insertions have been identified in families linked to this locus. All these mutations result in premature stop codons. Apparently, mutations are distributed over the entire coding sequence. Mutational analysis can be considered in patients with multiple cavernomas or a family history of cerebral hemorrhages/epileptic seizures. However, at-risk individuals may also be identified by MR-scanning.

Two additional loci CCM2 (MIM 603 284) and CCM3 (MIM 603 285) have been localized to chromosomes 7p13-15 and 3q25.2–27, respectively.

Stroke as a symptom of more complex Mendelian conditions

There are numerous Mendelian disorders, in which stroke occurs as part of a more complex phenotype (e.g. Marfan syndrome, Fabry's disease). These conditions are not discussed here.

Familial hemiplegic migraine (FHM, MIM 141 500)

Inheritance is autosomal dominant. Up to 40% of families are linked to a locus on chromosome 19p13. In some of these families point mutations have been found in a brain expressed calcium channel alpha1 subunit, CACNA1A. Point mutations in the same gene cause episodic ataxia type 2 (EA2), and the expansion of a CAG-repeat in this gene is responsible for spinocerebellar ataxia type 6 (SCA6) (see chapter on *Inherited ataxias*). There seems to be some overlap between all three conditions both clinically and genetically. About 40% of FHM families linked to this locus exhibit cerebellar signs. As in other disorders caused by a large number of different point mutations in a large gene, molecular diagnosis is rarely provided as a routine procedure and usually limited to a research setting.

About 8% of families are linked to a second locus on chromosome 1q (Ducros *et al*., 1997). There are also families in whom linkage to both loci has been excluded, indicating that at least one additional FHM locus is to be located. For review see (Terwindt *et al*., 1998).

Molecular diagnosis of neurocutaneous syndromes
Thomas Gasser

Congenital or hereditary conditions that feature lesions of both the skin and nervous system have been traditionally subsumed under the term of neurocutaneous disorders ('phakomatoses'). Several of these disorders have in common that they are characterized by the occurrence of hamartomas and a variety of tumors. These have also been collectively termed 'inherited tumor syndromes'. The most important of these are *neurofibromatosis type 1* (*MIM 162 200*) and *2* (*MIM 101 100*) (NF1 and 2), the tuberous sclerosis complex (TS) and von Hippel–Lindau disease (MIM 193 300).

These disorders are caused by a disturbance in cell cycle regulation and usually the consequence of mutations in tumor suppressor genes. The 'two-hit model' initially suggested for the development of retinoblastomas is still the favored pathogenetic concept: patients with these disorders carry germ-line mutations (which are also present in each cell of the body) in one of the tumor-suppressor genes. A single functional copy of the gene is still sufficient for adequate control of cell proliferation and differentiation. However, a second, mutation, that occurs in a somatic cell leads to a complete loss of function of the encoded gene product, leading to unregulated cell growth.

Most tumor suppressor genes are large, and many different (point-)mutations have been described. In addition, de novo mutations are fairly common. Molecular diagnosis therefore is often time-consuming and expensive. It may be nevertheless indicated, for example, to search for a mutation in an index patient in order to be able to perform exclusion testing in (as yet) asymptomatic siblings or children.

Neurofibromatosis type 1

Neurofibromatosis type 1 (NF1) is characterized by multiple café au lait spots and associated cutaneous findings of neurofibromas, plexiform neurofibromas, and axillary or inguinal freckling. Other manifestations include an increased risk for optic glioma, osseous lesions, and learning disability. The diagnosis of NF1 is based on clinical findings. Molecular testing by sequencing the entire NF1 gene is available, but is rarely utilized for clinical or pre-natal diagnosis.

NF1 is one of the most common dominantly inherited genetic disorders, occurring once in every 3000–4000 people. Half of all cases are new mutations. The mutation rate for the NF1 gene (1/10 000) is amongst the highest known for any gene in humans. The cause of the unusually high mutation rate for NF1 is unknown. For review see (Carey and Viskochil, 1999).

Neurofibromatosis type 2

Neurofibromatosis type 2 (NF2) is characterized by bilateral vestibular schwannomas with associated symptoms of tinnitus, hearing loss, and balance dysfunction (Gutmann, 1997). Other findings include schwannomas of other cranial and peripheral nerves, meningiomas, and juvenile posterior subcapsular cataract. The tumors of NF2 are derived from Schwann cells, meningeal cells, and glial cells. They are uniformly benign.

