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EFNS GUIDELINES/CME ARTICLE

EFNS guidelines on the molecular diagnosis of neurogenetic disorders: general issues, Huntington's disease, Parkinson's disease and dystonias

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Background and purpose: These EFNS guidelines on the molecular diagnosis of neurogenetic disorders are designed to provide practical help for the general neurologist to make appropriate use of molecular genetics in diagnosing neurogenetic disorders. Since the publication of the first two EFNS-guideline papers on the molecular diagnosis of neurological diseases in 2001, rapid progress has been made in this field, necessitating an updated series of guidelines.

Methods: Literature searches were performed before expert members of the task force wrote proposals, which were discussed in detail until final consensus had been reached among all task force members.

Results and conclusion: This paper provides updated guidelines for molecular diagnosis of Huntington's disease, Parkinson's disease and dystonias as well as a general introduction to the topic. Possibilities and limitations of molecular genetic diagnosis of these disorders are evaluated and recommendations are provided.

Introduction

In 2001, the first two EFNS guideline papers on the molecular diagnosis of inherited neurological diseases were published [1,2]. Since then, the progress of the field has been nothing less than astounding, so an updated series of guidelines is needed. The aim of this paper is to provide a summary of the current possibilities and limitations of molecular genetic diagnosis of Huntington's disease, Parkinson's disease and dystonias and to provide recommendations for genetic testing.

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This is a Continuing Medical Education article, and can be found with corresponding questions on the internet at http://www.efns.org/content.php?pid=132. Certificates for correctly answering the questions will be issued by the EFNS.

Search strategy

To collect data about the molecular diagnosis of different neurogenetic disorders, literature searches were performed in various electronic databases, such as MEDLINE; OMIM, or GENETEST. Original papers and meta-analyses, review papers, and guideline recommendations were reviewed.

Method for reaching consensus

Consensus about the recommendations was reached by a step-wise approach. First, members of the task force met at the EFNS congresses in 2007 and 2008 to discuss the preparations of the guidelines. In a second step, experts in the specific topics wrote proposals for chapters for each group of disorders. In a third step, these chapters were distributed and discussed in detail amongst all task force members until a final consensus had been reached.

Results and recommendations

Recommendations are based on the criteria established by the EFNS [3], with some modifications to account for the specific nature of genetic tests. As genetic testing is by definition the gold standard to diagnose a genetically defined disease (barring the rare event of a lab error), its diagnostic accuracy can not be tested against another diagnostic method. Therefore, the level of recommendations will be based on the quality of available studies (for a definition see supplementary material [3]) which investigate a proportion of cases of a clinically defined group of patients, which are explained by a specific molecular diagnostic test. As practically all of these studies have been retrospective (i.e. looking for a specific mutation in a previously ascertained and clinically diagnosed cohort of patients) the highest level of recommendation will be at level B [3]. References for the studies forming the basis of our recommendations are given both in tables and in the separate chapters. If only small case-series studying genotype-phenotype correlations are available, the level of recommendation will be at level C. If only case reports could be found, but experts still felt that they could give a recommendation, the level of recommendation will be 'good practice point'.

General guidelines of molecular diagnosis of neurogenetics disorders

For the neurologist, the availability of molecular testing for an increasing number of diseases is the most challenging consequence of the recent progress in the molecular genetic sciences. In clinical practice, the benefits and limitations of molecular diagnosis depend 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 [4], and routine molecular diagnosis can be provided by a simple and cheap PCR-based assay. In other cases, such as in the spastic paraplegias, many different mutations in different genes may be causative ('allelic' and 'non-allelic' heterogeneity, respectively). Depending on the size and number of the gene(s) involved, this may render molecular diagnosis costly and time-consuming. The treating physician therefore has to be able to weigh the probability that a test which is ordered will actually detect a mutation against its costs.

Despite these caveats and despite the fact that today only a small percentage of neurogenetic disorders can be treated effectively, molecular diagnosis is increasingly important because it may provide valuable information for the affected individuals and their families on prognosis and recurrence risks and may help to make informed decisions on life and family planning.

Today, molecular testing will usually be helpful only if a 'monogenic' disease or a rare monogenic variant of an otherwise common disease is suspected, although considerable progress has also been made in recent years in defining relevant genetic risk factors for the development of the more common 'genetically complex' diseases. Those variants will not be discussed here, despite their potential relevance for developing future therapies.

