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SPECIAL ARTICLE

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

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I. General section

Introduction

Recent progress in molecular genetics has greatly improved our understanding of the molecular basis of many inherited neurologic diseases. The chromosomal position of a large number of genes which, when mutated, can cause neurologic disorders, is now known. In many cases the genes themselves and their disease causing mutations have been identified. This increasing wealth of knowledge has allowed the reclassification of a number of formerly heterogeneous clinical syndromes, provides novel diagnostic possibilities, and allows characterization of the pathologic gene products, thereby providing further insight into the molecular pathogenesis of these disorders and eventually opening up new approaches towards therapy and prevention. Today and in the years to come, the rapid advances in neurogenetics will be a challenge to neurologists everywhere.

In clinical practice, the availability and the limitations of molecular diagnosis depend on our knowledge of the molecular genetic basis of the respective disease, but also on the degree of genetic complexity of the disorder under investigation. Some diseases, such as Huntington's disease, are caused by a specific mutation in a single gene (Huntington's Disease Collaborative Research Group, 1993), and routine molecular diagnosis can be provided by a simple polymerase chain reaction (PCR)-based assay. In other cases, however, many different mutations may underlie a disorder (allelic heterogeneity). Depending on the size of the gene(s), this may render molecular diagnosis very costly and time-consuming. In addition, molecular diagnosis may be further complicated by the fact that mutations in a number of different genes may cause similar or indistinguishable phenotypes (genetic heterogeneity).

Despite the fact that today only a small percentage of neurogenetic disorders can be treated efficiently, molecular diagnosis is increasingly important, because it may provide valuable information for the affected individuals and their families in order to make informed choices on life and family planning.

These *guidelines* 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. Genetic classification of disorders follows, if applicable, the most comprehensive catalogue of human hereditary diseases, the 'Online Mendelian Inheritance in Man (MIM)' (http://www.ncbi.nlm.nih.gov.omim), which is maintained by the National Center of Biotechnology Information (NCBI). 'MIM-numbers' are given in the text and in the Table 1 for easy reference.

General principles of molecular diagnosis

The primary goal of molecular diagnosis is to provide help for the individual patient, client and/or their families. Reducing the prevalence of inherited disorders in a population or in subsequent generations may be a

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Table 1 Inherited Ataxias

Clinical classification	SCA locus	Normal repeat size	Disease repeat size	MIM
ADCA I	SCA 1 (Chr 6p)	6–38	39–80	164 400
Ataxia with: ophthalmoplegia, optic atrophy,	SCA 2 (Chr 12q)	16–30	36–52	183 090
dementia, or extrapyramidal features (including	SCA 3 (Chr 14q)	14–40	60–85	109 150
Machado–Joseph's disease)	SCA 4 (Chr 16q)	Linkage only		600 223
ADCA II	SCA 7 (Chr 3p)	7–19	37–220†	164 500
Ataxia with: pigmentary. Maculopathy with or without ophthalmoplegia or extrapyramidal features				
ADCA III	SCA 5 (Chr 11p)	Linkage only		600 224
'Pure' ataxia	SCA 6* (Chr 19)	5–20 (calcium channel)	21–28	183 086
	SCA 11 (Chr 15q)	Linkage only		
	SCA 8	Repeat (see text)	? See text	603 680
Periodic autosomal dominant ataxia	EA 1 (Chr 12)	Potassium channel	Point mutations	160 120
	EA 2 (Chr 19)	Calcium channel	Point mutations	108 500

* SCA 6 can also be classified as ADCA I, as occasional additional features are found (see text).
† Intermediate allele 28–35 has been reported, these may expand in paternal transmission into the disease range.

secondary effect, but must never be allowed to guide the process of genetic counselling.

Genetic counselling

It must always be kept in mind that the molecular genetic diagnosis of an inherited disorder affects not only the patient, but also the entire family. Therefore, genetic counselling is an essential component of diagnosis of inherited disorders. Sensitive and informed counselling provides patients and families a foundation for decisions about testing. Patients should be counselled as to the clinical features and course of the respective disease as well as to potential consequences for the family, taking into consideration the most important genetic parameters such as mode of inheritance and penetrance. If the treating neurologist does not have thorough experience with inherited disorders, counselling by an experienced counsellor from a department of human genetics or another genetic counselling unit is strongly advised. In most situations, genetic testing should not be performed until adequate counselling has been provided.

In the case of predictive testing (see below), psychological counselling by appropriately trained persons is essential and mandatory before testing and after results have been disclosed.

Informed consent

As is true for all diagnostic procedures, the essential prerequisite for molecular diagnosis is the informed and voluntary consent of the patient. Therefore, the neurologist should establish that a patient or lawful surrogate is capable of comprehending relevant information and capable of exercising informed choices. Molecular genetic diagnostic tests should not be performed at the request of members of the patients' families or other third parties (e.g. insurers, employers) without the express written consent of the patient.

Confidentiality

Test results suggesting that patients or family members carry mutations that indicate or predict a major neurologic disorder or a susceptibility to a neurologic disorder are highly sensitive. Therefore, rigorous measures to ensure confidentiality should be taken. Test results should never be disclosed to a third party without explicit written consent from the patient or their lawful surrogates.

Pre-symptomatic diagnosis

The identification of disease genes allows pre-symptomatic (predictive) diagnosis in many cases. Guidelines for pre-symptomatic diagnosis have been issued by the International Huntington's Disease Society and the World Federation of Neurology for Huntington's disease (International Huntington Association and the World Federation of Neurology Research Group on Huntington's Chorea, 1994). These guidelines which include extensive pre- and post-test counselling and thorough psychological support during the extensive process should be followed in all cases of pre-symptomatic diagnosis. Generally, pre-symptomatic diagnosis should be provided within the setting of a department of Human Genetics. If no clear therapeutic consequences can be envisioned, pre-symptomatic diagnosis should not be performed in minors.

Principles of molecular diagnosis

'Direct' molecular diagnosis

If the gene causing a neurologic disorder is known, direct molecular diagnosis can be performed by mutational analysis. Only DNA from the affected individual is required. Usually, exons which are known to harbour mutations in the particular disorder will be amplified from DNA which has been extracted from peripheral blood leucocytes by PCR. Depending on its type, the mutation will then be detected either directly by gel electrophoresis (e.g. in the case of trinucleotide repeat expansions), digestion by restriction enzymes or by direct sequencing. If a gene is very large (genes with more than 30 exons are not uncommon) and mutations are scattered throughout the entire gene, direct mutational analysis can be very costly and time-consuming. In these cases, routine sequence analysis is sometimes offered only for portions of a gene, where mutations may be clustered (e.g. in CADASIL, where 70% of mutations are found in exons 3 and 4 of the Notch3 gene).

