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EFNS GUIDELINES/CME ARTICLE

EFNS guidelines on the molecular diagnosis of ataxias and spastic paraplegias

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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.

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 two particularly complex groups of disorders, the ataxias and spastic paraplegias. Possibilities and limitations of molecular genetic diagnosis of these disorders are evaluated and recommendations are provided.

Introduction

Since the publication of the first two EFNS guideline papers on the molecular diagnosis of neurological diseases in 2001 [1,2], rapid progress has been made in this field, necessitating an updated series of guidelines. This article provides updated guidelines for molecular testing of ataxias and hereditary spastic paraplegias. Criteria will be put forth which help to decide when a molecular diagnostic work-up should be initiated and tables will provide compact information about the genetic basis of the disorders discussed in the following sections.

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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/EFNSContinuing-Medical-Education-online.301.0.html. Certificates for correctly answering the questions will be issued by the EFNS.

For a more general introduction of molecular genetic testing including questions of genetic counselling, the reader is referred to our article 'EFNS guidelines on the molecular diagnosis of neurogenetic disorders: General issues, Huntington's disease, Parkinson's disease and dystonias' [3].

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, meta-analyses, review papers, and guideline recommendations were reviewed.

Method for reaching consensus

Consensus on recommendations was reached by a stepwise approach. First, members of the task force met at the EFNS congresses in 2007 and 2008 to discuss the

preparations of the guidelines. Second, experts in the specific topics wrote proposals for chapters for each group of disorders. In a third step, chapters were distributed and discussed in detail amongst all task force members until a final consensus was reached.

Results and recommendations

Recommendations are based on the criteria established by the EFNS [4], with 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 cannot be tested against another method. Therefore, the level of recommendations is based on the quality of available studies [4] which investigate the proportion of cases of a clinically defined group of patients which can be diagnosed by a specific test. As practically all studies have been retrospective (i.e. looking for a specific mutation in a previously ascertained diagnosed cohort), the highest level of recommendation will be at level B [4]. 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'.

Molecular diagnosis of hereditary ataxias

The hereditary ataxias are a heterogeneous group of neurological disorders (Table 1), characterized by imbalance, progressive gait and limb incoordination, dysarthria, and disturbances of eye movements. The phenotype is often complicated by the presence of additional neurological and systemic signs, which depend on both the specific genetic subtype and the individual characteristics. Approximately 10 loci for autosomal recessive phenotypes and more than 25 loci for the autosomal dominant forms [5,6] have so far been found. The estimated prevalence varies greatly between countries, from 1/50 000 for Friedreich ataxia (FRDA1) in Europe to 1/100 000 for ataxia telangiectasia (AT) and the dominant spinocerebellar ataxias (SCAs) worldwide. X-linked ataxias are rare. In rare instances, cerebellar ataxia may represent the main clinical finding of mitochondrial diseases [7]. Detailed family and medical history, physical examination, neuroimaging, and laboratory tests may guide molecular screening.

Autosomal dominant cerebellar ataxias

In the current genetic classification, the autosomal dominant cerebellar ataxias are designated as SCAs (*s*pino*c*erebellar *a*taxias) and EAs (episodic ataxias).

Spinocerebellar ataxias

At present, 27 genetic loci for SCAs have been identified: SCA1-8, SCA10-23, SCA25-30 [6,8]. In addition, dentato-rubro-pallidoluysian atrophy (DRPLA) is commonly included in this group [9] (Table 1). Three major classes of SCAs have been recognized. The first includes DRPLA and SCA1, 2, 3, 6, 7, 17 that are caused by a CAG repeat expansion in the coding region. This type of mutation represents the most common cause of dominantly inherited ataxias. Longer expansions are associated with earlier onset and more severe disease. A second category of repeat expansions is localized outside the protein-coding region. This group includes SCA8, 10, and 12. A third category is represented by the dominant ataxias SCA5, 11, 13, 14, 15/16, and 27, caused by conventional deletion, missense, nonsense, and splice site mutations in their respective genes. The clinico-genetic heterogeneity of the SCAs has led to some uncertainty concerning the assigned loci/genes: SCA15 and 16 are caused by mutations in the same gene; also SCA19 and SCA22 have been suggested to be the same disease, whilst two different non-allelic diseases have been mapped to the 16q22.1 locus [10,11] (Table 1).

