European Journal of Human Genetics (2000) 8, 4–18

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REVIEW

Clinical and molecular advances in autosomal dominant cerebellar ataxias: from genotype to phenotype and physiopathology

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Major advances have been made in the understanding of autosomal dominant cerebellar ataxias since genetic markers came into use in the 1980s. The subsequent mapping of nine genes, six of which have been identified, involved in this clinically diverse group of disorders highlighted their great genetic heterogeneity. Evidence is now accumulating that, except for SCA8, the same molecular and physiopathological processes underlie these diseases and other neurodegenerative disorders sharing the same mutational basis, the expansion of a (CAG)n-polyglutamine coding sequence. The clinical overlap among the different genetic entities makes prediction of the molecular origin impossible in a single patient so that molecular characterisation is necessary. However, extended clinical and neuropathological comparisons have shown that each genetic entity has a characteristic constellation of signs and symptoms that are related to CAG repeat size and disease duration. The combined genetic and clinical information form the basis of a new classification that will aid better understanding of disease evolution, assure follow up and permit genetic counselling by the clinician. *European Journal of Human Genetics* (2000) **8**, 4–18.

Keywords: autosomal dominant cerebellar ataxia; polyglutamine expansion; spinocerebellar ataxias; anticipation; genetic heterogeneity; clinical and genetic correlations

Introduction

The hereditary ataxias comprise a wide spectrum of diseases with different clinical and neuropathological profiles. Genetic advances during the last decade have shown that they constitute the majority of neurodegenerative disorders caused by trinucleotide repeat expansions, including Friedreich's ataxia (FA) and six forms of autosomal dominant cerebellar ataxias (ADCA).

The clinical and pathological heterogeneity that is found even among affected members of the same family makes accurate clinical diagnosis difficult. The first attempts to classify these disorders were unsatisfactory for several reasons. They were based on a small number of anatomopathological observations that were not helpful in clinical practice and did not take into account disease duration or mode of inheritance.[1–3] Furthermore, patients from the same family often fell into different pathological categories, whereas patients with different aetiologies presented with similar disorders. These difficulties led Harding to propose a classification, now widely accepted, based solely on the mode of inheritance and on clinical criteria.[4–6]

FA, the most frequent form of ataxia, is a recessive disorder[7,8] caused by a $(GAA)_n$ expansion in the first intron of the *X25* gene on chromosome 9.[9]

ADCA constitute a more clinical and molecular heterogeneous group of disorders. The recent demonstration that a coding CAG repeat expansion is common to five of the identified *ADCA* genes, is providing new insights into their physiopathology and partly explain their clinical heterogeneity. A new classification is now emerging from the molecular and clinical data (Table 1).

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Received 7 June 1999; revised 1 September 1999; accepted 3 September 1999

Table 1 Clinical[6] and molecular classification of ADCA

ADCA type	Signs associated with cerebellar ataxia	Gene	Locus	Frequency	Repeat number range Normal	Pathological
I	± Ophthalmoplegia ± optic atrophy ± dementia ± extrapyramidal signs ± amyotrophy	*SCA1*	6p23	5–40%	6–44	39–83
		SCA2	12q24.1	10–40%	13–33	32–77
		SCA3/MJD	14q32.1	11–84%	12–40	54–89
		SCA4	16q22.1	1 family		
		SCA12	5	1 family	7–28	> 65
		other	?	?		
II	Progressive macular dystrophy ± ophthalmoplegia ± dementia ± extrapyramidal signs	*SCA7*	3p12–13	5–8%	4–35	37–306
		other	?	1 family		
III	Pure cerebellar syndrome	*SCA5*	11cen	2 families		
		SCA6	19p13.1	1–16%	4–18	20/21–33
		SCA8	13q21	~5%	16–92	107–250
		SCA11	15q14–21.3	1 family		
	Ataxia and epilepsy	*SCA10*	22q13	2 families		
		TBP	6q27	1 case	25–42	63

Towards a unifying clinical and molecular classification of ADCA

ADCA are characterised by variable degrees of cerebellar and brainstem degeneration or dysfunction. Neuronal loss variably affects the pons, the olives, the basal ganglia, the cerebellum and its afferences and efferences. Onset is generally during the third of fourth decade but can also occur in childhood or in the elderly. Patients usually present with progressive cerebellar ataxia and associated neurological signs that define three distinct phenotypes according to Harding's clinical classification.[6]

ADCA type I is the most common subtype and variably combines cerebellar ataxia and dysarthria, ophthalmoplegia, pyramidal and extrapyramidal signs, deep sensory loss, amyotrophy and dementia. However, several other signs and symptoms can be associated as well, ie slow eye movements, sphincter disturbances, axonal neuropathy, fasciculations or swallowing difficulties. The genes for four different forms of ADCA type I have been localised: *spinocerebellar ataxia (SCA) 1* on chromosome 6p,[10] *SCA2* on 12q,[11] *SCA3* or Machado-Joseph disease (MJD) on 14q[12,13] and *SCA4* on 16q.[14] The association of progressive macular degeneration with cerebellar ataxia is characteristic of ADCA type II, the gene for which has been mapped to the *SCA7* locus on chromosome 3p.[15–17] Finally, ADCA type III denotes a 'pure', generally late onset, cerebellar syndrome. Two loci, designated *SCA5* and *SCA11* have been mapped to chromosomes 11cen[18] and 15q,[19] respectively, and a *CACNA1A* gene mutation, denoted *SCA6*, is also responsible for a pure cerebellar syndrome.[20] Furthermore, a locus (*SCA10*) has been mapped to chromosome 22q13 in two Mexican pedigrees presenting with ataxia and seizures.[21,22]

An polyglutamine-coding $(CAG)_n$ repeat expansion has been identified as responsible for the disease in five of these genes: *SCA1*,[23] *SCA2*,[24–26] *SCA3/MJD*,[27–29] *SCA6*[20] and *SCA7*.[30–32] These disorders share common properties, with few exceptions, with other polyglutamine diseases such as Huntington's disease (HD), spinal bulbar and muscular atrophy (Kennedy's disease), and dentatorubral and pallidoluysian atrophy:[33]

a) onset is mostly in adulthood, but some juvenile cases are observed, especially when transmitted by affected fathers;

b) the disease course is progressive, unremitting and usually fatal after 10–30 years of evolution;

c) the clinical symptoms appear above a threshold number of CAG repeats ranging from 20 in *SCA6* to 54 in *SCA3/MJD*;

d) there is a strong negative correlation between the number of CAG repeats and age at onset;

e) the repeat sequence is unstable and its increase in size during transmission results in genetic anticipation, except for *SCA6*;

f) the gene is expressed ubiquitously;

g) the pathological protein accumulates in ubiquitinated neuronal intranuclear inclusions in several affected but also in non-affected brain structures.