The main role of DNA-based testing is in early detection of at-risk individuals (primarily children of affected patients) for management reasons. Early recognition of NF2 may allow for earlier intervention and improved final outcome (Harsh *et al*., 1995), thus, surveillance of asymptomatic at-risk children for early

manifestations of NF2 is appropriate. Because the signs of NF2 are apparent in most individuals by the early 20s and because the age of onset is consistent within families in gene carriers. MRI scanning is usually begun in the teenage years and continued on an annual basis until at least the fourth decade of life.

Tuberous sclerosis complex (TSC)

Tuberous sclerosis complex (TSC) is characterized by abnormalities of the skin (hypomelanotic macules, facial angiofibromas, shagreen patches, fibrous facial plaques, ungual fibromas), brain (cortical tubers, subependymal nodules, seizures, mental retardation/developmental delay), kidney (angiomyolipomas, cysts), and heart (rhabdomyomas, arrhythmias) (Crino and Henske, 1999).

Two genes, TSC1 (MIM 191 100) on chromosome 9q34 and TSC2 on chromosome 16p13 have been identified. Between 60 and 80% of patients with TSC have an identifiable TSC mutation, with each of the TSC-genes being responsible for about half of the cases. The TSC1 and TSC2 (MIM 191 092) phenotypes are indistiguishable clinically, but a greater risk of renal malignancy and a higher frequency of mental retardation in patients with TSC2 has been reported.

Tuberous sclerosis complex (TSC) is inherited as an autosomal dominant trait. As in NF1, new mutations are common and found in approximately two-thirds of affected individuals. Because of the technical difficulties in molecular diagnosis caused by genetic and allelic heterogeneity, the causative mutation should be sought in an index patient first, if molecular diagnosis is desired in a family. This information can then be used to guide pre-symptomatic or pre-natal testing, given the appropriate counselling procedures.

Molecular diagnosis of mitochondrial diseases

Massimo Zeviani, Josef Finsterer and Thomas Klopstock

Classification

Mitochondrial diseases comprise a wide range of clinical phenotypes associated with failure of mitochondrial oxidative phosphorylation (OXPHOS). The best known morphological finding of these disorders is the transformation of scattered muscle fibers into 'ragged red fibers' (RRF), an accumulation of abnormal mitochondria under the sarcolemmal membrane. The identification of mutations of the mitochondrial DNA (mtDNA) has provided the basis for the current classification of mitochondrial disorders (Fig. 1). A first group of syndromes is characterized by the presence of sporadic or maternally transmitted mtDNA mutations. A second group is characterized by the association of mtDNA abnormalities with Mendelian transmission of the trait (defects of nucleo-mitochondrial signaling).

Finally, mutations in nuclear genes coding for mitochondrially located peptides may lead to mitochondrial disease in the presence of normal mtDNA. For review see Zeviani *et al.*, 1998.

Mutations of mtDNA

Under normal conditions, the mitochondrial genotype of an individual is composed of a single mtDNA species (homoplasmy). Mutations of mtDNA can lead to *heteroplasmy*, where the wild-type and the mutant genomes coexist intracellularly. Only when mutated copies of mitochondrial genomes accumulate over a certain threshold, the deleterious effects of the mutation will be expressed phenotypically. In addition,

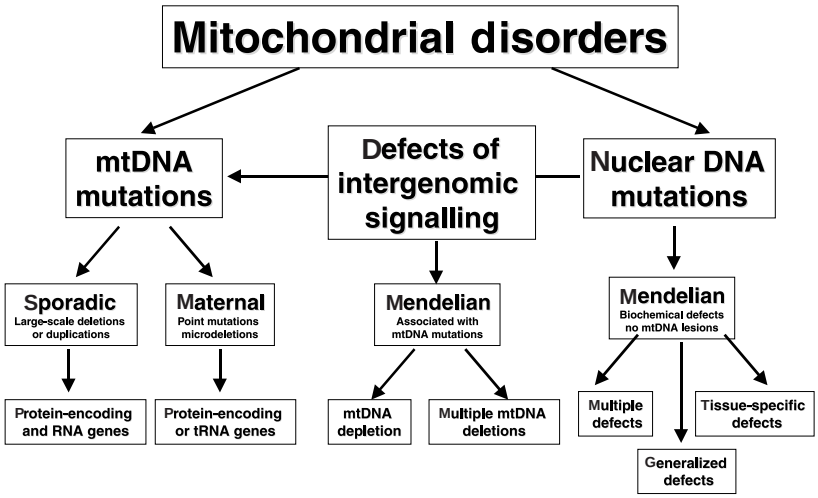

Figure 1 Classification of mitochondrial diseases.

phenotypic expression will depend upon the nature of the mutation, its tissue distribution, and the relative reliance of each organ system on the mitochondrial energy supply.