'Indirect' molecular diagnosis

Knowledge of the chromosomal position of a disease gene allows molecular support for the diagnosis, even if the disease gene itself is unknown or if analysis is unfeasible. 'Indirect' molecular diagnosis is limited to risk determination for an individual in whose family an inherited neurologic disease has already been diagnosed clinically. The method is based on the analysis of DNA markers known to be closely linked to the disease under investigation. Determination of these marker alleles in healthy and affected family members allows the identification of the disease-gene bearing chromosome in this particular family. It must be emphasized that accurate clinical diagnosis in at least one affected family member is an absolute prerequisite for this type of molecular diagnosis and there is a small but definite error rate with this sort of linkage analysis. Until the gene for Huntington's disease was identified in 1993, predictive testing for HD was the most widely used application for indirect molecular diagnosis. As more and more disease genes are identified, direct mutational analysis becomes increasingly important.

Practical approach to molecular diagnosis

The patient confirms his informed consent to the procedure in writing (see form attached). Usually, 10–20 ml of whole blood (EDTA) are sufficient. The blood can be mailed without freezing or refrigeration. Delay of 3 to 5 days before DNA extraction is acceptable. It is crucial, that the tubes are clearly labelled, and that the clinical information including family history and the informed consent are contained within the shipment.

Section II of these guidelines 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. References are limited to recent reviews and some key articles. This section will appear in two parts in this and the next issue of the *European Journal of Neurology*.

Table 2 Limb-girdle muscular dystrophies*

Gene	Chromosome	Protein	MIM
Autosomal dominant variants			
LGMD1A	5q22-q34	?	159 000
LGMD1B	1q11-21	?	159 001
LGMD1C	3p25	CAV3	601 253
LGMD1D	6q23	?	603 511
LGMD1E	5q31	?	
LGMD1F	7q	?	
Autosomal recessive variants			
LGMD2A	15q15.1-q21.1	CANP3	253 600
LGMD2B	2p13	DYSF	253 600
LGMD2C	13q12	γ – Sarcoglycan	253 700
LGMD2D	17q12-q21.33	α – Sarcoglycan	600 119
LGMD2E	4q12	β – Sarcoglycan	253 700
LGMD2F	5q33-q34	δ – Sarcoglycan	253 700
LGMD2G	17q11-q12	?	253 700
LGMD2H	9q31-q34.1	?	254 110

* From neuromuscular disorders 1999, 9, I.

A Table 2 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.

From a practical point of view, the availability of molecular diagnosis for a particular disorder will be classified as follows:

A: Routine diagnosis: The institution is able to provide a molecular diagnosis generally within 4 weeks. This applies particularly to diseases which are caused by expansions of repetitive trinucleotide sequences or other specific mutations.

B: Molecular diagnosis is a routine procedure but limited by the complexity of the disorder. This usually applies to diseases which may be caused by a number of different point mutations within a gene, requiring sequencing of a number of exons. This can be time consuming and expensive. Personal contact before requesting this type of diagnosis is advisable.

C: Molecular diagnosis within research projects: In these cases personal contact with the institution performing the DNA analysis is mandatory.

D: Molecular diagnosis is not yet available.

More important information on molecular diagnosis of inherited disorders can be found on the following internet pages:

'GeneClinics', a clinical information resource relating genetic testing to the diagnosis, management, and genetic counselling. University of Washington, Seattle. http://www.geneclinics.org/

Online catalogue of Mendelian disorders and traits in man (Online Mendelian Inheritance in Man, OMIM) http://omim.nih.org

MITOMAP: A Human Mitochondrial Genome Database. Center for Molecular Medicine, Emory University, Atlanta, GA, USA. http://http://www.gen.emory.edu/mitomap.html

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

Because of the large number of disorders covered, this section of the guidelines will be published in this and the next issue of the *European Journal of Neurology*. In the current issue, non-degenerative movement disorders, inherited ataxias, neurodegenerative disorders, dementias and myopathies and muscular atrophies will be covered. The upcoming issue will deal with skeletal muscle channelopathies, neuropathies, epilepsies, neurovascular disorders, neurocutanous syndromes and mitochondrial diseases.

Molecular diagnosis of non-degenerative movement disorders

Thomas Gasser, Huw Morris, Tim Lynch

The clinically and etiologically heterogeneous syndromes of non-degenerative movement disorders, that have traditionally been classified phenomenologically according to the predominant abnormal movement are now being reclassified on the basis of the underlying molecular defect. Because of this marked heterogeneity, however, molecular diagnosis is practically available only in a relatively small proportion of cases.

The primary dystonias

Primary dystonias are characterized by involuntary muscle contractions leading to twisting and repetitive movements with no discernible structural or metabolic cause. A number of distinct genetic forms have been identified, but only in some instances, molecular diagnosis is routinely available (Muller *et al.*, 1998).

Generalized torsion dystonia (MIM 128 000)

A deletion of the trinucleotide GAG (encoding glutamic acid) in the gene for torsin A on chromosome 9q34 has been identified in patients with autosomal-dominantly inherited *early onset generalized dystonia* (*DYT1*) (Ozelius *et al.*, 1997). The vast majority of patients with this mutation have dystonia beginning in the first or second decade, usually in an extremity, and progressing relatively rapidly to a generalized form. Approximately 50% of patients with this phenotype will carry a mutation, and molecular diagnosis is warranted and easily available in this group. In some instances, the mutations can also be found in patients with early onset (< 25 years) focal, segmental and multifocal dystonias with onset in an extremity, but occurs extremely rarely, if at all, in patients with typical adult-onset cervical or cranial dystonia. Important causes of secondary dystonia (perinatal hypoxia, Wilson's disease) should have been excluded. The reduced penetrance of about 30% has to be taken into account during the counselling process. This also means that a positive family history is supportive, but not a necessary prerequisite to consider DYT1-dystonia (Bressman *et al.*, 2000).