Molecular testing should be guided by taking into account the main associated symptoms (Table 1): SCA6 patients have relatively pure cerebellar ataxia; SCA1, 2, and 3 patients show variable involvement of the extrapyramidal, pyramidal, and peripheral nervous system, whilst those with SCA7 are typically distinguished by retinal degeneration [6,12–14]. An additional criterion guiding molecular tests is to consider the geographical origin of the family, because some SCA genotypes are more frequent in particular populations [6]. Genetic testing for SCA 1, 2, 3, 6, 7, and 17 is technically reliable and available today in many laboratories as a routine procedure. Large clinico-genetic series have been reported to support this selection of genes [12–14]. For the other SCAs, insufficient data do not allow general recommendations.

Recommendations for genetic testing in spinocerebellar ataxias

In case of a family history compatible with autosomal dominant inheritance, genetic testing should include SCA1, 2, 3, 6, 7, and 17, which represent the majority of the presently identified SCA genotypes in Europe (Level B) [12–14]. Amongst those, priorities should be chosen according to associated clinical features.

Episodic ataxias

The episodic ataxias (EA) are characterized by recurrent attacks of ataxia, giddiness, and vertigo. Attacks may last from minutes (EA1) or several hours

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Table 1 Molecular diagnosis of cerebellar ataxias

Disease	Gene	Inheritance[a]	Position	Mutation[b]	Main associated symptoms	Age at onset	Paraclinical test	MIM number
SCA1	ATXN1	AD	6p23	Trinuc	Dementia, nystagmus, slow saccades pyramidal signs, neuropathy	4–74		164400
SCA2	ATXN2	AD	12q24.1	Trinuc	Dementia, slow saccades, hyporreflexia, amyotrophy, neuropathy, myoclonus, rare parkinsonism	6–67		183090
SCA3	ATXN3	AD	14q24.3-q31	Trinuc	Nystagmus, diplopia, ophthalmoplegia, eye-lid retraction, parkinsonism, spasticity neuropathy	5–65		607047
SCA4[c]	Unknown	AD	16q22.1	Unknown	Cerebellar syndrome associated with axonal sensitive neuropathy	19–72		600223
16q-linked ADCA[c]	Puratrophin-1 PLEKHG4[a]	AD	16q22.1	Pm, C > T in the 5′UTR	Late-onset cerebellar syndrome, often associated with sensorineural hearing impairment	> 50		117210
SCA5	β-III Spectrin (SPTBN2)	AD	11q13	Pm; Del	Pure cerebellar syndrome, downbeat nystagmus, bulbar symptoms in juvenile cases. Slow progression	15–50		600224
SCA6	CACNA1A	AD	19p13	Trinuc	Pure cerebellar syndrome, downbeat positioning nystagmus, sometimes episodic ataxia at onset, double vision, pyramidal signs, deep sensory loss, migraine. (Disease is allelic to episodic EA2 and familial hemiplegic migraine)	19–77		601011
SCA7	ATXN7	AD	3p14-p21.1	Trinuc	Retinal degeneration, ophthalmoplegia, pyramidal signs	0.1–76		164500
SCA8	ATXN8 (Kelch-like)	AD	13q21	Trinuc	Sensory neuropathy, slow progression	0–73		608768
SCA10	ATXN10	AD	22q13	ATTCT expansion	Seizures, pyramidal and extrapyramidal signs	10–40		603516
SCA11	TTBK2	AD	15q14-21.3	Pm; Del; Ins	Pure cerebellar syndrome, hyperreflexia, very slow progression	17–33		604432
SCA12	PPP2R2B	AD	5q31-q33	Trinuc	Dementia, tremor of head and upper extremities, parkinsonism, hyperreflexia, neuropathy	8–55		604326
SCA13	KCNC3	AD	19q13.3-q13.4	Pm	Delayed motor development, mental retardation in some, seizures	4–60		605259
SCA14	PRKCG	AD	19q13.4-qter	Pm	Cognitive deficits, depression, facial myokymia, rare myoclonus and focal dystonia	10–59		605361
SCA15/SCA16	ITPR1	AD	3p26-p25	Del; Pm	Pure cerebellar syndrome or associated with head tremor. Slow progression	10–66		606658
SCA17	TBP	AD	6q27	Trinuc	Dementia, psychosis, chorea, seizures, Huntington disease-like symptoms	10–70		607136
SCA18	Unknown	AD	7q22-q32	Unknown	Limb weakness, axonal sensory neuropathy (EMG shows denervation)	13–27		607458
SCA19[d]	Unknown	AD	1p21-q21	Unknown	Dementia	20–45		607346
SCA20	Unknown	AD	11p13-q11	Unknown	Dysphonia, palatal tremor, spasmodic cough, bradykinesia, dentate calcification	19–64		608687
SCA21	Unknown	AD	7p21.3-p15.1	Unknown	Cognitive impairment, parkinsonism	6–30		607454
SCA22[d]	Unknown	AD	1p21-q23	Unknown	Pure cerebellar syndrome	10–46		607346
SCA23	Unknown	AD	20p13-12.3	Unknown	Pure cerebellar syndrome or associated with pyramidal signs, sensory loss	43–56		610245