Genetic counselling

The primary goal of molecular diagnosis is always to provide help for the individual patient, client (usually an affected or at risk individual) and/or their families. Reducing the prevalence of inherited disorders in a population or in subsequent generations may be a secondary effect, but must never be allowed to guide the process of genetic counselling.

A genetic diagnosis affects not only the patient, but the entire family. Therefore, genetic counselling is essential. Sensitive and informed counselling provides patients and families with a foundation for decisions about testing. Patients should be counselled as to the clinical features and course of the suspected disease as well as to potential consequences for the family, taking into consideration the most important genetic parameters such as mode of inheritance, penetrance or variability of clinical expression. Thorough experience in both the human genetics and the specific neurologic aspects of a disorder is necessary for qualified counselling.

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 of exercising informed choices. Genetic tests should not be performed at the request of members of the patients' families or other third parties (e.g. insurers, employers) without the expressed 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 disease 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 for 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 [5]. These guidelines, which include extensive pre- and post-test counselling, should be followed in all cases of pre-symptomatic diagnosis. Involvement of an experienced genetic counsellor is essential. If no clear therapeutic consequences can be envisioned, pre-symptomatic testing should not be performed in minors.

Other sources of information on genetic testing

Genetic classifications in these guidelines follow, if applicable, the most comprehensive catalogue of human hereditary diseases, the 'Online Mendelian Inheritance in Man (MIM)' (http://omim.nih.org), which is maintained by the National Center of Biotechnology Information (NCBI). 'MIM-numbers' are given for easy reference.

Further information can be obtained on several useful websites:

- http://www.geneclinics.org/: 'GeneClinics', a clinical information resource relating genetic testing to the diagnosis, management, and genetic counselling.
- http://www.eurogentest.org/ : 'EuroGentest', an EU-funded network of excellence that intends to harmonize genetic testing across Europe. The website provides information about availability and quality assurance of genetic test.
- http://www.orpha.net/: 'OrphaNet', a searchable database of over 5000 rare diseases, which includes information about genetic testing.
- http://omim.nih.org/: 'Online Mendelian Inheritance in Man, OMIM'. Online catalogue of Mendelian disorders and traits in man.
- http://www.mitomap.org/: 'MITOMAP', a human mitochondrial genome database.

Technical aspects of molecular testing

If the gene causing a neurological disorder is known, molecular diagnosis can be performed by mutational analysis. Only DNA from the affected or at-risk individual is required. Usually, exons that are known to harbour mutations (point mutations or small deletions or insertions) will be amplified by polymerase chain reaction (PCR) from genomic DNA, which has been extracted from peripheral blood leukocytes. Depending on its type, the mutation will then be detected either by gel electrophoresis (e.g. in the case of trinucleotide repeat expansions), or by DNA sequencing. Heterozygous deletions or multiplications of entire exons or even entire genes are increasingly recognized as a rather common type of pathogenic mutations. These mutations cannot be detected by routine sequencing, and must be sought by exon or gene dosage assays. If a gene is very large (genes with more than thirty exons are not uncommon) and mutations are scattered throughout the entire gene, mutational analysis can be very costly and time-consuming with current routinely used methods. In these cases, routine sequence analysis is sometimes offered only for portions of a gene where mutations are known to be clustered.

The patient confirms his informed consent to the procedure in writing. Usually, ten to twenty ml of whole blood (usually EDTA) is drawn. The blood can be sent to a laboratory without freezing or refrigeration. A delay of three 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 included in the shipment.