Other loci responsible for the different subgroups of ADCA will probably be discovered. A CAG repeat sequence of more than 40 units has been found by the Repeat Expansion

Detection method in several patients that are not linked to known loci.[34] There is, however, no evidence of another specific protein with polyglutamine expansions (encoded by codons CAA and CAG) in non-*SCA1–7* families suggesting that the repeat might be smaller than the detection threshold or different in nature.[35] Interestingly, a CTG triplet repeat expansion in a non-coding region of the *SCA8* gene has been implicated in a large family.[36] More recently, CAG expansions in the tata-binding protein gene (TBP) and in the 5' region of a regulatory subunit of protein phosphatase 2A (SCA12) have been implicated in a sporadic case and in a single family with ataxia, respectively.[177,178]

Frequency and origin of the mutations

The prevalence of ADCA is not precisely known but it appears to be less than 10/100 000.[37,38] Most reports concern populations with probable founder effects, such as *SCA2* in the province of Holguin in Cuba (4/10 000)[39] or *SCA3/MJD* in the Azores in Portugal (1/4000).[40]

The relative frequencies of the different CAG repeat expansions have been determined by molecular typing in several populations.[41–54] However, most of the large series do not clearly indicate the ethnic and/or geographical origin of the families. Although in most countries *SCA3/MJD* is the major locus, the relative frequencies of SCAs vary widely according to the geographical origin. *SCA3/MJD* represents 80% of the families in Portugal and is also frequent in France (30%), Germany (40%) and Japan (39%) but has not been detected so far in Italy.[55] *SCA6* is frequent in Japan (30%) and Germany (13%) but quite rare in neighbouring France[53] and Spain.[56]

These differences are probably accounted for by regional founder effects. Linkage disequilibrium, which reflects the existence of a single or a major founder effect, has been detected with flanking markers at the *SCA1* locus in Japan,[57] *SCA2* in Northern Europe,[58] *SCA3/MJD* in France, Portugal and Japan[59–63] and *SCA7* in Korea, North Africa, Continental Europe and Anglo-Saxon countries.[64] A strong linkage disequilibrium is also detected in German *SCA6* pedigrees which probably descend from a single founder, since the repeat is not unstable in this disease.[65] Conversely, intragenic polymorphisms in the *SCA3/MJD* gene showed that, in Portugal, where this locus is responsible for almost all ADCAs, several different founders existed.[63] These studies demonstrate that the *SCA3/MJD* mutation was not transmitted by Portuguese sailors or travellers to the rest of the world, as previously hypothesised,[40,66,67] since haplotype observed in black African and Jewish Yemenite *SCA3* patients has not been found in Portuguese patients.[63]

Genetic studies have not only revealed the existence of founder effects but they have also provided insight into the mechanism of the mutation. *De novo SCA* mutations are probably rare, except in *SCA7* where several cases have been observed or inferred from paternal transmissions.[68] These neomutations resulted from the expansion of large normal alleles, often designated as intermediate alleles (IA), that contain from 28 to 35 CAG units. It is interesting to note that *de novo* cases of ADCA have only been reported in *SCA7*, which presents the greatest degree of instability during transmission and the greatest anticipation among the polyglutamine repeat diseases. These observations suggest, as has been confirmed in Huntington's disease (HD)[69] and *SCA3/MJD*,[70] that the degree of instability increases with the size of the normal alleles even before they reach the pathological range. If this observation is extended to all *SCA*s, it can be expected that in a given population *de novo* mutations at a given locus occur once or recurrently on IA. Therefore the relative frequency of *SCA*s should correlate with the frequency of IA in a given population. A recent study on Japanese, French and American families provided results in agreement with this hypothesis.[50] For example, the proportion of IA at the *DRPLA* locus in Japanese (24%) and Caucasians (10%) reflects the relative disease frequency of 20% and less than 1%, respectively, in these populations. More evidence supporting the idea that IA represent a reservoir for *de novo* mutations comes from the study of a polymorphism within the *MJD1* gene. The haplotype segregating in all French *SCA3/MJD* families is present on only 25% of normal alleles but in all large normal alleles over 33 repeats.[63]

Distribution of normal and expanded alleles

Normal alleles carry a variable number of CAG repeats but the degree of polymorphism varies according to the locus. Normal *SCA1*, *SCA3* and *SCA6* alleles are very polymorphic, with heterozygosity rates of approximately 80%, whereas normal *SCA2* and *SCA7* heterozygotes represents only 24 and 35%, respectively. This is due to the high frequency, close to 80%, of alleles with 22 and 10 CAG repeats at the *SCA2* and *SCA7* locus, respectively.[30,71]

The size distribution of expanded alleles is even greater, except in *SCA6*, but a specific pathological threshold is observed for each locus, ranging from 20 repeats in *SCA6* to 54 in *SCA3/MJD*.[72,73] Only few expansions exceed 100 repeat units and are associated with infantile cases in *SCA2*[74] and SCA7.[75–78] Usually, normal and expanded alleles carry uninterrupted CAG repeats and there is no overlap between the normal and pathological range. There are, however, two exceptions, *SCA1* and *SCA2*, in which most of the normal alleles are interrupted by 1 to 3 CAT or CAA respectively, and can attain the size of small pathological expansions, albeit rarely.[71,79,80] It is believed that the interruptions stabilise these alleles which are transmitted without modification, even when they reach the size of pathological expansions. In these cases, sequencing distinguishes the interrupted large normal alleles from the uninterrupted small pathological expansions. These rare situations may be encountered during molecular testing.