Table 1 (Continued)

Disease	Gene	Inheritance[a]	Position	Mutation[b]	Main associated symptoms	Age at onset	Paraclinical test	MIM number
SCA25	Unknown	AD	2p15-p21	Unknown	Sensory neuropathy	1.5–39		608703
SCA26	Unknown	AD	19p13.3	Unknown	Pure cerebellar syndrome, slow progression	26–60		609306
SCA27	FGF14	AD	13q34	Pm	Tremor, dyskinetic movements, psychiatric signs	12–40		609307
SCA28	AFG3L2[e]	AD	18p11.22–q11.2	Pm	Ophthalmoplegia, hyperreflexia, slow progression	12–36		610246
SCA29	Unknown	AD	3p26	Unknown	Non-progressive, highly variable phenotype	Congenital		117360
SCA30[f]	Unknown	AD	4q34-q35	Unknown	Minor pyramidal signs	45–76		
DRPLA	DRPLA	AD	12p13.31	Trinuc	Dementia, chorea, myoclonus, seizures	10–59		125370
EA1	KCNA 1	AD	12p13	Pm; Del	Muscle spasms, interictal myokymia and jerking movements, chorea at onset. Attack duration: seconds to minutes	2–15		160120
EA2	CACNA1A	AD	19p13	Pm; Trinuc	Downbeat nystagmus, dysarthria, vertigo, muscle weakness, migraine. Interictal ataxia and nystagmus. Attack duration: hours to days	2–20		108500
EA3	Unknown	AD	1q42	Unknown	Myokymia, migraine, tinnitus, vertigo, dysarthria. Attack duration: 1m–6h	1–42		606554
EA4/PATX	Unknown	AD	Unknown	Unknown	Vertigo, diplopia. Interictal nystagmus and saccadic smooth pursuit. Attack duration: brief	23–60		606552
EA5	CACNB4β4	AD	2q22-23	Pm	Vertigo. Interictal nystagmus, ataxia, epilepsy. Attack duration: hours	3–19		601949
EA6	SLC1A3 (EAAT1)	AD	5p13	Pm	Cognitive impairment. Interictal epilepsy, migraine, ataxia, motor delayed milestones. Attack duration: hours/day	<20		600111
EA7	Unknown	AD	19q13	Unknown	Vertigo, dysarthria, muscle weakness. Attack duration: hours/days	13–19		611907
FRDA	FRDA1 (frataxin)	AR	9q13-q21.1	Trinuc; Pm	Saccadic smooth pursuit, fixation instability, saccadic dysmetria, dysarthria, Babinski sign, deep sensory loss, neuropathy cardiomyopathy, diabetes	2–55		229300
AVED	αTTP	AR	8q13.1-q13.3	Del; Ins; Pm	Head titubation, nystagmus, saccadic smooth pursuit, retinopathy	2–52	Low plasma levels of vitamin E	277460
ABL	MTP	AR	4q22–24	Pm	Steatorrhea, areflexia, sensory ataxia, retinal degeneration, dissociated nystagmus on lateral gaze, slow saccades, neuropathy	0–20	Acanthocytes, reduced serum LDL and VLDL	200100
AOA1	APTX	AR	9p13	Ins; Del; Pm	Oculomotor apraxia, fixation instability, saccadic pursuit, gaze-evoked nystagmus, hypometric saccades, neuropathy, choreoathetosis, mild mental retardation	1–29	Hypercholesterolemia, hypoalbuminemia	606350
AOA2	SETX	AR	9q34	Pm; Del	Oculomotor apraxia (rare), saccadic pursuit, slow saccades, fixation instability, choreoathetosis, motor neuropathy with amyotrophy	3–30	Increased AFP	608465
AT	ATM-gene	AR	11q22-q23	Del; Ins; Pm	Telangiectasia, immune deficiency, predisposition to cancer, oculomotor apraxia, increased latency of saccades	1–4	Chromosomal instability, increased AFP	208900

Table 1 (Continued)

Disease	Gene	Inheritance[a]	Position	Mutation[b]	Main associated symptoms	Age at onset	Paraclinical test	MIM number
ATLD	MRE11	AR	11q21	Pm	Similar to AT, milder course	1–7	Possible chromosomal instability, normal AFP	600814
FXTAS	FMR1	X-linked	Xq27.3	Trinuc	Intention tremor, nystagmus, mild parkinsonism, neuropathy	> 50		300623