Molecular diagnosis of Huntington's disease

Huntington disease (HD, MIM 143 100) is the 'prototypic' neurogenetic disorder (Table 1). It is usually characterized by the triad of choreic movements, cognitive decline and personality changes. Clinical manifestations may be highly variable, however, and particularly in juvenile patients, akinesia, rigidity, or epileptic seizures may occur. The disease is caused by the expansion of a CAG-triplet in the first exon of the HTT gene (formerly HD or IT15), which encodes huntingtin, leading to the formation of an elongated polyglutamine (polyQ) sequence within the protein [4]. This highly polymorphic CAG repeat ranges between 10 and 28 copies on normal chromosomes, but is expanded to a range of 36–121 on HD chromosomes. Adult onset patients usually have 40–55 repeats, with juvenile onset patients having over 60. CAG repeats above 40 are fully penetrant, although there is a

Table 1 Molecular diagnosis of Huntington's disease (HD) and HD-like disorders

Disease	Inheritance	Position	Mutation	Gene product	Reference	Remarks	MIM-number
Huntington's disease (HD)	AD	4p16.3	Trinuc	Huntingtin	[4]	In HD-cases with early onset, large expansions should be searched for by suited techniques	143 100
Huntington disease like (HDL) disorder 1 (HDL1)	AD	20pter-p12	Octapeptide expansion	Prion protein	[15]	Only one family, but octapeptide insertions in the PrP gene have been described in other HD-phenocopy series	603 218
Huntington's disease like (HDL) disorder 2 (HDL2)	AD	16q24.3	Trinuc	Junctophilin 3	[12]	Described to date only in patients of African ancestry	606 438
Spinocerebellar (SCA)17 (HDL4)	AD	6q27	Trinuc	TATA box-binding protein	[13]	Cerebellar atrophy	607 136
Dentato-rubro-pallido-luysian atrophy (DRPLA)	AD	12p13.31	Trinuc	Atrophin 1	[14]	Cerebellar atrophy	125 370

AD, autosomal dominant; Trinuc; Trinucleotid-repeat expansion.

borderline repeat range between 36 and 39 repeats with reduced penetrance. CAG repeat lengths vary from generation to generation, with both expansion and contraction, but there is a tendency for repeat lengths to increase, particularly when transmitted through the paternal lineage. The instability of the CAG expansion with the tendency to expand during transmission underlies the phenomenon of anticipation, i.e. increasing severity and earlier onset of an inherited disease in subsequent generations. CAG repeat instability during paternal transmission is important in the development of large expansions associated with juvenile HD and approximately 80% of juvenile HD patients inherit the HD gene from their father. There is a negative correlation between the CAG repeat size and age at onset. However, CAG repeat length does not completely explain variations in age of onset, clinical phenotype or rate of clinical progression, suggesting that other modifying genes may play an important role [6].

Diagnostic testing for HD is usually requested by neurologists when patients present with neurological signs and symptoms of the disease. Adequate genetic counselling and informed consent in these situations is important. In some instances there may be no previous known family history of HD, so the diagnosis comes as a shock to the person and their family. In these situations partners and other family members should be involved early in the diagnostic counselling process, as a confirmatory result of HD has profound implications for siblings and offspring.

Indications and consequences of diagnostic and presymptomatic molecular diagnosis have been studied widely in HD (reviewed in [7]). There are numerous issues relating to insurance, employment and genetic discrimination of persons at risk for HD [8]. In suspected HD patients with early onset it must be remembered that parents may carry smaller repeat expansions and thus may manifest the disease after their offspring. Molecular diagnosis in a young individual may therefore result in inadvertent presymptomatic testing in a parent [9].

The wide availability of genetic testing has allowed detailed genotype/phenotype studies in HD. It has also increased our understanding of disorders that present with a similar clinical picture to HD (HD phenocopies) with similar cognitive, psychiatric and motor features, but which are HD gene negative (reviewed in [10]). HD phenocopies occur in approximately 1% of large genetic screens of individuals with clinical signs of HD [11].

Expansions of CTG/CAG-triplets in a variably spliced exon of the JPH3 gene (junctophilin 3) are responsible for Huntington disease-like-2 (HDL2, MIM 606 438), but have been found only in rare patients of African ancestry [12] (Table 1). Spinocerebellar ataxia (SCA) 17 (MIM 607 136) is caused by a CAG repeat expansion in the TATA-binding protein (TBP) gene, which may resemble HD, and in fact has also been termed HDL-4 [13] (Table 1). Another autosomal dominant disease that may mimic HD is dentatorubro-pallidoluysian atrophy (DRPLA, MIM 125 370), which is caused by a CAG repeat expansion in the atrophin-1 gene [14] (Table 1).

