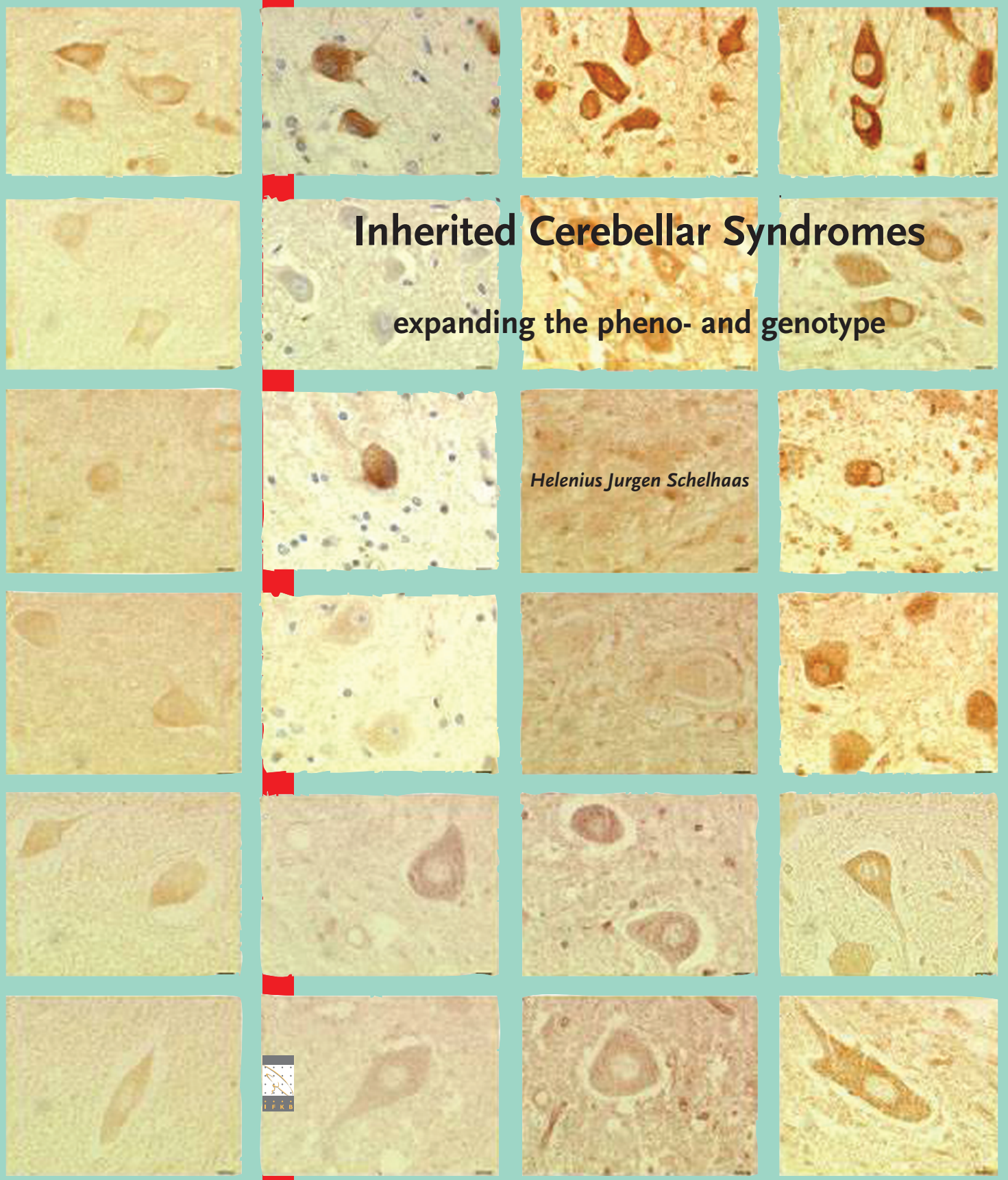


Inherited Cerebellar Syndromes

expanding the pheno- and genotype

Helenius Jurgen Schelhaas

I F K B

Inherited Cerebellar Syndromes

expanding the pheno- and genotype

Helenius Jurgen Schelhaas

H.J. Schelhaas, 2005
Inherited Cerebellar Syndromes
Expanding the pheno- and genotype