Other variants of dystonia

A rarer variant of dominant primary dystonia, *dopa-responsive dystonia* (*MIM 128 230*) is caused by point-mutations in the gene for GTP-cyclohydrolase I in the

majority of cases (Furukawa and Kish, 1999). Again, the phenotype is usually characterized by a childhood onset of dystonia, affecting the extremities first, but rarely, craniocervical dystonia may be the only manifestation. As a large number of different mutations have been described that are scattered over the entire gene (and could not even be detected in all cases within the coding region), the practical role of molecular diagnosis is limited. Fortunately, a suspicion of dopa-responsive dystonia can usually be confirmed by the excellent response to L-dopa treatment, so that molecular analysis is frequently not necessary.

In other forms of dystonia, such as the *myoclonus–dystonia syndrome* (*MDS, MIM 159 900*), or '*rapid-onset dystonia-parkinsonism*' (*MIM 128 235*), which are listed in the Table 3, genes have been mapped but not cloned, and molecular diagnosis is not available.

Other movement disorders

Sequence analysis of the ATP7B gene causing *Wilson's disease* (*MIM 277 900*) is possible in individual patients. Approximately 55% of cases in a Caucasian population harbour mutations exons 7, 8, 14, 15, or 18. The other mutations are scattered more or less evenly across the rest of the gene. Identification of a mutation in an index patient in a family allows pre-symptomatic testing in other at risk family members, which may be particularly important in this disease, because preventive and therapeutic measures can be taken. However in most cases, laboratory studies, particularly copper excretion in urine, is the diagnostic method of choice in Wilson's disease.

Although *essential tremor* (*MIM 190 300*) is the most prevalent movement disorder and autosomal dominant inheritance is common, only two genetic loci have been mapped, but no gene has been cloned so far. No locus is known for the *restless legs-syndrome* (*MIM 102 300*), another common movement disorder with frequent autosomal dominant inheritance. Problems of genetic heterogeneity or uncertainties in defining the clinical phenotype may be responsible.

Movement disorders in the setting of neurodegeneration (such as Huntington's disease, Parkinson's disease) are dealt with in the section on *Neurodegenerative disorders*.

Molecular diagnosis of inherited ataxias
Nicholas Wood

There has been rapid progress in our understanding of the molecular basis of the inherited ataxias (Klockgether and Dichgans, 1997). Although as single entities these disorders are rare, cumulatively they represent a major health burden. Moreover, the question often arises as to the role of the currently identified genes in idiopathic/sporadic ataxias.

This section is divided into two main parts the autosomal recessive and autosomal dominant ataxias. X-linked inheritance is exceedingly rare and the clinical picture is usually predominated by other features.

Autosomal recessive ataxias

Friedreich's ataxia (*FA, MIM 229 300*) is the most common of the autosomal recessive ataxias and accounts for at least 50% of the cases of hereditary ataxia in most large series reported from Europe and the United States. The prevalence of the disease in these regions is similar, between 1 and 2 per 100 000. The disease usually starts in the first or second decade, is relentlessly progressive and is characterized by additional features including pyramidal signs, large fibre neuropathy, skeletal abnormalities (pes cavus and scoliosis), cardiomyopathy, and more rarely optic atrophy, deafness and diabetes. Cases considered atypical for Friedreich's ataxia (e.g. with onset after age 25 or with retained reflexes) have now been recognized as manifestations of Frataxin gene mutations.

The gene for Friedreich's ataxia is located on chromosome 9q13. The predominant mutation is a trinucleotide repeat (GAA) in intron 1 of this gene. Expansion of both alleles was found in most patients. In a minority of patients point mutations are found on one allele and an expansion on the other. This is the first autosomal recessive condition found to be the result of a dynamic repeat. On normal chromosomes the number of GAA repeats varies from 7 to 22 units, whereas on disease chromosomes, the range is anything from around 100–2000 repeats.

The rarity of point mutations means that it is extremely improbable that a case of FA will have two point mutations and therefore a normal sized repeat length on both chromosomes argues strongly against a diagnosis of Friedreich's ataxia. Some laboratories offer sequencing of the *frataxin* gene as a service to complement the GAA repeat testing. It should be noted that one of the most frequent requests to a DNA lab is to test for carrier status in the partner of a Friedreich's patient. It should be emphasized to the partner that the test (i.e. the GAA repeat) is not perfect as the assay will not detect point mutations. If no abnormal repeat is detected the chance of an affected offspring is less than 1 : 10 000.

Other autosomal recessive ataxias

There is a long list of other inherited ataxic syndromes, most of which remain to be elucidated on the molecular

Table 3 Neurogenetic disorders

Disease	Symbol	Inheritance	Position	Gene product	Mutation	Molecular diagnosis	Reference	Remarks	MIM-number
Ataxias									
Friedreich's Ataxia	FRDA	AR	9q13-21.1	Frataxin	Trinuc/Pm	A	Campuzano *et al.*, 1996	Most common form of recessive ataxia	229 300
Spinocerebellar ataxias	SCA1	AD	6p21.3	Ataxin 1	Trinuc	A	Orr *et al.*, 1993	SCA1, 2, and 3 comprises approx. 60% of the dominant hereditary spinocerebellar atrophies	164 400
	SCA2	AD	12q23-24.1	Ataxin 2	Trinuc	A	Gispert *et al.*, 1993		183 090
	SCA3/MJD	AD	14q24	Ataxin 3	Trinuc	A	Kawaguchi *et al.*, 1994		109 150
	SCA4	AD	16q22.1	Unknown	Unknown	D	Flanigan *et al.*, 1996	Individual families	600 223
	SCA5	AD	11cen	Unknown	Unknown	D	Ranum *et al.*, 1994	Individual families	600 224
	SCA6	AD	19p13	Calcium-channel	Trinuc	A	Zhuchenko *et al.*, 1997	Allelic to FHM and EA2	183 086
	SCA7	AD	3p12-21.1	Ataxin 7	Trinuc	A	Benomar *et al.*, 1995		164 500
	SCA8	AD	13q21	Ataxin 8	Trinuc?	C	Koob *et al.*, 1999	Pathogenic role of expansion still controversial (Worth *et al.*, 2000)	603 680
	SCA10	AD	22q13	Unknown	Unknown	D	Zu *et al.*, 1999	Pure cerebellar ataxia	603 516
	SCA11	AD	15q	Unknown	Unknown	D	Worth *et al.*, 1999		
	SCA12	AD	5q31-q33	Protein phosphatase 2	Trinuc	A	Holmes *et al.*, 1999		604 326
Episod. ataxia with myokymia	EA1	AD	12p13	Potassium-channel	Pm	B	Browne *et al.*, 1994		160 120
Episod. ataxia without myokymia	EA2	AD	19p13	Calcium-channel	Pm	B	Ophoff *et al.*, 1996	Allelic to FHM and SCA6	108 500
Ataxia with vitamin deficiency	AVED	AR	8q13.1-13.3	Tocopherol transfer-protein	Pm	C	Ouahchi *et al.*, 1995		277 460
Movement disorders									
Huntington's chorea	HD	AD	4p16.3	Huntingtin	Trinuc	A	Huntington's Disease Collaborative Research Group, 1993	143 100	
Wilson's disease	WND	AR	13q14.1	Copper transport-protein	Pm/Del	B	Tanzi *et al.*, 1993		277 900
Primary torsion dystonia	DYT1	AD	9q34	Torsin A	GAG-deletion	A	Ozelius *et al.*, 1997	Early onset, generalized, rarely isolated writer's cramp	128 100
X-chromosomal dystonia-Parkinson-syndrome	DYT3	XL	Xq11.2	Unknown	Unknown	D	Graeber and Muller, 1992	Only found on the Philippines	314 250
Primary dystonia, mixed type	DYT6	AD	8cen	Unknown	Unknown	D	Almasy *et al.*, 1997	Presently only described in 2 families	602 629
Primary dystonia, focal type	DYT7	AD	18p13.1	Unknown	Unknown	D	Leube *et al.*, 1996	Founder effect in European populations	602 124
Dopa-responsive dystonia	DYT5, DRD	AD	14q22	GTP-cyclohydrolase I	Pm	B/C	Ichinose *et al.*, 1994	No mutations found in some cases	600 225
Dopa-responsive dystonia	DYT5, DRD	AR	11p15.5	Tyrosine hydroxylase	Pm	C	Knappskog *et al.*, 1995	Individual case reports	191 290