Other disorders that may more rarely resemble HD are SCA1 and SCA3. Inherited prion disorders may also cause HD phenocopies. Specifically, a 192-nucleotide insertion in the prion protein gene encoding eight octapeptide repeats was described to cause an HD-like disease, and was called HDL-1 (Table 1). It is essentially an early onset prion disease with prominent psychiatric features [15].

Other causes of HD-phenocopies include neuroacanthocytosis (MIM 200 150), where autosomal recessive cases have been associated with mutations in the chorein gene on chromosome 9 [16]. Lastly, a recently described disorder in the North of England, neuroferritinopathy (MIM 606 159), caused by mutations in the ferritin light chain polypeptide [17], has clinical features that overlap with HD. Despite the increasing number of recognized genetic disorders that resemble HD, achieving a genetic diagnosis in HD phenocopy cases is still difficult with currently less than 3% of cases having a confirmatory genetic result [18].

Summary of recommendations concerning molecular diagnosis of Huntington's disease

Diagnostic testing for HD is recommended (Level B) when a patient presents with an otherwise unexplained clinical syndrome of a progressive choreatic movement disorder and neuropsychiatric disturbances with or without a positive family history of the disease [11]. Previously established guidelines for pre-symptomatic molecular diagnosis should be followed [5]. In mutation-negative cases, no general recommendation can be given to test for any of the rare genes causing HD phenocopies.

Molecular diagnosis of inherited parkinsonian syndromes

Until recently, the role of genetic factors in the aetiology of Parkinson's disease (PD) has not been widely recognized. Today it is well established that mutations in several genes are able to cause monogenic forms of PD [19] (Table 2).

Table 2 Molecular diagnosis of Parkinson's disease and dystonias

Disease	Locus	Inheritance	Position	Gene product	Reference	Remarks	MIM number
Familial Parkinson disease, dominant	PARK1/4	AD	4q21	alpha-Synuclein	[20]	Point mutations as well as gene duplications and triplications found	601 508
	PARK8	AD	12p12	LRRK2, Dardarin	[24]	Most common form of dominant PD	607 060
Familial Parkinson disease, recessive	PARK2	AR	6q25–27	Parkin	[46]	Early-onset	602 544
	PARK6	AR	1p33	PINK1	[47]	Early-onset	605 909
	PARK7	AD	1p34	DJ-1	[48]	Early-onset	606 324
Familial parkinsonism, other	PARK9	AR	1p36	ATP13A2	[49]	Multisystem degeneration, Kufor-Rakeb syndrome	606 693
	GBA	AD	1q21	Glucocerebrosidase	[30,31]	Heterozygous carriers of pathogenic mutations in the Gaucher's associated gene GBA	
Primary torsion dystonia	DYT1	AD	9q34	Torsin A	[32]	A single GAG-deletion responsible for all cases	128 100
X-chromosomal dystonia-Parkinson-Syndrome	DYT3	XL	Xq11.2	TAF1	[50]	Very rare, only in Filippinos	314 250
Dopa-responsive dystonia	DYT5, DRD	AD	14q22	GTP-Cyclohydrolase I	[36]	Pharmacologic testing should precede genetic testing	600 225
Dopa-responsive dystonia	DYT5, DRD	AR	11p15.5	Tyrosine hydroxylase	[37]	Rare, often more complex phenotype	191 290
Myoclonus dystonia	DYT11, MD	AD	7q21	SGCE	[39]	Maternal imprinting causes reduced penetrance upon maternal transmission	159 900
Rapid-onset dystonia-parkinsonism	DYT12, RDP	AD	19q13	ATP1A3	[42]	Often *de novo* mutations	128 235
Paroxysmal dystonia, non-kinesiogenic	DYT8, PNKD	AD	2q35	MR-1	[43]	Attacks precipitated by coffee, alcohol, exertion	118 800
Paroxysmal exercise-induced dystonia	DYT17, PED	AD	1p35	Glut1	[44]	Treatable by ketogenic diet	612 126

AD, autosomal dominant; AR, autosomal recessive, XL, x linked.

Point mutations [20], but also duplications and triplications [21] of the gene for α-synuclein (SNCA) can cause an autosomal-dominant form of PD that can be clinically indistinguishable from the sporadic disease (MIM 168 601). Point mutations and triplications are rare and have mostly been found in cases with a strong dominant family history and a high prevalence of dementia, but SNCA-duplications can cause late-onset typical PD. Nevertheless, SNCA-mutations are very rare [22,23], so molecular diagnosis should be considered only for clearly familial cases.