Thesis Radboud University Nijmegen
ISBN no: 90-9019392-8

Cover: see figure 7.4
Design and layout: Marcel Janssen
Printed by: Praktijkwerk, Zutphen, the Netherlands

Financial support for publication of this thesis was generously provided by:
Baxter B.V., Boehringer-Ingelheim B.V., Instituut voor Fundamentele en Klinische Bewegingswetenschappen (IFKB), Ipsen Farmaceutica B.V., Lamepro B.V., Novartis Pharma B.V., Pfizer B.V., Pharmachemie B.V., Sanofi-Aventis B.V., Sanquin B.V., Stichting Het Remmert Adriaan Laan Fonds en de afdeling Neurologie van de Radboud Universiteit Nijmegen

Inherited Cerebellar Syndromes

expanding the pheno- and genotype

Een wetenschappelijke proeve op het gebied van
de Medische Wetenschappen

Proefschrift

Ter verkrijging van de graad van doctor aan de Radboud Universiteit Nijmegen,
op gezag van de Rector Magnificus Prof. Dr. C.W.P.M. Blom,
volgens besluit van het college van Decanen
in het openbaar te verdedigen op
donderdag 16 juni 2005,
des namiddags om 3.30 uur precies

door

Helenius Jurgen Schelhaas
Geboren op 15 oktober 1967
te Hoogeveen

Promotores:	Prof. dr. H.P.H. Kremer Radboud University Nijmegen
	Prof. dr. M.J. Zwarts Radboud University Nijmegen
Co-promotor:	Dr. G. Hageman, Department of Neurology Medisch Spectrum Twente, Enschede
Manuscriptcommissie:	Prof. dr. B.G.M. van Engelen Radboud University Nijmegen
	Prof. dr. N. van Slobbe-Knoers Radboud University Nijmegen
	Prof. dr. J.H. Wokke, Department of Neurology University Medical Centre Utrecht

Voor mijn ouders

Contents

Part I

Introduction

Introduction and aims of the study

Chapter 1

The inherited cerebellar syndromes show a striking genetic variability. With an estimated prevalence of 1/50,000, Friedreich's ataxia (FRDA) is the most common inherited degenerative ataxia and accounts for three-quarters of those with an onset before the age of 25. Of the autosomal dominant cerebellar ataxias (ADCAs), about two-thirds of the prevalence seems to be explained by five mutations only: spinocerebellar ataxia (*SCA*) type 1, 2, 3, 6 and 7. The prevalence of these mutations in the Netherlands is estimated to be 3/100.000.
This thesis starts with a brief historical overview of the clinical nosology of the inherited cerebellar syndromes (chapter 1). This historical overview is followed by an outline of the aims of the study, which is essentially to expand the knowledge regarding the pheno- and genotype of these disorders. Before tackling the issue of 'expanding the knowledge' an overview concerning the current state is provided. The current state of the autosomal dominant cerebellar syndromes is addressed in chapter 2, and the current state of the autosomal recessive cerebellar syndromes in chapter 3.

Historical overview

Nicolaus Friedreich was the first author who systematically described patients with a hereditary form of ataxia (Table 1.1). In five different papers, published between 1863 and 1877, he described a distinctive clinical syndrome in nine patients belonging to five sibships (Friedreich 1877; Friedreich 1876; Friedreich 1863a; Friedreich 1863b; Friedreich 1863c). The clinical features were dominated by an early onset (first or second decade), progressive cerebellar dysarthria and ataxia, sensory loss, muscle weakness, and in some, scoliosis, diabetes, and cardiomyopathy. Autopsy, in four patients, showed a uniform picture of degeneration of the funiculus dorsalis, posterior spinal roots, nuclei thoracicus, and spinal lateral funiculus.