Rapid-onset dystonia-parkinsonism	DYT12, RDP	AD	19q13	Unknown	Unknown	D	Kramer *et al.*, 1999	Only some families described	128 235
Paroxysmal dystonia	FPD1	AD	2q33-35	Unknown	Unknown	D	Fouad *et al.*, 1996		118 800
Paroxysmal kinesigenic choreoathetosis	PKD1	AD	16cen	Unknown	Unknown	D	Bennett *et al.*, 2000		128 200
Myoclonus dystonia syndrome	MDS	AD	7q	Unknown	Unknown	D	Nygaard *et al.*, 1999		159 900
Dentatorubropallidoluysian atrophy	DRPLA	AD	12p13.31	DRPLA-protein	Pm	A	Yazawa *et al.*, 1995	Rare in Europe	125 370
Familial Parkinson's disease	PARK1	AD	4q21	Alpha-synuclein	Pm	C	Polymeropoulos *et al.*, 1997	Very rare, mediterranian founder effect	601 508
Autosomal-recessive juvenile parkinsonism	PARK2, AR-JP	AR	6q25-27	Parkin	Del, Ins, Pm	B	Kitada *et al.*, 1998	No Lewy-body-pathology, relatively common in juvenile PD	602 544
Familial Parkinson's disease	PARK3	AD	2p13	Unknown	Unknown	D	Gasser *et al.*, 1998	North German founder effect	602 404
Familial hyperekplexia	STHE	AD	5q32	Glycine receptor	Pm	C	Shiang *et al.*, 1993		149 400
Essential tremor	ETM1	AD	3q13	Unknown	Unknown	D	Gulcher *et al.*, 1997		190 300
	ETM2	AD	2p14	Unknown	Unknown	D	Higgins *et al.*, 1997		602 134
Neuromuscular diseases and myopathies									
Spinal muscular atrophy	SMA I	AR	5q11.2-13	Survival motoneuron (SMN)	Del	A	Lefebvre *et al.*, 1995	SMAI, SMAII and SMAIII are allelic	253 300
infantile (Werdnig–Hoffmann)	SMA II	AR	5q11.2-13	SMN	Del	A	Roy *et al.*, 1995		253 300
juvenile (Kugelberg–Welander)	SMA III	AR	5q11.2-13	SMN	Del	A			253 300
Bulbospinal muscular atrophy	XBSN	X	Xq13-22	Androgen receptor	Trinuc	A	La Spada *et al.*, 1991		313 200
Duchenne	DMD	XL	Xp21.2	Dystrophin	Del/Dupl/Pm	A	Koenig *et al.*, 1987		310 200
Becker	BMD	XL	Xp21.2	Dystrophin	Del/Dupl/Pm				
Emery Dreifuss-Myopathy	EDMD	XL	Xq28	Emerin	Del/Ins/Pm	C	Bione *et al.*, 1994		310 300
	EDMD-AD	AD	1q11-q23	Lamin A/C	PM	D	Bonne *et al.*, 1999		181 350
Myotonic dystrophy (Curschmann's disease)	DM	AD	19q13.3	Myotonin	Trinuc	A	Brook *et al.*, 1992	Most common inherited myopathy	160 900
Facioscapulohumeral dystrophy	FSHD	AD	4qter	Unknown	Unknown	A	Wijmenga *et al.*, 1992		158 900
Limb–girdle myopathy	LGMD1A	AD	5q22-q31	Unknown	Unknown	D	Bartoloni *et al.*, 1998		159 000
	LGMD1B	AD	1q11-21	Unknown	Unknown	D	van der Kooi *et al.*, 1997		159 001
	LGMD2A	AR	15q15-q21	Calpain 3	Pm/Del	B	Richard *et al.*, 1995		253 600
	LGMD2B	AR	2p16-p13	Unknown	Unknown	D	Bashir *et al.*, 1998		253 601
	LGMD2C	AR	13q12	γ-Sarcoglycan	Pm	B	Noguchi *et al.*, 1995		253 700
	LGMD2D	AR	17q12-q21	Adhalin	Pm	B	Roberds *et al.*, 1994		600 119
	LGDM2E	AR	4q12	β-Sarcoglycan	Pm	B	Lim *et al.*, 1995		604 286
	LGMD2F	AR	5q33-q34	γ-Sarcoglycan	Pm	B	Moreira *et al.*, 1998		601 287
	LGMD2G	AR	17q11-q12	Unknown	Unknown	C	Moreira *et al.*, 1997		601 287
	LGMDH2H	AR	9q31-q33	Unknown	Unknown	C	Weiler *et al.*, 1998		254 110
Myotubular myopathy	MTM1	XL	Xq28	Myotubularin	Pm	C	Laporte *et al.*, 1996	Congenital myopathy	310 400

Table 3 (Continued)

Disease	Symbol	Inheritance	Position	Gene product	Mutation	Molecular diagnosis	Reference	Remarks	MIM-number
Central core diseases	CCD	AD	19q12-q13	Ryanodine receptor	Pm	C	Zhang *et al.*, 1993		117 000
Malignant hyperthermia	MH	AD	19q12-q13	Ryanodine receptor	Pm	C	Monnier *et al.*, 1997; Quane *et al.*, 1993		145 600

Note: This compilation of inherited neurologic disorders is not complete. The selection of the listed diseases was chosen relatively subjectively, 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; 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 as the result of occurrence of multiple mutations; results may take several months; C: Usually available only within research setting; D: Not yet available.

level. The genes responsible for *ataxia associated with vitamin E deficiency* (AVED, MIM 277 460) and that causing *ataxia telangiectasia* (AT, MIM 208 900) have been cloned. There is allelic heterogeneity (see above) and routine testing is not widely available.