Mutations in the gene for leucine-rich repeat kinase 2 (LRRK2; PARK8, MIM 607 060) are much more common, accounting for approximately 5–15% of familial and (due to reduced penetrance and late onset) 1–2% of apparently sporadic patients of PD [24–26]. In some genetically isolated populations, such as the Ashkenazi Jews or North African Arabs, the proportion of carriers of the most common mutation, G2019S, can be as high as 30–40% [27]. The clinical and pathologic picture is most commonly indistinguishable from idiopathic PD, as is the age of onset of around 60 years. As the gene is very large and the frequency of mutations varies between populations, the decision to seek molecular diagnosis will depend on the specific circumstances. In Europeans, molecular diagnosis will be feasible only in familial cases suggestive of dominant inheritance, whilst in some populations testing for specific high-prevalence mutations (e.g. the G2019S mutation in the Ashkenazim) is an already established clinical routine [28].

The most common cause of early-onset recessive parkinsonism are mutations in the parkin gene on chromosome 6 (MIM 600 116). The vast majority of patients with parkin mutations have disease onset before age 35, so genetic testing should be limited to early-onset cases [29]. As a substantial proportion of the mutations are whole exon or even whole gene rearrangements, genetic testing should include appropriate methods for the detection of these copy number variations. Mutations in the other recessive PD genes (PINK1, MIM 605 909 and DJ1, MIM 606 324) cause a clinically similar phenotype of early-onset parkinsonism; their prevalence is less well studied, but appears to be lower than that of parkin. Only homozygous or compound heterozygous mutations can be confidently considered to be pathogenic, as the role of heterozygous parkin mutations as risk factors for sporadic PD is still controversial.

Also, mutations in the gene for glucocerebrosidase (GBA), which cause Gaucher's disease in homozygous or compound heterozygous individuals clearly increase the risk for PD [30,31]. Again there is a markedly higher prevalence of these variants in Ashkenazi Jews. Penetrance of these variants however is not clear, making counselling difficult.

Summary of recommendations concerning molecular diagnosis of Parkinson's disease

In Europeans, molecular testing for LRRK2 is recommended (Level B) in familial cases with dominant inheritance of parkinsonian syndromes [25,26]. Testing for the LRRK2 G2019S mutation is recommended in familial and sporadic patients in specific populations, e.g. in the Ashkenazim or North African Arabs (Level B) [27].

Testing for mutations in recessive PD-genes (parkin, PINK1, DJ-1) is recommended (Level B) in families suggestive of recessive inheritance (affected sib pairs) or sporadic patients with very early onset (< 35 years) [29].

Molecular diagnosis of the dystonias

A growing number of genes are being found to cause familial forms of dystonias (Table 2). Consequently, molecular diagnosis of these disorders is becoming increasingly important, although in clinical practice it is still restricted to a relatively small proportion of patients with a clearly defined familial disease, whilst the contribution of genetic factors in the more common focal dystonias remains poorly defined.

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 specific mutation, a deletion of the trinucleotide GAG (encoding glutamic acid) in the gene for torsin A on chromosome 9q34, is the major cause of early-onset generalized dystonia (DYT1) [32]. Patients usually have disease-onset in an extremity before age 24, with relatively rapid progression to a generalized form. Molecular testing will identify the mutation in more than 90% of Ashkenazi Jewisch patients with this phenotype, due to a common founder mutation [33], and in about 30–50% of non-Jewish Caucasian patients [34], whilst no specific mutations are found in those with the much more common adult-onset cervical or cranial dystonias [35]. Due to the reduced penetrance of about 30% of the GAG-deletion, DYT1-mutation carriers often have a negative family history, and a positive family history is NOT a prerequisite for genetic testing in a patient

with a typical phenotype. This also must be taken into account during the counselling process.

Dystonia-plus syndromes

The genetic basis of several relatively rare forms of hereditary dystonia with specific additional clinical or biochemical features has been elucidated, providing the basis for molecular diagnosis. In clinically typical patients, mutations can be detected in about 40–80% of cases, whilst genetic testing is rarely helpful in clinically unclassifiable patients.