In 1893, Pierre Marie drew attention to four families with a clinical picture different from that of FRDA (Marie 1893). The age at onset was later, the tendon reflexes were increased, and neither scoliosis nor foot deformity was observed. Although Marie's 'heredo-ataxia cérébelleuse' is now known to be a heterogeneous group of disorders, his observation led to the important distinction between juvenile, and adult-onset, hereditary cerebellar ataxia. However, the significance of this distinction between early and late onset with respect to the Mendelian concept of autosomal recessive (usually early onset) and autosomal dominant inheritance (usually late onset) remained unrecognized until the beginning of the 20th century.

In 1900, Dejerine and Thomas described the clinical and post-mortem features of a sporadic case with symptoms that were characterized by progressive ataxic gait, dysarthria, hypotonia, and hyporeflexia (Dejerine-Thomas 1900). The symptoms started at the age of 30. Post-mortem examination revealed degeneration of the basis pontis, inferior olivary nuclei, and middle and inferior cerebellar peduncles. The post-mortem features they found were summarized in the descriptive term olivopontocerebellar atrophy (OPCA).

Holmes presented the first classification of the hereditary cerebellar syndrome in 1907 (Holmes 1907). He stated that the disease should be classified on the basis of morbid anatomy and pathogenesis. This approach was also taken by Greenfield (Greenfield 1954) and by Koningsmark and Weiner (Koningsmark and Weiner 1970) (for review see Pulst 2000).

However, several problems limited the usefulness of the clinicopathological classification systems. Because of pathological heterogeneity within families, patients in the same family were sometimes classified into different categories. Furthermore, the pathological classification system was not very helpful in everyday clinical practice - there was a need for a 'clinical' type of classification. In 1983, Harding achieved this task (Harding 1983). She proposed a classification based on clinical, genetic, and pathological features. Harding's classification soon became universally accepted. The subclassification of the ADCAs (a term

introduced by Harding) into type I, type II, and type III proved to be extremely useful for both clinical practice and research. In this classification, ADCA I was associated with additional features related to the optic nerve, (extra)pyramidal system, cerebral cortex, and peripheral nerves. ADCA II was associated with pigmentary macular dystrophy, and ADCA III was a relatively pure late-onset cerebellar syndrome.

While Harding's monograph remains a landmark in the history of hereditary cerebellar ataxia research, her classification has now been replaced by a new classification based on molecular genetic data. This new genetic classification system is known as 'SCA' classification.

Concerning the genetic SCA classification, linkage and assignment of the *SCA1* locus, centromeric to the HLA region on the short arm of chromosome 6, was achieved in 1989 (Zoghbi et al. 1989). The mutation for SCA1 was defined at the end of 1993 (Orr et al. 1993). They confirmed the earlier observation that *SCA1* mapped to 6p22-p23 and found a highly polymorphic CAG repeat in this region. This CAG repeat was found to be unstable and expanded in individuals with SCA1. Moreover, the size of the CAG repeat expansion was correlated with the age of disease onset. Larger alleles occurred in those patients with a juvenile disease onset, and anticipation was present in subsequent generations. A similar pathological mechanism had previous been described in Huntington and Kennedy disease and was later also reported for SCA2, 3, and 7 (Huntington research group 1993; La Spada et al. 1992; Pulst et al. 1996; Kawaguchi et al. 1994; David et al. 1997).

The number of a particular SCA is based on the sequence of finding the new locus. Currently, 25 different types of SCA have been identified (see chapter 2; table 2.1). Not all of these SCAs are CAG repeat disorders.