Autosomal dominant ataxias (ADCA)

The autosomal dominant ataxias (ADCAs) are a clinically and genetically complex group of neurodegenerative disorders. ADCA type I is characterized by a progressive cerebellar ataxia, variably associated with other neurological features such as ophthalmoplegia, optic atrophy, peripheral neuropathy, pyramidal and extrapyramidal signs. The presence and severity of these signs is, in part, dependent on the duration of the disease. Mild or moderate dementia may occur but it is usually not a prominent early feature. ADCA type II is clinically distinguished from the ADCA type I by the presence of pigmentary macular dystrophy, whereas ADCA type III is a relatively 'pure' cerebellar syndrome and generally starts at a later age. This clinical classification is still useful, despite the tremendous improvements in our understanding of the genetic basis, because it provides a framework which can be used in the clinic and helps direct the genetic evaluation.

The nomenclature of the genes has been based on the term *spinocerebellar ataxia* (SCA). The SCAs are numbered in chronological order.

To date molecular diagnosis is routinely available for SCAs 1, 2, 3, 6, and 7. These disorders have a number of common features including onset in adult life (predominantly) and a progressive course. Additional features (see above) may vary both within and between families. At a molecular level, although the genes encode very different proteins, the mutation is basically same, namely an expanded trinucleotide repeat sequence of CAG (Brice, 1998). All so far described are the results of a relatively modest expansion within the coding region. Although the exact number of repeats on both the normal and the abnormal allele varies between the different diseases, the normal range of repeats is in the 20 s, whereas for the disease carrying allele it tends to be over 40. The codon CAG encodes the amino acid glutamine and therefore all these disorders have an expanded polyglutamine tract within the relevant protein. The exact pathogenic mechanism remains unknown.

The assays are PCR based and relatively simple and quick to perform. With only some exceptions it is unusual to find a positive SCA result in a sporadic case but if clinically suspected then it is still reasonable to include SCA testing in such patients.

Recently SCA 8 has been reported to be caused by a much larger repeat sequence which is not translated into protein (i.e. different from the other SCAs). It still remains to be established if this repeat is definitely pathogenic, as expansions have been found in several asymptomatic individuals.

The rare autosomal dominant *periodic* (*episodic*) *ataxias* are ion channel disorders and require sequencing of the entire gene(s) to identify the mutations. As the genes for ion channels are usually very large, routine sequencing is prohibitively expensive. However, sequencing may be done by research groups who are interested in these disorders (see chapter by Jurkat-Rott and Lehmann-Horn on *Molecular diagnosis of channelopathies*).

Molecular diagnosis of neurodegenerative disorders
Thomas Gasser, Huw Morris, Tim Lynch

Huntington's disease

Huntington's disease (HD, *MIM 143 100*) is the 'prototypic' neurogenetic disorder. It is usually characterized by the triad of a choreic movement disorder, cognitive decline and personality changes. Clinical manifestations may be highly variable, however, and particularly in juvenile cases, akinesia, rigidity, or epileptic seizures may occur. The disease is caused by the expansion of a CAG-triplet in the first exon of the gene for huntington, leading to the formation of an elongated polyglutamine domain within the protein (Huntington's Disease Collaborative Research Group, 1993; Brice, 1998) (analogous to the SCAs). Molecular diagnosis is technically simple, as with other trinucleotide repeat diseases.

The indications and consequences of diagnostic and pre-symptomatic molecular diagnosis have been studied widely in HD. Based on these experiences, guidelines have been developed, that should be followed, in adapted form, also in the genetic diagnosis of other neurologic disorders (The American College of Medical Genetics/American Society of Human Genetics Huntington Disease Genetic Testing Working Group, 1998). In particular, a thorough counselling process must ensure that only patients and families who can deal appropriately with their knowledge are selected. This is of course particularly relevant for pre-symptomatic testing.

As in other disorders caused by trinucleotide repeat expansions, onset of age and severity of the disease is inversely correlated to the size of the expansion. In suspected HD-cases with early onset it must be remembered, that parents may carry smaller size repeat expansions and may manifest the disease after their offspring. Molecular diagnosis in a child may therefore result in inadvertent pre-symptomatic testing in a parent (Scheidtmann *et al*., 1997).

Parkinson's disease

Until recently, the role of genetic factors in the etiology of Parkinson's disease (PD), has not been widely recognized. Today we know that mutations in several genes are able to cause monogenically inherited forms of Parkinson's disease (Gasser, 1999). The discovery that mutations in the gene for α-synuclein can cause an early onset form of PD (MIM 168 601) is particularly interesting in the light of the fact that the α-synuclein protein is one of the major components of the histopathologic hallmark of PD, the Lewy body. Mutations in this gene are exceedingly rare, so that sequencing is not routinely warranted, except in early onset cases with a clear dominant family history.

Mutations in the parkin gene on chromosome 6, which have been found in patients with an autosomal–recessive syndrome of juvenile parkinsonism appear to be causative in a substantial portion of patients with L-dopa responsive parkinsonism and juvenile onset of the disease. Mutations can be found in up to 75% of those with disease onset under 20, and still about 5% with onset under 40 (Lücking *et al*., 2000). Genetic diagnosis may be technically difficult, as point mutations as well as homozygous and heterozygous exon deletions have been described, necessitating full sequence analysis as well as gene dosage studies. Other genes or loci associated with PD still have no role in molecular diagnosis.