Dopa-responsive dystonia (DRD) (MIM 128 230) is most commonly caused by point-mutations or exon deletions in the gene for GTP-cyclohydrolase I (dominant with reduced penetrance) [36], but can rarely be also due to recessive mutations in the genes for tyrosine hydroxylase [37]. Given these genetic parameters, family history is not a good predictor for a positive test result, so a convincing response to levodopa treatment should be documented before molecular testing is initiated. Other than by their dopa-response, these patients cannot reliably be distinguished from DYT1 patients. Conspicuous diurnal fluctuations of symptom severity (getting worse during the day) may be a clue. The clinical picture may also mimic cerebral palsy (CP), which is why any patient with CP should be given a trial with levodopa. In a patient with typical DRD, mutations can be found in up to 80% of cases [38].

If the phenotype is characterized by very rapid ('lightning-like') myoclonic jerks affecting predominantly the muscles of the trunk, neck and proximal extremities, a diagnosis of *myoclonus-dystonia (M-D) (MIM 159 900)* should be considered [39]. If the family history is positive, mutations are identified in the gene for ϵ-sarcoglycan (SGCE) in a significant proportion of cases [40,41].The genetic basis of some other dystonia-plus syndromes, such as '*rapid-onset dystonia-parkinsonism*' (MIM 128 235) [42], or some of the paroxysmal dystonias [43] has also been elucidated, but these disorders are exceedingly rare and mutational analysis is usually only offered in a research setting.

Summary of recommendations concerning molecular diagnosis of dystonia

Molecular testing for the GAG-deletion in the TOR1A gene is recommended (Level B) in patients with early (< 26 years) and limb-onset generalized dystonia regardless of family history. Testing for GCH1-mutations including gene dosage studies is recommended (Level B) in patients with early-onset generalized dystonia with a clear response to levodopa, regardless of the family history. Sequencing and gene dosage studies of the SGCE-gene is recommended (Level B) only in patients with a typical clinical picture of myoclonus-dystonia syndrome and a suggestive family history. No genetic tests can be recommended in more common focal dystonias (good practice point).

Other movement disorders

Sequence analysis of the ATP7B gene causing *Wilson's disease* (WD) (MIM 277 900) can confirm the diagnosis in a patient with a diagnosis of WD for family counselling purposes [44]. Blood and urine chemistry, particularly copper excretion in urine, is still the diagnostic method of choice in the majority of cases. Approximately 55% of patients in a Caucasian population harbour mutations in exons 7, 8, 14, 15, or 18. Identification of a mutation in an index patient allows pre-symptomatic testing in other at-risk family members, which may be particularly important in this disease, because preventive and therapeutic measures are of help.

Although *essential tremor (MIM 190 300) and the restless legs-syndrome (MIM 102 300)* are the most common movement disorders, no disease-causing mutations that would allow genetic testing have been identified so far. Identified risk-alleles cannot be used for individual diagnosis.

Conclusion

The presented guidelines on the molecular diagnosis of Huntington's disease, Parkinson's disease and dystonias have been created in response to the increasing amount of data on the genetic background of these disorders, the increasing need of the clinical neurologist to learn about the genetic perspective, and the increasing availability of commercial molecular diagnosis for the daily routine.

Conflicts of interest

Member of this Task Force have no conflicts of interest related to the recommendations given in this paper.

References

1. Gasser T, Dichgans M, Finsterer J, *et al*. EFNS task force on molecular diagnosis of neurologic disorders: guidelines for the molecular diagnosis of inherited neurologic diseases. Second of two parts. *Eur J Neurol* 2001; **8:** 407–424.
2. Gasser T, Dichgans M, Finsterer J, *et al*. EFNS task force on molecular diagnosis of neurologic disorders: guidelines for the molecular diagnosis of inherited neurologic diseases. First of two parts. *Eur J Neurol* 2001; **8:** 299–314.
3. Brainin M, Barnes M, Baron JC, *et al*. Guidance for the preparation of neurological management guidelines by