The identification of genetic abnormalities might be considered as the first step in the elucidation of the processes of neurodegeneration. For the SCAs, a major second step forward came with the identification of the neuronal intranuclear inclusions (NII), pathological structures found in Huntington disease, SCA1, 2, 3, 7 and 17 (for review see Davies et al. 1998). These two steps are essential to elucidate the biochemical alterations that are induced by DNA mutation, followed by altered transcription and protein translation, worsened by abnormal protein folding and protein segregation (NII) and ultimately leading to selective cell degeneration.

But what about Friedreich? In 1996, Campuzano et al. reported the molecular basis of FRDA (Campuzano et al. 1996). Most patients with FRDA are homozygous for a new GAA triplet repeat expansion, but some patients show an expansion in one allele and a point mutation in the other. With the discovery of the *frataxin* gene, it was recognized that the clinical spectrum of FRDA was broader than previously thought. Even so, the clinical features of most of our patients with 'DNA-confirmed' FRDA are similar to those described by Friedreich in 1863.

Table 1.1 Historical overview of the hereditary cerebellar ataxias.

1863: Friedreich	Concept of hereditary ataxia
1893: Marie	Late, versus early onset hereditary ataxia
1900: Dejerine and Thomas	Concept of 'Olivopontocerebellar ataxia'
1907: Holmes	First description of a classification system
1954: Greenfield	'Classical' pathological classification
1970: Koningsmark and Weiner	Heterogeneity in OPCA
1983: Harding	Classic 'ADCA classification'
1993: Orr and Zoghbi	CAG expansion in the *SCA1* gene
1996: Campuzano	GAA repeat expansion in the *frataxin* gene

Aims of the thesis

Hereditary cerebellar ataxia is a complex neurological syndrome. At present, age at onset and mode of transmission are key elements in the differential diagnosis. However, in order to develop a clinical algorithm further, more needs to be known about the phenotype at a clinical, pathophysiological, and pathological level, and about the different genotypes.

The aims of the study are:

With respect to the autosomal dominant spinocerebellar ataxias:

- to identify a new SCA locus that might help to explain those families that cannot be assigned to any of the known loci.
- to unravel the relevance of neuronal intranuclear inclusions (NII) to the pathophysiology of the CAG trinucleotide repeat disorders.
- to determine whether there is peripheral nervous system involvement in SCA6, in order to introduce a new element that might be useful for phenotype recognition and pathophysiological research.

With respect to the autosomal recessive cerebellar ataxias:

- to contribute to phenotype recognition, in particular with respect to the peripheral aspects of these disorders.
- to establish a clinical classification system for early-onset cerebellar ataxia (EOCA) that is based on clear clinical features and nerve conduction studies, and which can be used for counseling and DNA research.

Outline of the thesis

After the introduction (**Section I**), the different aspects are covered in three sections.
Section II covers the study of the phenotype and genotype of a large Dutch ADCA family that could not be linked to any of the known loci.
Section III describes an important aspect of the pathophysiology of CAG trinucleotide repeat disorders, focusing on the pattern of neurodegeneration in relation to the presence of NII in the brains of five patients with SCA3. The clinical relevance of *CACNA1A* (the *SCA6* gene), which is expressed in both the central nervous system (CNS) and the peripheral nervous system (PNS), is also addressed.
Section IV describes studies performed with the aim of expanding the phenotype and genotype (including clinical work-up and clinical classification) of the autosomal recessive cerebellar syndromes.
Ultimately, in the discussion we will propose an expanded clinical algorithm generated on the basis of the literature, our experience, and our findings.

Similarities and differences in the phenotype, genotype and pathogenesis of different spinocerebellar ataxias; a review

H.J. Schelhaas, P.F. Ippel, F.A. Beemer and G. Hageman

Chapter 2

Adapted from: European Journal of Neurology 2000; 7:309-314.