[a]AD, Autosomal dominant; AR, Autosomal recessive.
[b]Pm, Point mutations; Del, Deletions; Ins, Insertions; Trinuc, Trinucleotide-repeat expansions.
[c]See Ref. [10].
[d]SCA19 and SCA22 may be the same disease (see Ref. [11]).
[e]Di Bella D, *et al. AFG3L2* mutations cause autosomal dominant SCA28 and reveal an essential role of the m-AAA AFG3L2 homocomplex in the cerebellum. (Abstract N.216, presented at the annual meeting of The American Society of Human Genetics, 11–15 November, 2008, Philadelphia, Pennsylvania). Available at: http://www.ashg.org/2008meeting/abstracts/fulltext/.
[f]Not yet catalogued in OMIM; see Ref. [8].
AFP, Alpha-Fetoprotein; AT, ataxia telangiectasia; ATLD, Ataxia-Telangiectasia-like Disorder; ATM, AT-mutated gene; ATTP, α-Tocopherol Transfer Protein; ATXN 1/2/3/7/8/10, Ataxin1/2/3/7/8/10; AVED, Ataxia with vitamin E deficiency; CACNA1A, Calcium Channel, Alpha-1a Subunit; CACNB4β4, Calcium Channel, Beta-4 Subunit; DRPLA, dentato-rubro-pallidoluysian atrophy; EA, episodic ataxias; EAAT1, Excitatory Amino Acid Transporter 1; FGF14, Fibroblast Growth Factor 14; FMR1, Fragile X Mental Retardation; FXTAS, Fragile X tremor/ataxia syndrome; ITPR1, Inositol 1,4,5-Triphosphate Receptor, Type 1; KCNA 1, Potassium Voltage-Gated Channel; KCNC3, Potassium Channel, 3; MRE11, Meiotic Recombination 11; MTP, Microsomal Transfer Protein; PLEKHG4, Pleckstrin Domain-Containing Protein; PPP2R2B, Protein Phosphatase 2, Subunit B; PRKCG, Protein Kinase Cγ; SLC1A3, Glial High-Affinity Glutamate Transporter 3; SPTBN2, Spectrin, Beta, Non-erythrocytic 2; TBP, TATA Box-Binding Protein; TTBK2, Tau Tubulin Kinase 2.

(EA2), can be provoked by rapid movements in EA1 and by exercise, emotional stress, alcohol and caffeine in EA2. In the interval, myokymia is present in hand muscles when analysed by EMG in EA1 whereas EA2 patients present with interictal gaze-evoked nystagmus. The current classification recognizes seven distinct subtypes (Table 1) [15]. Four genes have been identified, including the potassium channel gene *KCNA1* (EA1), the calcium channel genes *CACNA1A* (EA2) and *CACNB4* (EA5), and the glutamate transporter gene *SLC1A3* (EA6) [15,16]. EA2 is allelic to SCA6 and familial hemiplegic migraine (FHM1). In these disorders, clinical phenotypes are highly overlapping: both ataxia and hemiplegic migraine may occur in the same patient, and EA2 phenotype or SCA6 features have been described in the same family [16].

Severity and frequency of attacks may be reduced by acetazolamide.

Recommendations for genetic test in episodic ataxias

Genetic testing for EA1 and EA2, available in specialized or research laboratories, is recommended in patients with family history suggesting dominant inheritance and recurrent attacks of ataxia and vertigo (Level C) [16].

Autosomal recessive cerebellar ataxias

Autosomal recessive ataxias are a heterogeneous group of rare neurodegenerative diseases, mostly characterized by early onset cerebellar ataxia associated with various neurological, ophthalmologic, or systemic signs (Table 1). Neurological features include optic atrophy, extrapyramidal and pyramidal signs, peripheral neuropathy, cognitive impairment, or epilepsy. These diseases are usually caused by a '*loss of function*' of specific cellular proteins involved in metabolic homeostasis, cell cycle, and DNA repair/protection [5,17]. The more common recessive forms in Europe are as follows: Friedreich ataxia (FRDA), ataxia telangiectasia (AT), and ataxia with oculomotor apraxia (AOA).