According to the molecular and genetic features of the mutation of mtDNA, clinical syndromes can be the result of:

1. Large-scale rearrangements of mtDNA.
2. Point mutations of mtDNA.

Rearrangements of mtDNA

Large-scale rearrangements of mtDNA can be either single partial mtDNA deletions or, more rarely, partial duplications. Both types of mutation are heteroplasmic (i.e. they coexist with wild-type mtDNA). Three main clinical phenotypes are associated with these mutations: *Kearns–Sayre syndrome* (KSS), *sporadic progressive external ophthalmoplegia* (PEO), and *Pearson's syndrome*.

Kearns–Sayre syndrome is a sporadic, severe disorder characterized by the invariant triad of PEO, pigmentary retinopathy, and onset before age 20. Frequent additional symptoms are a progressive cerebellar syndrome, heart block and increased protein content in the cerebro-spinal fluid (CSF).

Sporadic PEO is characterized by bilateral ptosis and ophthalmoplegia, frequently associated with proximal muscle weakness and exercise intolerance.

Pearson's bone marrow–pancreas syndrome is a rare sporadic disorder of early infancy characterized by sideroblastic anemia with pancytopenia and exocrine pancreatic insufficiency. Interestingly, infants surviving into childhood may develop the clinical features of KSS.

In most cases, the disorders caused by mtDNA rearrangements occur sporadically. Most probably, they originate during oogenesis in the patient's mother. Rarely maternal transmission of heteroplasmic partial duplications of mtDNA, but not of deletions, has been reported in association with familial diabetes mellitus and deafness. For diagnostic purposes, muscle biopsy is recommended because in both KSS and PEO, RRF are numerous, and mtDNA deletions can often be detected only in muscle DNA, but not in DNA isolated from peripheral blood lymphocytes.

Point mutations of mtDNA

Point mutations of mtDNA are usually maternally inherited, and affect mRNA, tRNA or rRNA genes. Four mutations are by far the most frequent. These are the A3243G 'MELAS/PEO', the G8344A 'MERRF', the T8993G 'NARP', and the A11778G 'LHON' mutations. However, many other mutations may occur, and some have been described in only single individuals or families.

Mitochondrial encephalomyopathy, lactic acidosis and strokelike episodes (MELAS) is defined by the presence of (i) stroke-like episodes as a result of focal brain lesions often localized in the parieto-occipital lobes, (ii) lactic acidosis and/or RRF. Other signs of central nervous system involvement include dementia, recurrent headache and vomiting, focal or generalized seizures, and deafness.

MELAS was first associated with a heteroplasmic point mutation in the $tRNA^{Leu(UUR)}$, an A → G transition at position 3243. Other MELAS-associated point mutations were later reported, although the A3243G remains by far the most frequent. The genotype-phenotype correlation of the A3243G mutation is rather loose because the A3243G mutation has been detected in several patients with maternally inherited PEO, isolated myopathy, cardiomyopathy, or in pedigrees with maternally inherited diabetes mellitus and deafness.

Myoclonus epilepsy with ragged-red fibers (MERRF) is a maternally inherited neuromuscular disorder characterized by myoclonus, epilepsy, muscle weakness and wasting, cerebellar ataxia, deafness and dementia.

The most commonly observed mutation of mtDNA associated with MERRF is an A → G transition at np 8344 in the $tRNA^{Lys}$ gene. Although the genotype–phenotype correlation between MERRF syndrome and the A8344G mutation is tighter than that of other mutations, the A8344G transition has also been reported in phenotypes as different as Leigh syndrome, isolated myoclonus, familial lipomatosis, and isolated myopathy.