The size range of the untranslated CTG repeat at the *SCA8* locus is wider than in the polyglutamine diseases.[36] Normal alleles commonly carry from 16 to 37 repeats but chromosomes with up to 92 repeats have been detected in controls. In ataxic patients, pathological chromosomes have from 107 to 127 CTG repeats.

Instability and anticipation

Instability is a major characteristic of mutations caused by trinucleotide repeat expansions and is observed at both the somatic and the gonadal levels. Normal alleles are usually transmitted to progeny without modification. Most expansions (except *SCA6*), however, are unstable during transmission, with a tendency to increase in successive generations. At the *SCA1*, *SCA2* and *SCA7* loci, there is a tendency for greater instability during paternal than during maternal transmissions (Table 2), particularly for the largest expansions (> 20 CAG units). The result of instability is an increase in the mean size of the expansion over successive generations. However, the mean increase per generation varies greatly depending on the locus, ranging from approximately +0.7 for *SCA3/MJD* to +12 for *SCA7*.[81] Except in *SCA7*,[75] there is no correlation with the size of the repeat sequence, suggesting that flanking sequences may account for interloci differences in instability.

Instability during transmission results from gonadal mosaicism which can be easily detected in the sperm of patients. Analysis of whole or single sperm reveals much greater mosaicism of the expansion at the *SCA7* locus than at the *SCA3/MJD* locus, which is in accordance to the differences observed during transmissions.[28,75] Mosaicism is also detected in somatic tissues, including the central nervous system, but is always much less pronounced than gonadal mosaicism.[82–86]

CAG repeat instability is thought to result from slippage during DNA replication or from the formation of stable hairpin structures. In the latter case, this will result in large expansions or contractions depending on their location on the leading or lagging strand, respectively.[87–89] Genetic and epigenetic factors, such as the position and orientation with regard to the origin of replication, can modify repeat instability in *Escherichia coli*[88] and *Saccharomices cerevisiae*.[90–92] Instability is also influenced by the size of the repeat required to form stable structures as demonstrated in HD and *SCA7*.[75,93] In *SCA3/MJD*, the analysis of polymorphisms located close to the CAG repeat showed that they act both in *trans* and in *cis*[70,94] Sex-dependent instability is thought to be due to the difference between spermatogenesis and ovogenesis. The latter stops early during embryogenesis, whereas the former continues throughout life, resulting in a much larger number of mitoses. This may explain why the CAG repeat in HD is more unstable when the age at conception increases.[95] The human mismatch repair 2 protein (MSH2), which binds specifically to CAG repeats, might also interfere with repeat stability.[96] It is interesting to note that triplet repeats are located within regions with a high GC content.[97]

Instability at the *SCA8* locus, caused by an untranslated CTG repeat, is even more marked since parent–child differences in the number of repeats, ranging from –86 to +600, have been observed.[36] Furthermore, a maternal bias in this disease results in greater instability during mother–child than father–child transmissions. These observations are reminiscent of the group of diseases, including myotonic dystrophy, caused by untranslated trinucleotide repeats.[33,98]

Gonadal instability is the molecular basis of a major feature of ADCAs – the phenomenon of anticipation, ie the earlier onset and/or more severe course of the disease in successive generations. Due to the increase in size of the expansion from generation to generation and to the negative correlation between expansion size and the age at onset (Figure 1), the mean age at onset of ADCAs decreases with successive generations. The greatest anticipation is therefore found in *SCA7* families in which the expansion is very unstable. However, anticipation is usually overestimated because of observation biases. We have shown that the expected anticipation in *SCA2*, determined from the CAG expansion size/age at onset correlation curve was 12 years, whereas it was 20 years when calculated from the ages at onset in parent–child pairs.[71] Likewise, in *SCA3*, the expected and observed anticipations were 5 and 12 years, respectively.[52] In *SCA7*, however, expected and observed anticipations were similar.[75] Although anticipation was reported to be greater in paternal than in maternal *SCA7* transmissions,[99,100] this has not been confirmed in recent reports,[31,75,76,78] although all juvenile cases are transmitted by affected fathers. Surprisingly, anticipation has also been reported in *SCA6* families in which no instability occurs[44,54,101] This also probably results from an observation bias.[53] Observation of slight anticipation is therefore not sufficient to conclude that an unstable mutation is involved in a disease.

In the absence of meiotic distortion, anticipation should lead to extinction of the disorder in carrier families after a variable number of generations because the disease is not transmitted by infantile or juvenile patients. If the disease

Table 2 Comparison of CAG repeat instability during transmission to progeny at loci *SCA1, SCA3/MJD* (G Stevanin, A Dürr, A Brice, unpublished data, 1998), *SCA2*[26,71], *SCA7* [81], *DRPLA*[167,168], *SBMA*[169–171] and *HD*[172, 173]

	Gender of the transmitting parent	
	Male	*Female*
SCA1	+2.0 (–2 to +8, *n*=16)	+0.2 (–1 to +1, *n*=5)
SCA2	+3.5 (–8 to +17, *n*=33)	+1.7 (–4 to +8, *n*=23)
SCA3/MJD	+0.9 (–3 to +5, *n*=26)	+0.6 (–8 to +3, *n*=34)
SCA7	+12.1 (0 to +85, *n*=34)	+4.8 (–6 to +18, *n*=34)
DRPLA	+7.0 (0 to +28, *n*=33)	+0.3 (–4 to +4, *n*=9)
SBMA	+1.8 (–2 to +5, *n*=11)	+0.2 (–4 to +2, *n*=20)
HD	+6.1 (–4 to +74, *n*=156)	+0.6 (–4 to +16, *n*=160)