Friedreich's ataxia (FRDA)

FRDA (MIM 229300) is the most common hereditary ataxia in Caucasian populations. The classical clinical features are onset before age 25, progressive gait and limb ataxia, dysarthria, absent deep tendon reflexes, sensory loss, and pyramidal weakness. Ataxia is of the afferent, rather than the cerebellar type. Cardiomyopathy is present in the majority of patients. Axonal sensory neuropathy, distal wasting, scoliosis, sensorineural deafness, optic atrophy, and diabetes are common features. In most cases, the disease is caused by a

GAA-trinucleotide-repeat expansion in the first intron of the *FRDA* gene on chromosome 9q13-21 [17]. FRDA patients carry expanded alleles with 90–1300 repeats. Over 90% of FRDA patients carry the expansion on both alleles. Only a few patients are compound heterozygous, harbouring point mutations or microdeletions on one allele, and the GAA expansion on the other. The expansion size has been shown to be inversely correlated with age of onset and age of confinement to wheelchair, and directly correlated with the incidence of cardiomyopathy.

Ataxia with oculomotor apraxia type 1 and type 2 (AOA1-AOA2)

The disease is characterized by ataxia, oculomotor apraxia, and choreoathetosis. AOA has been recently associated with two distinct genetic forms: AOA 1 and AOA 2 [18]. AOA 1 (MIM 208920) has an early age at onset and is characterized by cerebellar ataxia, sensorimotor neuropathy, nystagmus, variable oculomotor apraxia, extrapyramidal signs, and mild cognitive impairment. Patients may present hypoalbuminaemia, hypercholesterolaemia, and normal alpha-fetoprotein. The disease is caused by mutations in the aprataxin gene, *APTX,* on chromosome 9p13. The protein probably plays a role in DNA repair.

Ataxia with oculomotor apraxia type 2 (MIM 606002) presents with a similar phenotype as type 1, but age at onset is in the early teens, and laboratory studies show normal albumin and high serum alpha-fetoprotein. The disease is caused by mutations in the gene encoding senataxin, *SETX,* on chromosome 9q34. Although the functional role of human senataxin is unknown, its yeast orthologue, *Sen1p*, is implicated in DNA transcription, repair, and processing.

Ataxia telangiectasia (AT)

Ataxia telangiectasia (MIM 208900) has a prevalence estimated between 1:40 000 to 1:100 000. The disease is characterized by cerebellar ataxia, ocular apraxia, telangiectasias, immune defects, and a predisposition to malignancy. Patients present in early childhood with progressive cerebellar ataxia and later develop telangiectasias and progressive neurological degeneration. Choreoathetosis and/or dystonia occur in 90% of patients. High serum alpha-fetoprotein is typical. The disease results from mutations in the AT-mutated gene (ATM) on chromosome 11q22–23. The protein is involved in the DNA damage response pathway. The phenotype can vary in severity depending on the amount of the ATM protein expressed [19]. A very important feature is susceptibility to cancer, with a risk of approximately 40%. Most frequent malignancies are leukaemia or lymphoma. Patients and heterozygote relatives should be advised to avoid any kind of radiation, including unnecessary X-ray.

Ataxia with vitamin E deficiency Abeta-lipoproteinaemia

Ataxia with vitamin E deficiency (AVED, MIM 277460) presents with a phenotype similar to Friedreich's ataxia with age at onset before 20, yet the concentrations of vitamin E in serum are typically reduced. Most patients are from the Mediterranean area. Decreased visual acuity or retinitis pigmentosa may occur. The disease is caused by mutations of the alpha-tocopherol transfer protein gene on chromosome 8q13. Supplementation with vitamin E slows the progression of the disease [20].

Abeta-lipoproteinaemia (ABL, MIM 200100) is caused by mutations in the gene for the large subunit of the microsomal triglyceride transfer protein, on chromosome 4q22–24, which functions in the assembly of apolipoprotein-B containing very low-density lipoproteins and chylomicrons. The neurological phenotype is similar to that observed in vitamin E deficiency, but it is associated with lipid malabsorption, hypocholesterolaemia, and acanthocytosis. Treatment involves dietary modification and vitamin E replacement, which may prevent neurological complications [5].

Recommendations for genetic test in autosomal recessive ataxias

In cases presenting with early onset ataxia, peripheral sensory neuropathy, and absence of marked cerebellar atrophy at MRI, genetic test for FRDA mutation is recommended (Class B) [5,17].

Molecular testing for ATM, AOA1, and AOA2 is recommended when guided by positive biochemical findings such as reduced levels of albumin, and increased levels of cholesterol or alpha-fetoprotein (Level C) [18,19]. AVED can usually be diagnosed reliably by measuring vitamin E levels in serum. Molecular diagnosis can be helpful for early detection in siblings of patients with an established diagnosis. (Level C) [20].