Neurogenic weakness, ataxia and retinitis pigmentosa (NARP) is a maternally-inherited syndrome associated with a heteroplasmic T → G transversion at position 8993 in the ATPase 6 subunit gene. RRF fibers are absent in the muscle biopsy. When the percentage of mutant mtDNA is more than 95%, patients show the clinical, neuroradiologic and neuropathologic findings of Leigh syndrome (hence called MILS, maternally inherited Leigh syndrome). The two phenotypes of NARP and MILS may coexist in the same family.

Leber's hereditary optic neuropathy (LHON) is characterized by bilateral, acute or subacute loss of central vision because of optic atrophy. The visual defect can occasionally be associated with cardiac conduction abnormalities (pre-excitation syndrome). Penetrance of LHON is much higher in males than in females, and onset is usually in the second and third decade.

A total of 17 mtDNA mutations are now reported to be associated with LHON. However, based on numerous genetic, clinical and biochemical parameters, mutations at three nucleotide positions, np 11 778 (subunit 1 of complex I, ND1), np 3460 (subunit 4 of complex I, ND4), and np 14 484 (subunit 6 of complex I, ND6) encompass most of LHON families in all

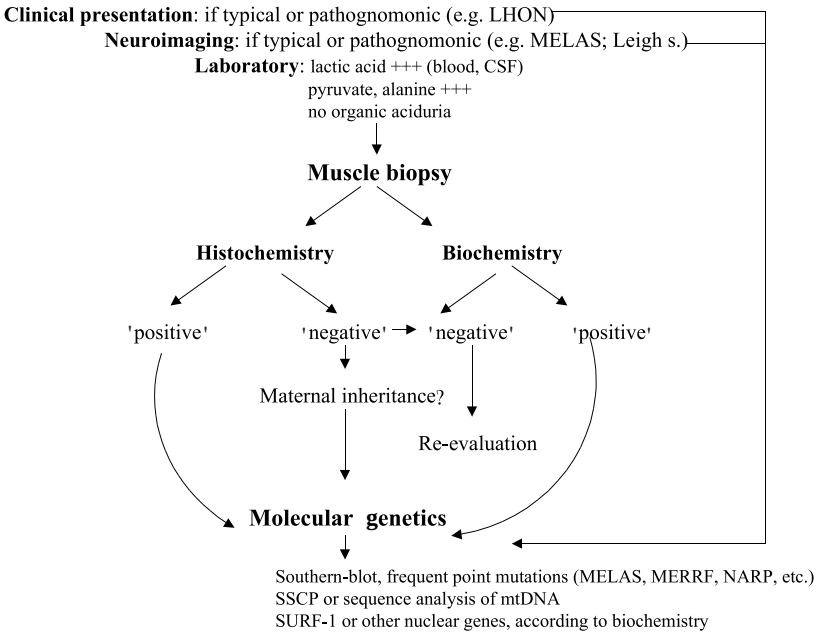

Figure 2 Diagnostic algorithm in mitochondrial disorders.

human populations, and are considered as high-risk and primary LHON mutations. A combination of LHON and dystonia has for long been recognized as a distinct entity. A pathogenic G → A transition at np 14 459 in the ND6 gene (complex I) has been reported in three independent LHON-dystonia pedigrees.

Other mtDNA point mutations

Several other point mutations of mtDNA have been detected in single patients or in pedigrees affected with different phenotypes. For instance, *myopathy and cardiomyopathy* (MMC) is associated with a number of different tRNA gene mutations, and aminoglycoside-induced or spontaneous, non-syndromic progressive deafness has been associated with a homoplasmic A → G transition at np 1555 in the 12S rRNA gene.

Defects of nucleo-mitochondrial signaling (Mendelian traits associated with mtDNA lesions)

Two groups of nucleus-driven mtDNA abnormalities have been described: *qualitative alterations* of mtDNA, i.e. multiple large-scale deletions of mtDNA and *quantitative decrease* of the mtDNA copy number, i.e. tissue-specific depletion of mtDNA.