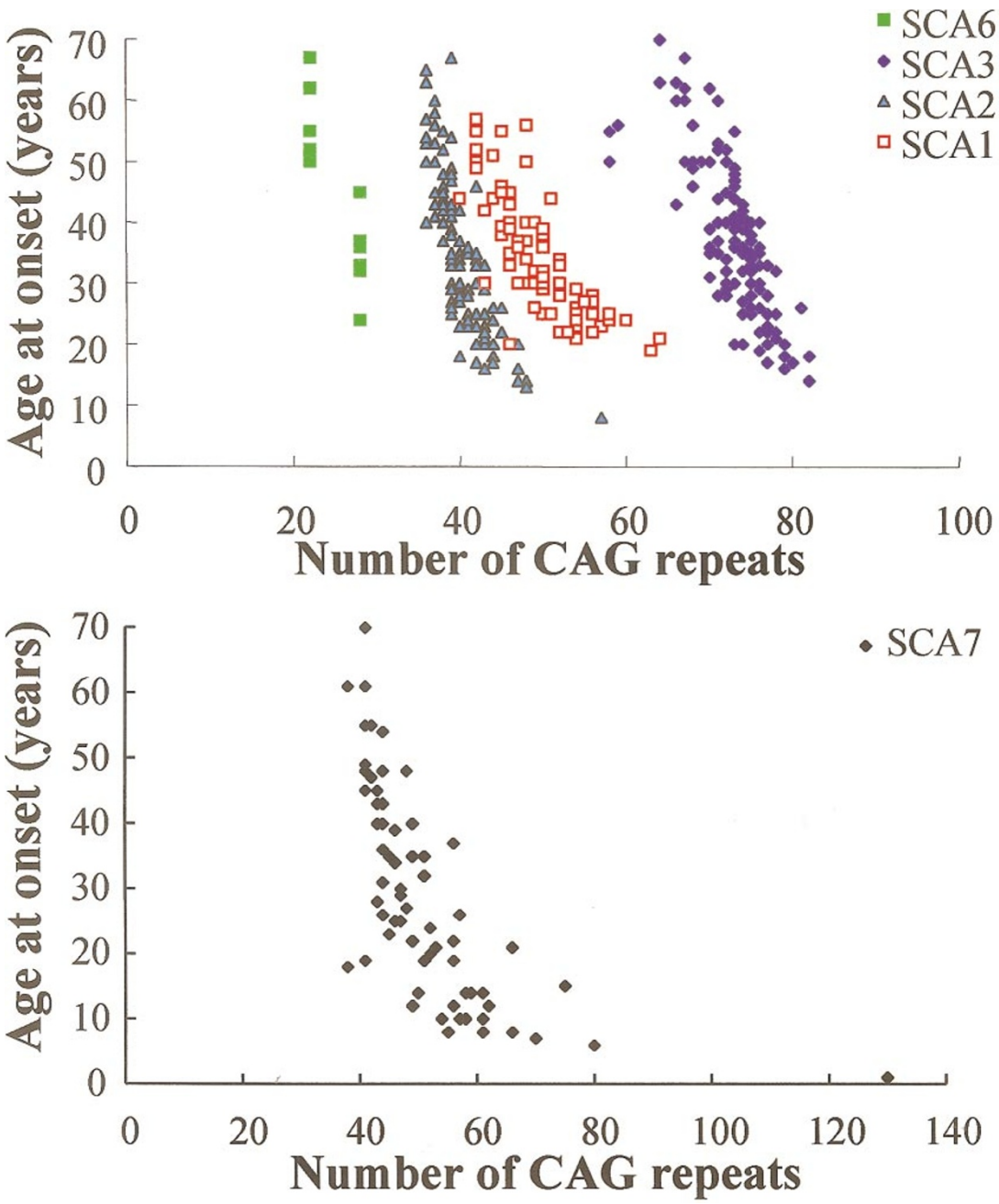

Figure 1 Age at onset/CAG repeat number correlation curves in spinocerebellar ataxias.[52,53,71,75,174,175]

remains in equilibrium, *de novo* mutations must occur to replace the non-transmitted pathological alleles. This seems to be the case in *SCA7* in which there is marked anticipation, frequent *de novo* mutations and IA in unaffected branches of the *SCA7* pedigrees.[68] On the contrary, the number of *de novo* mutations is probably very low in *SCA6* in which the repeat sequence is stable. The strong linkage disequilibrium in German *SCA6* kindreds is consistent with this hypothesis.[65]

Phenotypes and neuropathological features according to the locus

Clinical presentation in patients

The phenotypes of large series of genetically homogeneous patients has become possible through molecular screening. The major clinical features for *SCA1*, *SCA2*, *SCA3/MJD*, *SCA6* and *SCA7* patients are given in Table 3. Age at onset varies widely within and among groups. There is a tendency for the

Table 3 Frequency of neurological signs associated with SCA mutations[52,53,71,75,174,175]

	SCA1	*SCA2*	*SCA3/MJD*	*SCA6*	*SCA7*
Mean age at onset (years)	34	35	38	45	30
Onset after 55 years	–	–	±	++	–
Cerebellar syndrome	+++	+++	+++	+++	+++
Dysarthria	+++	+++	+++	++	+++
Babinski sign	++	+	++	0	++
Brisk reflexes	++	+	++	+	+++
Diminished or abolished reflexes	+	++	++	++	0
Spasticity in lower limbs	++	±	++	±	++
Amyotrophy	+	+	++	–	++
Extrapyramidal syndrome/dystonia	±	±	+	0	+
Myoclonus	–	++	±	–	
Nystagmus	++	+	+++	++	+
Ophthalmoplegia	++	++	++	0	++
Decreased saccade velocity	+	++	+	0	+++
Decreased visual acuity	0	0	0	0	+++
Bulging eyes	+	+	+	0	+
Myokymia	+	++	+	0	+
Decreased vibration sense	++	++	+	++	++
Dysphagia	++	++	++	++	++
Sphincter disturbances	++	++	++	++	++
Dementia	+	+	+	0	+
Tremor	–	+	±	±	+
Axonal neuropathy	++	+++	++	0	+
Decreased hearing acuity	0	0	0	0	+

mean age at onset to be earlier in *SCA7* and later in *SCA6*, compared with the other forms of ADCA. The only sign that is specific for a single locus is decreased visual acuity leading to blindness due to progressive macular dystrophy in most *SCA7* patients. No other clinical sign is specifically associated with a given genotype. However, group differences in the frequency of several signs, and their characteristic combination observed in more than one family member, can be suggestive of the genetic subtype in some cases.