X-linked ataxias

Fragile X tremor/ataxia syndrome (FXTAS**,** MIM 300623) is caused by an expanded CGG-trinucleotide-repeat in the FMR1 gene, ranging in size from 55 to 200 repeats ('premutations'). Full repeat expansions (> 200 repeats) result in fragile X mental retardation syndrome. Men with a fragile X premutation present in the sixth decade with progressive intention tremor, ataxia, and parkinsonism, cognitive decline, peripheral neuropathy [21]. Symmetric regions of increased T2 signal intensity in the middle cerebellar peduncles and adjacent cerebellar white matter are considered typical of this neurological condition.

Recommendations for FXTAS genetic testing
Genetic testing for the X-linked FXTAS is recommended when there is a clinical suspicion, and it is readily available in many laboratories (Class B) [21].

Molecular diagnosis of hereditary spastic paraplegias

The hereditary spastic paraplegias (HSP) are a heterogeneous group of neurodegenerative disorders (Table 2), characterized by a slowly progressive pyramidal tract dysfunction, occurring either in 'pure' or in 'complicated' forms, the latter being associated with a variety of other neurological signs and symptoms [22]. Classifications based on phenotypes have been gradually replaced by a genetic classification [23]. To date, about 40 genetically distinct forms are recognized, and 17 of the genes have been identified. There are about as many autosomal dominant (AD) as recessive (AR) forms, in addition to at least 3 X-linked HSPs (see Table 2). Most of the autosomal dominant-hereditary spastic paraplegias (AD-HSPs) present clinically as pure HSP, whilst most of the AR–HSPs are complicated forms. If the family history is negative, a thorough work-up of the differential diagnosis is mandatory. Adrenomyeloneuropathy (AMN), mitochondrial disorders, multiple sclerosis, vitamin B12 deficiency, and myelopathy because of cervical pathology are the most important.

X-linked forms of HSP
Symptoms caused by mutations in the spastic paraplegia gene 1 (SPG1) (MIM 303350) start in infancy. SPG1 is allelic to MASA (mental retardation, aphasia, shuffling gait, adducted thumbs) and X-linked hydrocephalus, caused by different mutations in the *L1-CAM* gene, encoding a neural cell adhesion molecule. Mutations in the *proteolipid protein (PLP)* gene (MIM 312920) cause SPG2. PLP mutations are disturbing myelination and are associated with a wide variety of phenotypes, ranging from severe, infantile forms of Pelizaeus–Merzbacher disease (PMD, MIM 312080) with complex oculomotor disturbance, spastic weakness, ataxia, choreoathetosis, optic atrophy and psychomotor retardation to relatively mild pure spastic paraplegia of adult onset.

For both SPG1 and SPG2, clinical diagnosis depends on the typical neurological findings, X-linked inheritance pattern, and abnormal myelination on MRI [24].

AD forms of HSP
The most frequent form of HSP reported worldwide is SPG4, where either point mutations or deletions in the *SPAST* gene are found (MIM 182601). It accounts for approximately 50% of AD-HSP [25]. Most patients carry point mutations or small deletions or insertions, but in about 20% of SPG4 families the disease is caused by deletions of one or more entire exons in the *SPAST* gene [26]. Interestingly, a large number of *SPAST* variants have been reported in HSP patients without a family history of gait disorders, although there appear to be very few *de novo* mutations. This may be because of the presence of 20% asymptomatic mutation carriers and to the remarkable heterogeneity in the clinical presentation with regard to age at onset and severity of disease. Onset has been reported from 1 to 76 years of age, mostly in early adulthood (20–40 years). As a rule, there are no additional neurological symptoms except for urinary urgency and leg cramps that are considered to be part of pure HSP. A few SPG4 families with complicated HSP (neuropathy, mild ataxia, and dementia) have been described. Progression tends to be faster with later onset.

The second most frequent AD-HSP is SPG3, caused by mutations in the *SPG3A* gene, encoding the protein atlastin (MIM 182600). SPG3 is a pure form, sometimes associated with neuropathy with onset in childhood or adolescence (< 20 years old). The course of the disease is often mild but may be severe. SPG3 represents about 40% of young-onset AD-HSP when SPG4 has been excluded [27].

SPG31 with mutations in the *REEP1* gene is the next frequent form of HSP and accounts for 8% of all AD-HSP [28].

SPG10 with mutations in *KIF5A* coding for the kinesin heavy chain is less frequent, and association with an axonal neuropathy is reported [29]. The other forms of AD-HSP (Table 2) have only been reported in one or a few families, and for many forms, the gene is not yet identified. A variant in the gene *ZFYVE27*, which was hypothesized to cause SPG33 [30], is probably a rare polymorphism [31].

AR forms of HSP
About 15 forms of AR-HSP are described (see Table 2), and for most of them, no gene has been identified yet. As a rule, most recessive forms are complicated.