EFNS scientific task forces – revised recommendations 2004. *Eur J Neurol* 2004; **11:** 577–581.
4. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993; **72:** 971–983.
5. International Huntington Association (IHA) and the World Federation of Neurology (WFN) Research Group on Huntington's Chorea. Guidelines for the molecular genetics predictive test in Huntington's disease. *Neurology* 1994; **44:** 1533–1536.
6. Wexler NS, Lorimer J, Porter J, *et al.* Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. *Proc Natl Acad Sci U S A* 2004; **101:** 3498–3503.
7. Hayden MR, Bombard Y. Psychosocial effects of predictive testing for Huntington's disease. *Adv Neurol* 2005; **96:** 226–239.
8. Harper PS, Gevers S, de WG, Creighton S, Bombard Y, Hayden MR. Genetic testing and Huntington's disease: issues of employment. *Lancet Neurol* 2004; **3:** 249–252.
9. Scheidtmann K, Schwarz J, Holinski E, Gasser T, Trenkwalder C. Paroxysmal choreoathetosis – a disorder related to Huntington's disease? *J Neurol* 1997; **244:** 395–398.
10. Wild EJ, Tabrizi SJ. Huntington's disease phenocopy syndromes. *Curr Opin Neurol* 2007; **20:** 681–687.
11. Kremer B, Goldberg P, Andrew SE, *et al.* A worldwide study of the Huntington's disease mutation. The sensitivity and specificity of measuring CAG repeats. *N Engl J Med* 1994; **330:** 1401–1406.
12. Holmes SE, O'Hearn E, Rosenblatt A, *et al.* A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. *Nat Genet* 2001; **29:** 377–378.
13. Stevanin G, Fujigasaki H, Lebre AS, *et al.* Huntington's disease-like phenotype due to trinucleotide repeat expansions in the TBP and JPH3 genes. *Brain* 2003; **126**(Pt 7)**:** 1599–1603.
14. Koide R, Ikeuchi T, Onodera O, *et al.* Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nat Genet* 1994; **6:** 9–13.
15. Moore RC, Xiang F, Monaghan J, *et al.* Huntington disease phenocopy is a familial prion disease. *Am J Hum Genet* 2001; **69:** 1385–1388.
16. Ueno S, Maruki Y, Nakamura M, *et al.* The gene encoding a newly discovered protein, chorein, is mutated in chorea-acanthocytosis. *Nat Genet* 2001; **28:** 121–122.
17. Curtis AR, Fey C, Morris CM, *et al.* Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. *Nat Genet* 2001; **28:** 350–354.
18. Wild EJ, Mudanohwo EE, Sweeney MG, *et al.* Huntington's disease phenocopies are clinically and genetically heterogeneous. *Mov Disord* 2008; **23:** 716–720.
19. Gasser T. Update on the genetics of Parkinson's disease. *Mov Disord* 2007; **22**(Suppl 17)**:** S343–S350.
20. Polymeropoulos MH, Lavedan C, Leroy E, *et al.* Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 1997; **276:** 2045–2047.
21. Singleton AB, Farrer M, Johnson J, *et al.* {alpha}-Synuclein Locus triplication causes Parkinson's Disease. *Science* 2003; **302:** 841.
22. Berg D, Niwar M, Maass S, *et al.* Alpha-synuclein and Parkinson's disease: implications from the screening of more than 1,900 patients. *Mov Disord* 2005; **20:** 1191–1194.
23. Ibanez P, Lesage S, Janin S, *et al.* Alpha-synuclein gene rearrangements in dominantly inherited parkinsonism: frequency, phenotype, and mechanisms. *Arch Neurol* 2009; **66:** 102–108.
24. Zimprich A, Biskup S, Leitner P, *et al.* Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 2004; **44:** 601–607.
25. Berg D, Schweitzer K, Leitner P, *et al.* Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*. *Brain* 2005; **128**(Pt 12)**:** 3000–3011.
26. Gilks WP, Abou-Sleiman PM, Gandhi S, *et al.* A common LRRK2 mutation in idiopathic Parkinson's disease. *Lancet* 2005; **365:** 415–416.
27. Ozelius LJ, Senthil G, Saunders-Pullman R, *et al.* LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 2006; **354:** 424–425.
28. Orr-Urtreger A, Shifrin C, Rozovski U, *et al.* The LRRK2 G2019S mutation in Ashkenazi Jews with Parkinson disease: is there a gender effect? *Neurology* 2007; **69:** 1595–1602.
29. Lucking CB, Durr A, Bonifati V, *et al.* Association between early-onset Parkinson's disease and mutations in the parkin gene. *N Engl J Med* 2000; **342:** 1560–1567.
30. Aharon-Peretz J, Rosenbaum H, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 2004; **351:** 1972–1977.
31. Mata IF, Samii A, Schneer SH, *et al.* Glucocerebrosidase gene mutations: a risk factor for Lewy body disorders. *Arch Neurol* 2008; **65:** 379–382.
32. Ozelius LJ, Hewett JW, Page CE, *et al.* The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. *Nat Genet* 1997; **17:** 40–48.
33. Bressman SB, Sabatti C, Raymond D, *et al.* The DYT1 phenotype and guidelines for diagnostic testing. *Neurology* 2000; **54:** 1746–1752.
34. Valente EM, Warner TT, Jarman PR, *et al.* The role of DYT1 in primary torsion dystonia in Europe. *Brain* 1998; **121:** 2335–2339.
35. Grundmann K, Laubis-Herrmann U, Bauer I, *et al.* Frequency and phenotypic variability of the GAG deletion of the DYT1 gene in an unselected group of patients with dystonia. *Arch Neurol* 2003; **60:** 1266–1270.
36. Ichinose H, Ohye T, Matsuda Y, *et al.* Characterization of mouse and human GTP cyclohydrolase I genes. Mutations in patients with GTP cyclohydrolase I deficiency. *J Biol Chem* 1995; **270:** 10062–10071.
37. Knappskog PM, Flatmark T, Mallet J, Ludecke B, Bartholome K. Recessively inherited L-DOPA-responsive dystonia caused by a point mutation (Q381K) in the tyrosine hydroxylase gene. *Hum Mol Genet* 1995; **4:** 1209–1212.
38. Hagenah J, Saunders-Pullman R, Hedrich K, *et al.* High mutation rate in dopa-responsive dystonia: detection with comprehensive GCHI screening. *Neurology* 2005; **64:** 908–911.
39. Zimprich A, Grabowski M, Asmus F, *et al.* Mutations in the gene encoding epsilon-sarcoglycan cause myoclonus-dystonia syndrome. *Nat Genet* 2001; **29:** 66–69.
40. Asmus F, Zimprich A, Tezenas Du MS, *et al.* Myoclonus-dystonia syndrome: epsilon-sarcoglycan mutations and phenotype. *Ann Neurol* 2002; **52:** 489–492.