An early decrease in saccade velocity and reduced tendon reflexes without extrapyramidal signs is suggestive of *SCA2*.[45,71,102,103] *SCA3/MJD* and *SCA6* patients present frequently with cerebellar oculomotor signs such as saccadic smooth pursuit, gaze-evoked nystagmus and diplopia. Extrapyramidal signs, myokimia and bulging eyes have been reported to characterise Machado-Joseph disease[104] but are not frequent in non-Portuguese Western European *SCA3/MJD* patients and might, therefore, be related to ethnic background.[13,29,52,105] *SCA3/MJD* patients also frequently have ophthalmoplegia or amyotrophy.[52,60,105–109] *SCA6* patients, however, usually have later onset, slower disease progression and few neurological signs in addition to cerebellar ataxia, at least during the first decade,[53] a profile that closely resembles that of SCA5 patients.[18,110] Episodic ataxia has been described as the presenting sign in some *SCA6* patients.[72] The clinical signs associated with the *SCA1* mutation are in general broader and homogeneous, and the patients have usually a pyramidal syndrome, often with hyperreflexia.[111]

Paraclinical investigations can also help to identify group differences. Schöls *et al*[45] have shown that increased conduction times in the central (> 10 ms) and peripheral (> 18 ms) nervous system are distinctive of the *SCA1* phenotype. Recording of ocular movements might also be useful, but there is some overlap of phenotypes.[112] Hypermetria is frequent in SCA1, slowing of saccades in *SCA2* and *SCA7*, gaze-evoked nystagmus and a tendency to hypometria in *SCA3/MJD*. Gaze-evoked nystagmus is also seen in SCA6. Cerebral MRI of SCA6 patients show pure and severe atrophy of the cerebellar *vermis* and hemispheres (Figure 2), whereas brainstem and cerebral hemispheres are spared.[44,53,113,114] This is strikingly similar to that observed in SCA5 patients.[110] SCA1, SCA2 (Figure 3) and *SCA7* patients also present with various degrees of cerebellar atrophy. *SCA3/MJD*, however, is characterised by severe pontine and spinal atrophy with moderate cerebellar atrophy. These features correlate well with the neuropathological observations.

Neuropathological lesions

Each genetic sub-form has a strikingly different neuropathological profile (Table 4).[115] *SCA6* patients have severe Purkinje cell loss with moderate degeneration of cells in the granular layer and inferior olives. *SCA1* patients have the most widespread cell loss. Their neuropathological profile can resemble OPCA. Olivocerebellar and dentatorubral tracts as well as the posterior column are affected. Atrophy of cranial nerves (mostly the third and 12th) and severe Purkinje cell loss (*vermis*) are also noted.[115] Cell loss is mild in the *substantia nigra*, the *locus coeruleus* and the Clarke's column.[52,116] The SCA2 profile is considered typical of OPCA since the inferior olive, *substantia nigra*, cerebellum (severe Purkinje cells loss) and pontine nuclei are affected.[117] It can be distinguished from SCA1, however, since the superior cerebellum peduncles are spared and the *substantia nigra*

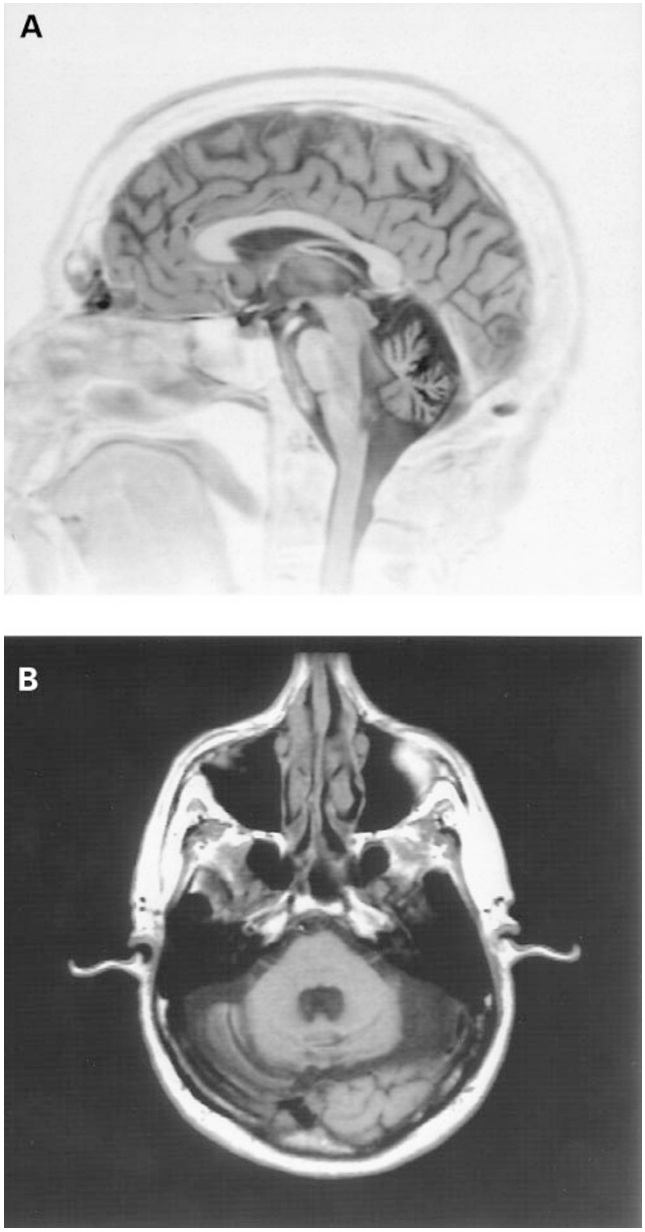

Figure 2 MRI of a SCA6 patient after 10 years' disease[53] **A** Inversion Recovery T2 weighted (TR 4000 ms; TE 17 ms) image on a strictly mid-sagittal section with major cerebellar atrophy and preserved brainstem and cerebrum; **B** Spin Echo T1 weighted (TR 640 ms; TE 11 ms) image on axial section through the pons and the cerebellum. The cerebellar vermis and cortex are severely atrophied, the pons is spared. (by permission of Dr Ayman Tourbah)