SPG7 (MIM 607259) is caused by mutations in the gene encoding paraplegin. SPG7 has a wide range of onset (8–42 years) and mainly presents as pure HSP but may also include cerebellar and cerebral atrophy, optic atrophy and bulbar symptoms. 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. Analysis of muscle biopsies from two patients carrying paraplegin mutations showed typical signs of mitochondrial OXPHOS defects, thus suggesting a mitochondrial mechanism for neurodegener-

Table 2 Hereditary spastic paraplegia (Autosomal dominant AD, recessive AR, X-linked)

Name	Locus/Gene/Protein	Heredity	Onset (years)	Phenotype	MIM number
SPG1	Xq28/L1-CAM[a]	X	1–5	MASA[b], MR[c]	303350
SPG2	Xq21/PLP1[d]	X	1–18	Hydrocephalus, allelic with Pelizaeus–Merzbacher	312920
SPG3A	14q11.2/atlastin	AD	2–50	Pure HSP, neuropathy	182600
SPG4	2p22- p21/SPAST/spastin	AD	1–74	Pure HSP, Rarely cognitive impairment, neuropathy, thin corpus callosum	182601
SPG5A	8p12-q13/CYPB1	AR	1–30	Pure HSP	270800
SPG6	15q11.1/NIPA1	AD	12–35	Pure HSP	600363
SPG7	16q24.3/SPG7/paraplegin	AR	8–42	Complex or pure HSP: Optic atrophy, ophthalmoplegia, bulbar symptoms, scoliosis, cortical and cerebellar atrophy	607259
SPG8	8q23-q24/KIAA0196/strumpellin	AD	20–40	Pure HSP	603563
SPG9	10q23.3-q24.1	AD	1–40	Cataract, distal amyotrophy, neuropathy, short stature, gastrooesophageal reflux	601162
SPG10	12q13/KIF5A/kinesin heavy chain	AD	2–51	Neuropathy	604187
SPG11	15q13-q15/KIAA1840/spatacsin	AR	4–21	Thin corpus callosum, white matter lesions, cognitive impairment, dysarthria	604360
SPG12	19q13	AD	1–22	Pure HSP	604805
SPG13	2q24-q34/HSPD1/HSP60	AD	17–68	Pure HSP	605280
SPG14	3q27-q28	AR	30	Mild MR[c], distal neuropathy, pes cavus	605229
SPG15	14q22-q24/ZFYVE26/spastizin	AR	8–35	*Kjellin*'s syndrome, cognitive impairment, macula pigmentation, cerebellar ataxia, neuropathy and distal amyotrophy, cerebral atrophy, thin corpus callosum	270700
SPG16	Xq11.2	X	1–5	Complex HSP: Severe	300266
SPG17	11q12-q14/BSCL2/seipin	AR-AD	8–40	Silver's syndrome, distal amyotrophy. Allelic with DSMAV	270685
SPG19	9q33-q34	AD	36–55	Pure HSP:	607152
SPG20	13q12.3/SPG20/spartin	AR	1–10	Troyer's syndrome, distal amyotrophy	275900
SPG21	15q22.31/SPG21/maspardin	AR	1–30	Progressive dementia, cerebellar and extrapyramidal symptoms, thin corpus callosum	248900
SPG23	1q24-q32	AR	1	Mild MR[c], postural tremor, pigmentary skin abnormalities,	270750
SPG24	13q14	AR	1	Pure HSP	607584
SPG25	6q23-q24.1	AR	30–46	Pain, discs prolapses, spondylosis	608220
SPG26	12p11.1-12q14	AR	6–11	Mild cognitive impairment, dysarthria, atrophy of hand muscles	609195
SPG27	10q22.1-10q24.1	AD	25–45	Dysarthria	609041
SPG28	14q21.3-q22.3	AR	6–15	Pure HSP	609340
SPG29	1p31.1-21.1	AD	11–30	Hypacusis, paraoesophageal hernia and vomiting	609727
SPG30	2q37.3	AR	12–21	Mild cerebellar ataxia and neuropathy	610357
SPG31	2p12/REEP1	AD	1–60	Pure HSP	610250
SPG32	14q12-q21	AR	6–7	Mild MR[c], pseudobulbar symptoms, slow progression, cerebral atrophy, thin corpus callosum, hypoplastic pons	611252
SPG33?	See text				610244
SPG35	16q21-q23	AR	6–11	Cognitive impairment, epilepsy	612319
SPG37	8p21.1-q13.3	AD	8–60	Pure HSP	611945
SPG38	4p16-p15	AD	7–23	Hand muscle atrophy	612335
SPG39	19p13.3/PNPLA6	AR	Childhood	Distal amyotrophy	612020
SPG42	3q24-q26/SLC33A1	AD	4–42	Pure HSP	612539
SPAX1	12p13	AD	10–20	Spastic ataxia	108600
ARSACS[e]	13q12/SACS/sacsin	AR	1–70	Spastic ataxia, retinopathy, neuropathy	270550
IAHSP[f]	2q33/ALS2/alsin	AR	1–2	Tetraplegia, dysphagia, anarthria	607225
AHDS	Xq13.2	X	Infancy	Allan–Herndon syndrome	300523

[a]Neural cell adhesion molecule L1 gene.