41. Grunewald A, Djarmati A, Lohmann-Hedrich K, *et al*. Myoclonus-dystonia: significance of large SGCE deletions. *Hum Mutat* 2008; **29:** 331–332.
42. de Carvalho AP, Sweadner KJ, Penniston JT, *et al*. Mutations in the Na+/K+ -ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. *Neuron* 2004; **43:** 169–175.
43. Rainier S, Thomas D, Tokarz D, *et al*. Myofibrillogenesis regulator 1 gene mutations cause paroxysmal dystonic choreoathetosis. *Arch Neurol* 2004; **61:** 1025–1029.
44. Suls A, Dedeken P, Goffin K, *et al*. Paroxysmal exercise-induced dyskinesia and epilepsy is due to mutations in SLC2A1, encoding the glucose transporter GLUT1. *Brain* 2008; **131**(Pt 7)**:** 1831–1844.
45. Tanzi RE, Petrukhin K, Chernov I, *et al*. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993; **5:** 344–350.
46. Kitada T, Asakawa S, Hattori N, *et al*. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998; **392:** 605–608.
47. Valente EM, bou-Sleiman PM, Caputo V, *et al*. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 2004; **304:** 1158–1160.
48. Bonifati V, Rizzu P, van Baren MJ, *et al*. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* 2003; **299:** 256–259.
49. Ramirez A, Heimbach A, Grundemann J, *et al*. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet* 2006; **38:** 1184–1191.
50. Makino S, Kaji R, Ando S, *et al*. Reduced neuron-specific expression of the TAF1 gene is associated with X-linked dystonia-parkinsonism. *Am J Hum Genet* 2007; **80:** 393–406.