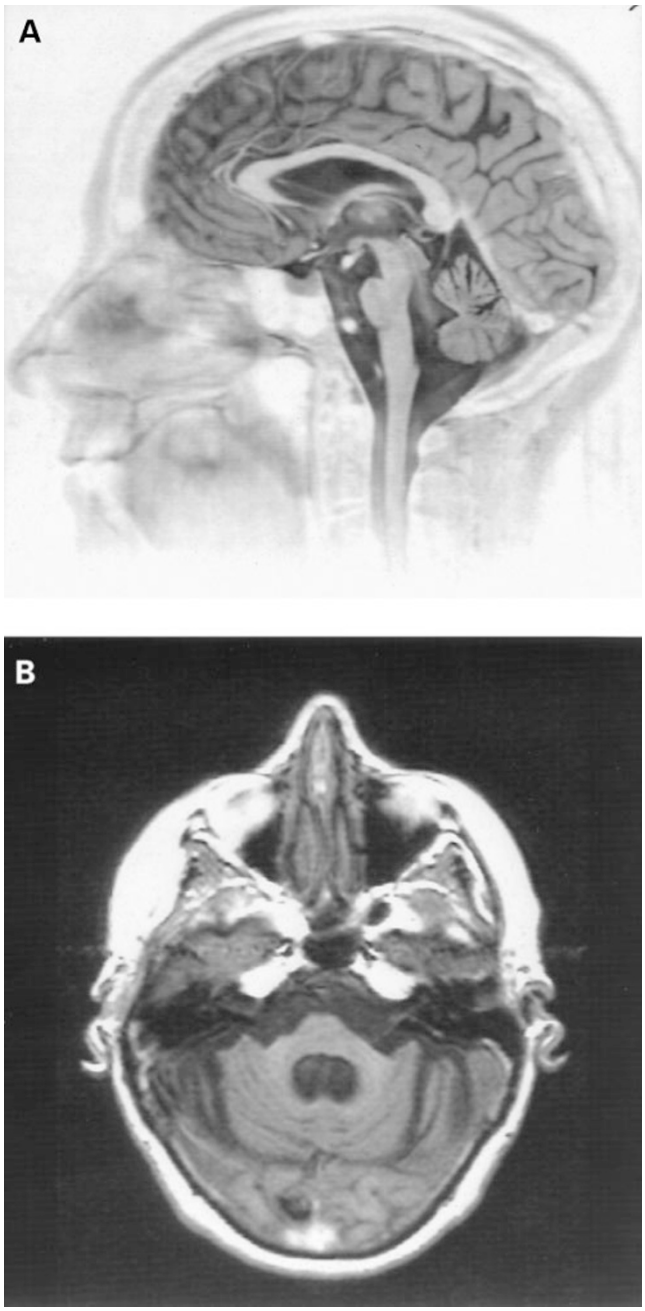

Figure 3 Cephalic MRI of a SCA2 patient with 10 years' disease. **A** Sagittal plane, inversion Recovery T2 weighted (TR = 5000 ms, TE = 17 ms, TI = 140 ms) showing a global atrophy of the pons, predominantly posterior, associated to a discrete bulbar and cerebellar vermian superior atrophy. **B** Spin Echo T1 weighted (TR = 2000 ms, TE = 11 ms, TI = 800 ms) image on axial section through the middle of the pons showing its atrophy as well as on the cerebellar hemispheres associated with the dilatation of the fourth ventricle. (by permission of Dr Ayman Tourbah)

Table 4 Neuropathological features associated with SCA mutations (A Dürr, C Duyckaerts, unpublished data, 1999 and[52,115,117,147,176])

Structure	*SCA1*	*SCA2*	*SCA3/MJD*	*SCA6*	*SCA7*
Cerebral cortex	–	+	–	–	(+)
White matter	–	+	–	–	–
Globus pallidus	+external	+	++internal	–	+
Sub-thalamic nucleus	+	+	++	–	++
Substantia nigra	+	++	++	–	++
Pontine nuclei	+	+++	++	–	+
Inferior olivary	+++	+++	–	(+)	+++
Purkinje cells	+	++	(+)	+++	++
Dentate nucleus	++	–	++	(+)	++
Spinocerebellar tracts	++	–	+++	–	+
Cortico-spinal tracts	–	–	(+)		+
Anterior horn	+	+	+	–	+
Posterior column	+	+++	+	–	+

– spared; (+) slight alteration; + discrete; ++ moderate; +++ severe atrophy.

severely lesioned. The cerebral cortex is also often affected. In SCA3/MJD, lesions of the basal ganglia (internal *pallidum*, *sub-thalamic nucleus* and *substantia nigra*), the intermediolateral column and Clarke's column are more severe than in SCA1, but the Purkinje cells, the inferior olives and posterior column are spared. This profile varies as a function of CAG repeat size.[52] Given the differences between the neuropathological profiles of SCA1 and SCA3/MJD patients, it is surprising that patients with these mutations present with the same variety of clinical signs associated with cerebellar ataxia.[52]

In SCA7 (ADCA type II) patients, spinocerebellar, olivocerebellar and efferent cerebellar tracts are severely affected. Purkinje cells, granule cells and neurons in the dentate nuclei also degenerate. Cell loss is also observed in the inferior olive, the *substantia nigra* and basis pontis, whereas the *thalamus* and *striatum* are spared. The distinctive features of ADCA II are involvement of optic pathways and the retina. The pregeniculate visual pathways are affected, probably as a consequence of retinal degeneration. Pathological examination of the retina shows early degeneration of the photoreceptors, the bipolar and granular cells, particularly in the foveal and parafoveal regions. Later, the inner retinal layers are affected with patchy loss of epithelial pigment cells and penetration of pigmented cells into the retinal layers.[118]

Factors influencing intrafamilial clinical variability

The major factors that influence phenotype are the size of the repeat expansion and disease duration at examination.

Factors influencing age at onset

In all subforms of SCA, age at onset varies among patients from the same family and correlates negatively with CAG repeat size, as in other trinucleotide repeat disorders. The repeat length explains 50–80% of the variability of age at onset (Figure 1), indicating that other factors influence pathogenesis. Its effect (the slope of regression curve), however, differs for each mutation and suggests that the protein context also affects pathogenesis.