Qualitative abnormalities: multiple familial mtDNA deletions

Autosomal-dominant or recessive PEO are examples of this group of disorders. Wild-type mtDNA coexists with several deletion-containing mtDNA species. It is thought that mutations in nuclear genes affect mtDNA replication. Linkage analysis has revealed the existence of two distinct adPEO disease loci on chromosome 10q and chromosome 4q. Recently, it has been established that the cause of the latter type of adPEO are mutations of ANT1, a gene encoding a muscle-specific ADP/ATP mitochondrial translocator. Mutations of the gene encoding thymidine phosphorylase, a key enzyme in the control of the cellular nucleoside pool, have been found in MNGIE (*myo-neuro-gastro-intestinal encephalomyopathy*), another autosomal-recessive disorder characterized by the presence of multiple deletions and/or partial depletion of mtDNA in muscle and other tissues.

Quantitative abnormalities: mtDNA depletion

Depletion of mtDNA is a disorder with Mendelian inheritance, whose clinical manifestations fall into three groups: (1) a fatal infantile congenital myopathy with or without a DeToni–Fanconi renal syndrome; (2) a fatal infantile hepatopathy leading to rapidly progressive liver failure; (3) a late infantile or childhood myopathy, with onset after 1 year of age, characterized by a progressive myopathy causing respiratory failure and death in late infancy. Southern blot analysis is diagnostic, demonstrating the severe reduction of mtDNA in affected tissues (up to 98% in the most severe forms). The presence of affected siblings born from healthy parents suggested an autosomal recessive mode of inheritance, possibly affecting a nuclear gene involved in the control of the mtDNA copy number.

Defects of nuclear genes resulting in mitochondrial phenotypes

In the recent past, important progress has been made in the identification of nucleus-encoded genes associated with OXPHOS defects.

These genes can be classified into two groups. The first group comprises nuclear genes encoding structural components of the respiratory chain complexes. Examples are genes encoding several subunits of complex I or complex II, whose mutations have been found in single patients affected by early onset OXPHOS failure, leading in most of the cases to Leigh syndrome.

The second group includes nuclear disease genes encoding factors involved in the formation, turnover, and function of the respiratory chain complexes, although they are not part of the protein components of any complex. The most important gene of this group is SURF-1, whose mutations are responsible for the most common type of *Leigh syndrome*, namely that associated with severe deficiency of cytochrome c oxidase (LS^{COX}). SURF-1 encodes an assembly factor of cytochrome c oxidase, the Surf-1 protein is absent in all tissues of affected LS^{COX} individuals (Tiranti *et al*., 1999).

Leigh syndrome (LS, MIM 256 000), or *subacute necrotizing encephalomyelopathy*, is an early onset progressive neurodegenerative disorder. Affected infants show severe psychomotor delay, cerebellar and pyramidal signs, dystonia, respiratory abnormalities, poor co-ordination of ocular movements, and recurrent vomiting. Focal symmetric lesions are found by MRI in the brainstem, thalamus and posterior columns of the spinal cord. RRF are consistently absent in the skeletal muscle. LS is a genetically heterogeneous entity. In some cases it is attributable to mtDNA mutations (e.g. the T8993G 'NARP/MILS' mutation), in others to an autosomal recessive defect of a nuclear gene. In still other cases, the defect is X-linked or sporadic, as in the case of the defect of the E1-alpha subunit of PDH. In any case, all defects described to date in patients with LS affect the terminal oxidative metabolism and probably impair energy production.

Diagnostic considerations

The diagnosis of mitochondrial disorders relies upon clinical, morphological, biochemical and molecular genetic studies (Fig. 2). A careful clinical examination is necessary to correctly address the subsequent diagnostic protocol.

Neuroimaging investigations have identified several patterns of involvement of the CNS. The observation of abnormalities within the brainstem, basal ganglia, white matter, or the presence of multiple cortico-subcortical areas of abnormal signal intensity may suggest the correct diagnosis, particularly in patients with atypical presentations. MRI spectroscopy is a non-invasive technique that can detect increased lactate levels in the brain, and low ATP production in both brain and muscle. Useful laboratory findings, especially in infants and children, include the presence of elevated levels of lactic acid and pyruvic acid in blood and CSF, and the absence of organic acidurias. The absence of RRF, or other morphological signs of mitochondrial pathology, does not necessarily exclude the diagnosis. The availability of muscle tissue is mandatory in patients with phenotypes associated with large-scale rearrangements of mtDNA, that can be easily missed in leukocytes. By contrast, point mutations of mtDNA can be detected also in blood samples. However, in maternally inherited phenotypes associated with heteroplasmic point mutations the percentage of mutant mtDNA is often significantly higher in muscle than in other tissues.

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