Amyotrophic lateral sclerosis

Less than 10% of cases with ALS (*MIM 105 400*) show dominant inheritance, and only about 20% of those are caused by mutations in the gene for superoxide dismutase 1 (SOD1, *MIM 147 450*) on chromosome 21. Despite the low prevalence of these mutations, sequencing of the relatively small SOD1-gene should be considered in patients from families with clear dominant inheritance, in order to be able to offer pre-symptomatic or pre-natal diagnosis, if this is wanted by family members.

Familial spastic paraplegias

The familial spastic paraplegias are a heterogeneous group of neurodegenerative disorders that are characterized by a slowly progressive pyramidal tract dysfunction, which occurs in some cases in isolation, in

other cases in association with a variety of other neurologic signs and symptoms (Reid, 1999). As in the cerebellar ataxias, classifications based on these additional symptoms have not been very helpful, and are now being replaced by a genetic classification. To date, eight genetically distinct forms are recognized, and it is clear that there are still families that are unlinked to any of these loci. Three of the genes have been identified.

Mutations in the *proteolipid protein* (PLP) gene on the X-chromosome are associated with a wide variety of phenotypes, ranging from severe, infantile forms of Pelizaeus–Merzbacher disease (PMD, *MIM 260 600*) to relatively mild pure spastic paraplegia of adult onset.

Pelizaeus–Merzbacher disease (PMD) typically manifests in infancy or early childhood with nystagmus, hypotonia, and cognitive impairment. Neurologic deficits progress to include severe spasticity and ataxia and death often occurs in childhood. Other mutations in the same gene, however, may manifest as a relatively pure spastic paraparesis (SPG2, *MIM 312 920*) with usually a normal lifespan. Female carriers may show mild to moderate signs of the disease. Still other mutations may lead to 'complicated' spastic paraparesis, often including autonomic dysfunction (such as spastic bladder disturbance), ataxia, and nystagmus. A clear distinction cannot be drawn on objective criteria between complicated spastic paraplegia and relatively mild PMD (such as the 'PLP null syndrome').

Clinical diagnosis depends on the typical neurologic findings and disease progression, X-linked inheritance pattern, and abnormal myelination on MRI (in cases with CNS involvement). Molecular diagnosis by sequence analysis is offered by many laboratories.

A rare form of adult onset of pure spastic paraplegia with recessive inheritance (SPG7, *MIM 602 783*) was found to be caused by a gene on chromosome 16 called paraplegin. Interestingly, the encoded protein is highly homologous to the yeast mitochondrial ATPases, which have both proteolytic and chaperon-like activities at the inner mitochondrial membrane. Immunofluorescence analysis and import experiments showed that paraplegin localizes to mitochondria. Analysis of muscle biopsies from two patients carrying paraplegin mutations showed typical signs of mitochondrial OXPHOS defects, thus suggesting a mechanism for neurodegeneration in HSP-type disorders. This appears to be a rare cause of spastic paraplegia and molecular diagnosis is not offered at this time.

The gene underlying the most common form of spastic paraplegia, the one linked to chromosome 14, has recently been identified (SPG4, spastin, *MIM 604 277*). It accounts for approximately 50% of all cases with a pure SPG-phenotype, adult-onset, and a family history compatible with autosomal dominant inheritance. Again, complete sequencing of this relatively large gene is necessary, but justifiable, in suspected cases as a result of the high prevalence of this disorder.

Molecular diagnosis of inherited dementias
M. Rossor

The majority of inherited degenerative dementias occurs as autosomal dominant disorders with similar phenotypes to sporadic disease. The frequency of familial occurrence varies from being a rarity (e.g. in dementia with Lewy bodies) to relatively frequent, as with the frontotemporal lobe degenerations. A number of pathogenic mutations associated with familial degenerative dementias have now been identified providing opportunities for specific diagnoses in affected patients and pre-symptomatic testing in at risk individuals.

It is particularly important in dementing disorders to ensure adequate genetic counselling prior to any attempt at molecular genetic diagnosis and to obtain consent from the patient and/or family carer (Post *et al.*, 1997). It should also be pointed out that a post mortem diagnosis of the cause of a familial degenerative dementia can provide critically important information in the future counselling for the family and should be discussed.

Alzheimer's disease

Alzheimer's disease is the most common type of the primary degenerative dementia. Mutations in three genes have been found to be associated with a form of the disease that is inherited in an autosomal dominant fashion with high penetrance. Overall, less than 5% of all cases can be accounted for by mutations in these genes. Amongst them, mutations in the gene for presenilin 1 (PS1, MIM 104 311) on chromosome 14 is most common, whereas mutations in the gene for presenilin 2 (PS2, MIM 600 759, chromosome 1) and amyloid precursor protein (APP, MIM 104 760, chromosome 21) are exceedingly rare (Mayeux and Ottman, 1998). An early onset (in the 40s and 50s) is common to all these monogenic forms, and along with a clearly positive family history, should act as an indication for molecular genetic diagnosis. Mutational screening in individuals without a family history is rarely indicated unless there is a very characteristic phenotype and inadequate pedigree history.

In addition to the mutations mentioned above, which can cause AD with high penetrance, an expanding list of genetic risk factors is being identified for dementias of which the *apolipoprotein E4* allele as a risk factor for AD is the best established. However, as the E4 allele is neither necessary nor sufficient to cause AD, there is a

wide consensus that at present there is no clear benefit in apolipoprotein E genotyping to assist with diagnosis (McKeith and Morris, 1996) nor with pre-symptomatic risk assessment. Other risk factors, such as the α2-macroglobulin and the interleukin-6 gene are suspected, but their role is less well established. It is conceivable, however, that response to certain forms of treatment will be found to vary, according to the combination of genetic susceptibilities in a given individual, so that their knowledge may be of clinical importance in the future.

Other degenerative dementias

A group of familial disorders characterized by a predominantly frontotemporal distribution of cortical cerebral atrophy and a clinical picture of prominent behavioural changes and dementia, variably associated with a variety of other neurologic deficits, such as parkinsonism or motoneuron degeneration has recently come into focus. These cases have in common a pathologic deposition of the microtubule associated protein tau (MAPTau). Historically, at least a subset of these patients had been subsumed under the heading of Pick's disease. The currently accepted terminology for the *inherited* forms is that of *frontotemporal dementia with parkinsonism linked to chromosome 17* (Foster *et al*., 1997). Mutations in the gene for MAPtau on chromosome 17 have been identified in a number of families with several different clinical presentations, such as 'classic' frontotemporal dementia, familial subcortical gliosis, corticobasal degeneration and even progressive supranuclear palsy. The prevalence of tau-mutations in a population of sporadic patients with the phenotype of frontotemporal degeneration from a dementia clinic however, is well below 10%, so that molecular diagnosis should be restricted to those cases with a clear family history.