[b]Mental retardation, aphasia, 'shuffling gait', 'adducted thumbs'.

[c]Mental retardation.

[d]Proteolipid protein gene.

[e]Spastic ataxia of Charlevoix–Saguenay.

[f]Infantile ascending hereditary spastic paraplegia.

AOA, ataxia with oculomotor apraxia; AR, Autosomal recessive; ARSACS, Autosomal-recessive spastic ataxia of Charlevoix–Saguenay; HSP, hereditary spastic paraplegias; MASA, mental retardation, aphasia, shuffling gait, adducted thumbs; PLP, proteolipid protein.

ation in HSP-type disorders. SPG7 was found with a frequency of 7% in sporadic Dutch cases of HSP with adult onset [32].

SPG11 (MIM 182601) appears now to be the most frequent form of AR-HSP in the European population (21%) [33]. The gene *KIAA1840* codes for the protein spatacsin, but its function is not yet known. It is a complicated form of HSP, with early onset, generally before 25 years, and moderate to severe disability. Typical associated findings are thin corpus callosum (TCC), cognitive impairment, dysarthria, and atrophy of hand muscles.

SPG15 (MIM 270700) may be the second most frequent form of complicated HSP with TCC [34]. It is similar to SPG11 with cognitive impairment in most patients, neuropathy and distal amyotrophy, mild cerebellar signs, pigmentary retinal degeneration and TCC, and/or white matter hyperintensities. This clinical presentation was previously described in the literature as Kjellin syndrome. The gene *ZFYVE26* coding for the protein spastizin has been identified recently [34].

Autosomal-recessive spastic ataxia of Charlevoix–Saguenay (ARSACS, MIM 270550) was described as a common type of ataxia in northeastern Quebec. It has also been reported in other countries, including Africa and Japan, and showed a high frequency in Dutch patients with early onset spastic ataxia [35]. The *SACS* gene codes for the protein sacsin. Clinical features suggesting ARSACS are spasticity, cerebellar atrophy, neuropathy, and retinopathy.

Summary of recommendations concerning molecular diagnosis of HSP

Dominant forms. Patients with pure HSP and a family history of spastic paraparesis should be tested for SPG4 (level B). If direct sequencing of the *SPAST* gene is negative, a multiplex ligation-dependent probe amplification assay (MLPA) should be applied to assess genomic deletions (level B). As a third step, sequencing of *Atlastin* (SPG3) in subjects with a pure form and onset under 20 years is recommended (level B). Sequencing of REEP1 and KIF5A can be considered in remaining mutation-negative dominant families, the latter particularly when a neuropathy is present (level C).

X-linked and recessive forms. Testing for mutations in *L1-CAM* and *PLP* (SPG 1 and SPG2) should be proposed in early onset complex forms of HSP with typical radiological findings (level B). Molecular testing first for SPG11 and second SPG15 is recommended in recessive HSP and thin corpus callosum (level B). SPG7 may be tested especially when cerebellar features are present (level C). For other recessive and X-linked HSP forms, no general recommendation can be given.

Apparently sporadic spastic paraplegia. Sporadic subjects with progressive spastic paraparesis where other causes of spasticity have been carefully excluded should be tested for SPG4 mutations including MLPA (level B) [36]. In negative cases, sequencing of SPG7 may be proposed (level C).

Conflicts of interest

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

Web-links

- http://www.geneclinics.org/ 'GeneClinics', a clinical information resource relating genetic testing to diagnosis, management, and genetic counselling. University of Washington, Seattle.
- http://www.eurogentest.org/ 'EuroGentest', an EU-funded network of excellence intends to harmonize genetic testing across Europe. The website provides information about availability and quality assurance of genetic tests.
- http://www.orpha.net/ 'OrphaNet' is a searchable database of over 5000 rare diseases, which includes information about genetic testing (methods, laboratories) on most neurogenetic diseases.
- http://omim.nih.org 'Online Mendelian Inheritance in Man, OMIM'. Online catalogue of Mendelian disorders and traits in man)

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