Several attempts have been made to identify other factors influencing age at onset. A gender effect[119] was reported to be a contributing factor in SCA3/MJD, but this observation was not found by other groups.[120,121] At least one other familial factor is postulated in this disease[122] and the number of CAG repeats of the normal allele has also been reported to influence age at onset.[52] Homozygosity is reported to cause earlier onset in SCA2,[26] SCA3/MJD[119,123,124] and SCA6,[101,125,126] suggesting that allelic dosage influences clinical onset. This was not observed, however, in HD.[127,128]

Factors underlying variability of phenotype and progression rate

Part of the variability in phenotype has been explained by a bias resulting from clinical evaluation of patients with different disease durations. The frequencies of decreased vibration sense, Babinski sign, ophthalmoplegia, amyotrophy and sphincter disturbances are positively correlated with disease duration in ADCA-I families.[129] Clinical signs such as dysphagia or sphincter disturbances increase with disease duration in SCA2,[71] SCA3/MJD[52] and SCA7,[75] as does dysarthria in SCA6 patients.[53]

CAG repeat size, not only affects age at onset, it also has a major effect on phenotype expression. The rate of progression until death, of SCA1,[130] SCA3/MJD[131] and SCA7[75] patients, is negatively correlated with repeat size. Indeed, large *SCA7* expansions are associated with juvenile forms of the disease that are more severe and progress more rapidly than adult forms.[56,75,76,78] The number of CAG repeats also affects the frequency of several clinical signs and partly accounts for phenotypic variability among patients. In SCA3/MJD patients, the frequency of pyramidal signs increases with the size of the expanded repeat, whereas the frequency of altered vibration sense decreases.[52] Late-onset SCA3 patients often present with peripheral neuropathy (areflexia and amyotrophy) and have small CAG repeats.[52] Some patients with small *SCA3/MJD* expansions can present with late onset DOPA-responsive Parkinsonism.[132] The presence of mild axonal neuropathy helps to distinguish these patients

from idiopathic Parkinson's disease. On the other hand, SCA3 patients with large expansions are mostly dystonic. Some patients with amyotrophic lateral sclerosis-like presentation are diagnosed SCA1 and carry large expansions. They constitute an extreme clinical presentation of SCA1. In SCA2, myokimia, myoclonus, dystonia and fasciculations are more frequently observed in patients carrying large expansions.[71] SCA2 patients with large expansions manifest chorea and dementia as prominent features. In SCA7, the frequency of pyramidal signs, ophthalmoplegia and decreased visual acuity increases with repeat size.[75] In SCA6, given the slow progression of the disease, only patients with the largest expansions, associated with earlier onset, develop associated signs that can overlap with those found in ADCA type I.[53] Interestingly, cardiac failure was observed in patients with very large *SCA2*[74] and *SCA7*[78] expansions, indicating the possibility of extra-neurological involvement in extreme cases.

Physiopathology of ADCA and other neurodegenerative diseases caused by polyglutamine expansions

The expression of both mutated and normal proteins, and the dominant nature of the mutation suggest that the disease is the result of a gain of function that occurs at the protein level and increases with repeat size after a threshold of approximately 40 glutamines. The mechanism of this gain of function is still not elucidated, but evidence suggests that the pathological expansion confers a toxic property on the protein.

Animal and cellular models have been very useful for exploring the pathophysiology of triplet repeat disorders. Direct expression of a human cDNA encoding the *SCA1* and *SCA3* genes with expanded CAG repeats, and expression of an isolated expanded CAG repeat, cause Purkinje cell degeneration and ataxia in transgenic mice.[133,134] That expansion alters the conformation of polyglutamine tracts as initially suggested by the detection of long repeats using the 1C2 antibody.[135] This could explain the formation of insoluble intranuclear aggregates in an animal model of HD[136] and in transgenic models of ataxia. Neuronal intranuclear inclusions (NI) have also been detected in the brains of patients with several polyglutamine diseases (HD, DRPLA, SCA1, SCA3/MJD, SCA7) as well as in animal or cellular models,[137] and appear to constitute a common signature of this group of disorders (Figure 4). Cytoplasmic non-ubiquitinated aggregates have also been observed in SCA6 brains and in cultured cells transfected with full length CACNA1A with pathological expansion.[138]

How are NI formed? A number of hypotheses have been proposed, but none has been demonstrated *in vivo*: non-convalent interactions with other proteins, transglutamination,[139,140] formation of multimeric aggregates by hydrogen-bonded polar-zippers.[141] *In vitro*, the fibrillary appearance of

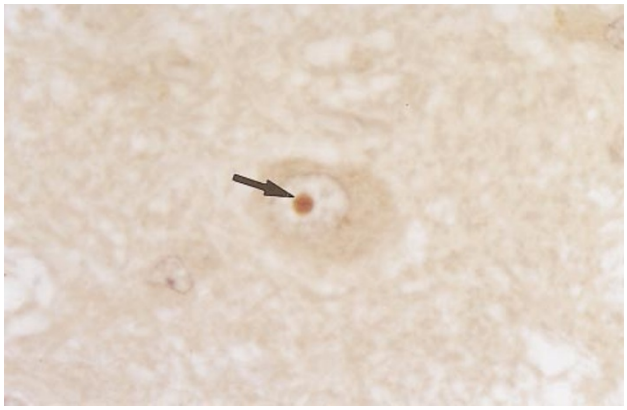

Figure 4 Intranuclear inclusions (NIs) in the inferior olive of a SCA7 patient carrying 85 CAG repeats ($\times$ 250). The inclusion has been labelled with the 1C2 antibody[135] and revealed by the peroxidase/anti-peroxidase technique, with diaminobenzidine as the chromogen. Staining of the nucleus by Harris haematoxylin. These NIs are also detected with an anti-ubiquitine antibody (data not shown). (by permission of Professor Charles Duyckaerts)

inclusions on electron microscopy and the green birefringence after staining with Congo red, both of which are reminiscent of amyloid, are consistent with the polar zipper hypothesis. Polyglutamine expansions are also good substrates for transglutaminases *in vitro*,[142] and the presence of transglutaminase inhibitors prevented aggregate formation in COS-7 cells transiently transfected with truncated forms of the *DRPLA* or *HD* gene with expansions.[143,144]