Prion diseases (spongiform encephalopathies, in humans usually as *Creutzfeldt-Jakob disease*, *CJD*, *MIM 123 400*) may occur in idiopathic, acquired, or inherited disorders. A clearly positive family history is found in 10–20% of cases. Numerous different mutations in the prion protein gene have been identified in hereditary cases. Complete sequencing of the prion protein gene is provided by several centres and should be offered, given the appropriate counselling, in all cases with a strong clinical suspicion of CJD.

Other degenerative disorders associated with dementia

Many familial disorders may be associated with cognitive impairment which is overshadowed by other clinical features and many metabolic disorders with recessive inheritance. These may present to adult neurologists (Coker, 1991), are not discussed further here. Mutations in the synuclein, superoxide dismutase, huntingtin, and ataxin genes occur in familial neurological disorders in which cognitive disturbance may also occur (see also chapters on *Inherited degenerative movement disorders* and *Inherited ataxias*).

Dementia, along with recurrent ischemic events, is also a major feature of 'cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy' (CADASIL, *MIM 125 310*), which is caused by mutations in the Notch 3 gene (see also chapter on *Neurovascular disorders*).

Molecular diagnosis of myopathies and muscular atrophies

I. Hausmanowa-Petrusewicz, J. Zaremba

Proximal spinal muscular atrophy of childhood

Proximal spinal muscular atrophy (SMA) is one of the most common and severe autosomal-recessive diseases of children. The frequency is between 1 : 8000 and 1 : 10 000. The pathology involves dysfunction and loss of anterior horn cells, leading to muscle atrophy and weakness. Three forms of SMA are recognized: SMA I (infantile, Werdnig-Hofmann type, *MIM 253 300*), II (intermediate, *MIM 253 550*), and III (juvenile, Kugelberg-Welander type, *MIM 253* 400 (Zerres *et al*., 1997; Biros and Forrest, 1999).

All three forms are caused by mutations in a single gene. In 95–98% of cases, a homozygous deletion of exon 7 of the telomeric copy of the *survival motor neurone* gene (SMN1), which maps to chromosome 5q13, can be demonstrated by PCR. In the remaining 2–5% of cases the disease is caused by point mutations or small deletions or insertions in this gene. Most of these cases are compound heterozygotes with the common SMN1 deletion on one chromosome and another mutation on its homologue. The role of the gene product, the SMN protein, which is present in motoneurons and in other tissues is not yet clear. It is most probably that it is involved in RNA metabolism.

The risk for parents who already have had a child with SMA to have another affected child is 1 in 4. The risk for heterozygous healthy siblings of an SMA patient of having an affected child is $2/3 \times 1/50 \times ¼ = 1/300$, and the risk for having an affected child for mildly affected or non-manifesting individuals with a homozygous mutation of the SMN1 gene is 1/100 (assuming a frequency of heterozygotes in the general population of 1/50).

Genetic testing including pre-natal diagnosis should be offered to all couples having a genetic risk of ¼, but should also be available to those of the 1/100–1/300 risk group. An important prerequisite, however, is the previous identification of the mutation(s) in an affected individual from the family. If the proband is already deceased, which is common in SMA, the clinician should bear in mind that DNA can also be obtained from biological material such as samples of organs kept in formalin or in paraffin blocks, dried drops of blood on blotting paper (obtained during new born screening for PKU), dried fragments of umbilical cords or milk-teeth sometimes in the possession of parents.

Even if the diagnosis in the index patient cannot be confirmed molecularly, the demonstrated absence of a homozygous deletion in a proband can reduce the recurrence risk of SMA from 25 to 2%.

Inherited disorders with predominant involvement of skeletal muscle

Duchenne/Becker muscular dystrophy (DMD/BMD, MIM 310 200)

Duchenne/Becker muscular dystrophy is an X-linked recessive condition. The frequency of DMD is 1 in 3000 and BMD 1 in 20.000. The onset of DMD in most of the cases is below 5 years. Characteristic traits of the clinical picture are progressive muscular weakness – mainly proximal, calf pseudohypertrophy, features of myopathy in muscle biopsy and in EMG as well as markedly elevated serum creatine kinase. In BMD the onset is later and the course of the disease is generally milder, but there is a remarkable variability of clinical expression.

Immunohistochemistry of the muscle biopsies shows absence of dystrophin in DMD and markedly reduced dystrophin in BMD.

The huge dystrophin gene, which spans 2.4 Mb of DNA, maps to chromosome Xp21 and includes 79 exons. DMD and BMD are caused by mutations in the dystrophin gene. Deletions of a variable number of exons are the most common mutation in approximately 60% of cases, duplications are found in 5–10%. Point mutations are responsible in the remainder.

The molecular diagnosis of DMD/BMD can be done from blood DNA or by examining the gene product, dystrophin, in a muscle biopsy. DNA analysis detects deletions and most duplications. Because of enormous size of the gene, point mutations are difficult to detect. If a PCR-based assay for deletions is negative, it is often worthwhile to perform dystrophin analysis based on immunohistochemistry and on western blot (immunoblot). If dystrophin analysis confirms the diagnosis of DMD/BMD, detection of asymptomatic carriers of the mutated gene or pre-natal diagnosis can be carried out by genetic linkage analysis even if no mutation can be detected (see chapter on *indirect DNA-testing* in Section I).

Pre-natal diagnosis is offered not only to the confirmed carriers but also to women who previously gave birth to affected boys (isolated cases) and DNA analysis has shown that they are not the carriers, as there is a high probability of gonadal mosaicism. For review, see Kissel and Mendell, 1999.

Facioscapulohumeral muscular dystrophy (FSHD, MIM 158 900)

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant condition. The frequency is 1 : 20.000. Distribution of muscle weakness and wasting reveals descending progression involving the face, shoulder girdle, hip girdle and peroneal muscles (Kissel, 1999).

The gene for FSHD is located on chromosome 4q35. Molecular diagnosis in FSHD is complicated by the fact that the gene itself and the precise nature of the mutation is still not known. It is known, however, that in normal controls, southern blots of DNA digested with *Eco*RI reveal a large fragment of over 30 kb, whereas in most FSHD patients, the respective fragment is smaller (between 12 and 30 kb). This is probably because of a deletion of variable size in a 3.3-kb repetitive sequence (D_4Z_4). Interpretation of molecular studies in FSHD is not straightforward, because 30% of the subjects with the deletion are asymptomatic. On the other hand, a deletion has been detected only in 95% of the patients affected with FSHD. There is also evidence for genetic heterogeneity in FSHD (linkage to another gene) and for the occurrence of germinal mosaicism: as many as 20% of the reported sporadic patients have a mosaic parent. Nevertheless, molecular testing is widely available and should be performed if there is a clinical suspicion of the disease.

Myotonic Dystrophy (DM, MIM 160 900)

Myotonic dystrophy is one of the most frequent muscular dystrophies affecting adults and children (Thornton, 1999).

Besides wasting and myotonia in skeletal muscles in a characteristic distribution (facial muscles, mostly temporal, masseter and sternocleidomastoid, as well as distal limb muscles) the disease also affects several other organ systems. Additional features are male baldness, cataract, cognitive changes, hormonal disturbances, cardiomyopathy, and visceral symptoms.

The genetic basis for DM is the expansion of a CTG repeat in the DMPK gene on 19q13. Normal alleles vary from 5 to 37 CTGs. The range 38–49 is considered

as pre-mutation; expansion over 50 CTGs is usually associated with clinical symptoms. As in other diseases with repeat expansions, molecular diagnosis is easily done with a PCR-based assay or southern blot.

Another autosomal dominant disorder that closely resembles DM except that muscle weakness is predominantly proximal and less pronounced whilst hypertrophy of calves is frequent, has been described under the name of *proximal myotonic myopathy (PROMM, MIM 600 109)*.

Congenital myopathies

This is a large group of diseases steadily increasing as a result of development of research techniques and diagnostic methods. The common clinical denominators for the whole group are: congenital floppiness, muscle weakness, slimness, frequent skeletal dysmorphism.

The diagnosis is based on morphological abnormalities specific for each myopathy. These conditions are usually stationary or slowly progressive. Of the large group of congenital myopathies, only a few will be discussed.

Central core disease (CCD) is transmitted as an autosomal dominant trait (MIM 117 000). The characteristic feature of muscle histopathology is an amorphous area in the centre of the fibre. CCD is caused by mutations in the rhyanodine receptor on chromosome 19q13, and is allelic (different mutations in a single gene) to one form of *malignant hyperthermia susceptibility* (*MHS, MIM 145 600*). Patients with CCD are at risk for malignant hyperthermia and both conditions may appear in the same family. MHS is genetically heterogeneous. It has been estimated that approximately 50% of the cases are the result of mutations in the rhyanodine receptor, but four other loci have been identified. Identification of a particular mutation in a family with an individual known to be susceptible to malignant hyperthermia may be helpful to counsel family members and may obviate a muscle biopsy to rule out the disorder in family members.

The characteristic histopathological feature for *nemaline myopathy* (MIM 161 800) is the presence of small rods, originating from the Z-band of the muscle fibre, staining red by the Gomori technique. The disease is transmitted as an autosomal recessive (maps to 2q21.2–2q22) or autosomal dominant trait (maps to 1q 21–23). The gene product is α-tropomyosine. The course of the disease is very variable. Three forms of the disease are recognized: neonatal (very severe, usually lethal), childhood and adult ones.

In *myotubular* or *centronuclear myopathy* (MIM 310 400) the nuclei are situated centrally, surrounded by a pale halo. The muscle fibers show signs of immaturity. There are several different forms of *myotubular myopathy*, transmitted in an X-linked fashion (mapped to Xq28), as well as in an autosomal recessive and autosomal dominant form.

Emery-Dreifuss type muscular dystrophy (MIM 310 300)

The clinical features are: joint contractures mostly in elbows, knees, ankles, neck; moderate weakness and wasting of muscles, mostly of a proximal distribution in the upper extremities and a distal pattern in the legs; cardiac symptoms also occur. The first symptoms start usually in childhood as contractures. Cardiac symptoms may occur, also in otherwise asymptomatic heterozygous female carriers.

The disease is genetically heterogeneous – the main mode of transmission is X-linked. In these cases, deletions are found in a small gene on Xq28 (Emerin). Most mutations are private, i.e. different in each affected family. Therefore complete sequencing is usually necessary.

Another type of ED is transmitted as an autosomal dominant. The gene has been localized to chromosome 1, and identified as lamin A/C.

Limb-girdle muscular dystrophies

The limb–girdle muscular dystrophies (LGMDs) are a group of genetically determined disorders with the common feature of a progressive proximal muscle weakness. As a result of genetic heterogeneity and clinical similarity the precise LGMD diagnosis is one of the most difficult to make. Eight autosomal recessive LGMD variants are known. Four of them (sarcoglycanopathies, SGPs) result from mutations in the genes coding for dystrophin associated proteins, sarcoglycans (SG) α, β, γ, δ. In SGPs cardiac involvement is possible. The subset of autosomal recessive LGMD without SG involvement is represented by four variants. One of them is caused by mutations in the calpain 3 (CANP3) gene, another variant results from mutations in the dysferlin (DYSF) gene. The genes for the remaining two forms have not yet been identified.

5–10% of LGMDs are inherited in an autosomal dominant fashion. At present six loci have been mapped. Of these only the gene encoding caveolin-3 (CAV3) has been identified so far. Cardiac involvement has been reported in two dominant LGMD phenotypes.

The diagnosis of autosomal recessive LGMD is usually accomplished by immunohistochemistry using of specific antibodies recognizing SG complex in muscle tissue. The mutation in any gene encoding one of the SGs would result in a primary deficiency of this particular SG and a secondary deficiency in the

remaining ones. This approach allows to identify sarcoglycanopathies.

If SGs appear normal, the use of antibodies recognizing CANP3 and DYSF in muscles is recommended. Deficiency of theses proteins indicate dystrophy subtype and permit direct mutations search at particular LGMD genes.

X-linked bulbospinal muscular atrophy, Kennedy disease (MIM 313 200)

X-SBMA is an X-linked recessive, slowly progressive disorder of adult onset caused by the loss of lower motoneurons. Muscle wasting is accompanied by a variable degree of gynecomastia and reduced fertility. The mutation is the expansion of a CAG repeat in the first exon of the androgen receptor (AR) gene (abnormal range over 35 repeats; normal alleles – 9–34 repeats). This disorder may be an important differential diagnosis to ALS or adult forms of SMA. Molecular analysis is based on PCR amplification of the CAG repeat region and routinely available.

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