The major question remains whether nuclear aggregates are a cause or a consequence of the pathogenic process. They appear before neuronal loss and before expression of the phenotype in a mouse model of HD.[136] It was then suggested that they are a good hallmark of the disease, since their density is correlated to the expansion size.[145] However, they are also present in epithelial cells of a *drosophila* model of SCA3 in which there is no neuronal death or phenotype,[146] and have been detected in non-affected neuronal tissues in an SCA7 patient,[147] and in peripheral tissues in an HD mouse model,[148] indicating that their presence is not sufficient to cause death and/or phenotype. A recent although controversial[149] study showed, however, that visible aggregates are not a prerequisite for pathogenesis in a SCA1 mouse model[150] and that neuronal death is not correlated with NI formation in animal and cellular models of HD.[144,151] It was consequently proposed that NI may reflect a cell defence rather than a pathogenic mechanism.[152]

Is targeting to the nucleus a crucial step in pathogenesis and how does it occur? Neuronal degeneration does not occur when mutant ataxin-1[150] or mutant huntingtin[144] are targeted to the cytoplasm – two experiments again criticised.[149] The nucleus may therefore be a primary site of the degenerative process. The pathological proteins might be targeted to the nucleus either by nuclear transport or passive

transport following proteolytic cleavage. Evidence for aberrant proteolysis come from experiments in which truncated proteins with polyglutamine expansions appeared more prone than full length proteins to aggregate or cause cell death by apoptosis.[134,153–155] In addition, truncated fragments that may result from caspase-1 cleavage[156] have been detected in nuclear aggregates of HD patients.[157] The question remains whether this cleavage refers to the normal function of the proteins, to abnormal degradation, or to a protective response of the cell to these proteins. Elucidation of the proteolytic processing mechanisms would be desirable.

Interestingly, several neurodegenerative diseases including polyglutamine diseases as well as Alzheimer's disease, Parkinson's disease and prion-generated diseases result from the accumulation of misfolded proteins and associated cytotoxicity.[158] Indeed, experiments have revealed a characteristic that is reminiscent of prions, the recruitment of full-length proteins by truncated molecules in HD, DRPLA and SCA3.[143,153,154] More recently, expanded ataxin 1, atrophin and androgen receptor have been co-localised to the proteasome, a proteolytic system, and several studies have shown redistribution of the proteasome complex to NI,[159–161] which are ubiquitinated, one of the primary events in the proteasome degradation pathway. This might lead to the disorganisation of the nuclear machinery, perhaps by sequestering of transcription factors such as the TATA-binding protein,[162] other regulatory proteins or RNA,[163,164] and finally to cell death. The redistribution of the promyelocytic leukaemia protein in the nucleus of SCA1 patients is consistent with this hypothesis.[164]

The proteins with expanded polyglutamine tracts are widely expressed in the nervous system of patients, contrasting with the relatively selective pattern of degeneration observed in each disorder. Several studies have revealed that the degree of somatic mosaicism detected in the nervous system does not account for the selectivity of neuronal death,[86] which might result from specific interactions of the mutated protein with protein partners expressed preferentially in the regions affected by the respective diseases and/or from abnormal expression levels. Several protein partners have been identified, some of which have greater affinity for the expanded protein than the normal form. This is particularly the case with the leucine-rich acidic nuclear protein that interacts with ataxin-1 and co-localises with NI.[163] Selectivity is also a function of the progression of the disease, since infantile and adult cases can have different patterns of degeneration.

Diagnostic implications

Identification of *ADCA* genes and their mutations has now changed clinical practice because of the possibility of direct molecular diagnosis without which correct classification and diagnosis is extremely difficult. Molecular analysis for translated CAG repeat expansions permits routine diagnostic testing of individuals who already present with symptoms of the disease. Molecular analysis is also useful to distinguish disorders which are clinically similar or which can be confused with other diseases because of their extremely variable clinical presentation.

DNA testing in asymptomatic at-risk individuals, however, raises many difficult ethical issues for severe adult-onset disorders for which no treatment can be proposed. The international guidelines elaborated for Huntington's disease should also be followed for ADCA.[165] The number of CAG repeats on the expanded allele is a major factor which accounts for 50–80% of the variability of the age at onset. However, individual variations are important and age at onset cannot be precisely predicted from the number of CAG repeats.

Molecular diagnosis of isolated cases is also crucial. SCA6 and SCA2 were first to provide a molecular basis for some cases with idiopathic sporadic ataxia.[45,71] Family histories can be missed if the transmitting parent dies before onset of symptoms or is still asymptomatic because of marked anticipation. *De novo* mutations, as in SCA7,[68] also appear as isolated cases. Finally, care should also be taken to deduct alleles with more than 100 repeats, reported in juvenile or infantile forms of SCA2 and SCA7,[56,74–76,78,166] that might be difficult to amplify and visualise.

Conclusions

Discovery of the mutations underlying ADCA and the correlations between CAG repeat length and clinical or neuropathological features of genetically specific sub-forms of the disease have simplified the molecular diagnosis and permit analysis of uniformly classified patients, a necessary step to the development of a precise nosology and a better follow-up of the patients. Although it is often impossible to identify the SCA mutation on the basis of clinical criteria since phenotype depends on the locus, the size of the repeat expansion, the duration of the disease and other unknown factors, analysis of the molecular and clinical characteristics have revealed group differences that will be helpful for understanding the history and evolution of patients with ADCA.

The cause of the molecular instability and the pathophysiological consequences of the expanded polyglutamine tract remain unknown, and therapeutic intervention requires elucidation of the underlying pathogenic mechanism. Animal and cellular models are helping to understand processing of the pathological proteins and to identify their molecular partners. The recent report of a new type of mutation, an untranslated CTG repeat sequence at the *SCA8* locus, has complicated this picture and suggests that a different mechanism of toxicity might be involved. A dominant negative effect, a deleterious effect on the transcription of another gene close to this locus, or an abnormal interaction at the

RNA level are possible mechanisms but nothing is known as yet on this novel form of ADCA.

Acknowledgements

The authors are grateful to Dr Merle Ruberg and Anne-Sophie Lebre for critical reading of the manuscript. The authors' work is financially supported by the VERUM foundation, a BIOMED Concerted Action of the European Economic Community, the Institut National de la Santé et de la Recherche Médicale and the Association pour le Développement de la Recherche sur les Maladies Génétiques Neurologiques et Psychiatriques. GS held a fellowship from the Fondation pour la Recherche Médicale.

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