Introduction

The autosomal dominant cerebellar ataxias are a heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar dysfunction in combination with various associated features. Since 1993 these disorders have been increasingly characterized in terms of their genetic mutation and are referred to as SCA. These mutations have been identified for SCA1, 2, 3, 6, 7, 8, 10, 12, 14, 17 and fibroblast grow factor type 14 (FGF14) whereas the genes for SCA4, 5, 11, 13, 15, 16, 18, 19, 20, 21, 22, 23 and 25 remain to be isolated (Table 2.1) (http://www.gene.ucl.ac.uk/cgi-bin/nomenclature). The status of SCA8 is still being discussed (Schols et al. 2003). Some families with autosomal dominant cerebellar syndromes cannot be assigned to any of the known loci, implying further genetic heterogeneity.
Despite of the genetic heterogeneity, the clinical syndromes are similar to each other. This clinical overlap is possibly caused by similar mutations in different genes (Hardy and Gwinn-Hardy 1998). For example *SCA1, 2, 3* and *7* involve an unstable CAG repeat, which codes for glutamine (Orr et al. 1993; Pulst et al. 1996; Kawaguchi et al. 1994; David et al. 1997; Zhuchenko et al. 1997). In this review we focus on the clinical presentation of the different SCAs and discuss the pathway from genetic mutation, abnormal protein to common neuropathology in these disorders.

ADCA and SCA: progress in the understanding of autosomal dominant ataxias

In 1983, Harding proposed a clinical classification for 'late onset' ADCA (Harding 1983). In ADCA I, cerebellar ataxia was associated with additional features related to the optic nerve, (extra)pyramidal system, cerebral cortex and peripheral nerves. ADCA II was associated with pigmentary macular dystrophy. ADCA III was a pure late onset cerebellar syndrome while ADCA IV was associated with myoclonus and deafness. In 1993 Harding adapted this classification, the main difference being that ADCA IV was no longer seen as a distinct entity (Harding 1993). Nowadays, ADCAs are characterized in terms of their genetic locus and are referred to as SCA. The 'SCA-classification' and the 'ADCA-classification' are not congruous. Since the ADCA-classification was purely clinical, it could be applied to individuals only. For example, in a family with the *SCA6* mutation, there could be some family members with ADCA I and some with ADCA III. For this, at least for those patient in which the mutation is known, the term ADCA should be considered obsolete.

Genotype/phenotype relation

The overlap between the SCA phenotypes and the high variability within SCA subgroups mean that the underlying mutation cannot be predicted reliably for individual patients. Therefore, diagnosis has to be made on the genotype. Still, experienced neurologists may appreciate some fairly characteristic symptoms and signs and may try to deduce the underlying mutation on the basis of the clinical, electrophysiological, and magnetic resonance imaging (MRI) results (Schols 1997b). SCA4, 5, 11, 13, 15, 16, 18, 19, 21, 22, 23, 25 are acquired on the study of one or two families, can only be analyzed in a research setting, are probably extremely rare, and, except for SCA19 (Verbeek et al. 2002), and SCA23 (Verbeek et al. 2004), have never been found in the Netherlands. SCA9 is reserved but has, until now, not been claimed and SCA24 is a SCA with autosomal recessive inheritance (Swartz et al. 2002). The pheno- and genotype of SCA8 is not fully established and current evidence suggests that SCA8 merely reflects a risk factor for ataxia (Zeman et al. 2004). For SCA8, diagnostic tests should not be performed until its etiologic role is fully clarified (Schols et al. 2003). The occurrence of SCA10, relatively pure cerebellar ataxia with epilepsy, and SCA12, cerebellar ataxia with an intention tremor, seems to be confined to specific populations (Matsuura et al. 2002; Fujigasaki et al. 2001b).
Therefore, in most Western European countries, DNA analysis is available (and useful) for SCA1, 2, 3, 6, 7, 14 and 17 only. For the clinical approach of these SCAs an algorithm can be helpful (Figure 2.1; Table 2.2).

Table 2.1 Current state: the autosomal dominant spinocerebellar ataxias.

Disease	Locus	Gene	CAG repeats (normal range)	CAG repeats (disease range)	Anticipation	Gene product	NII	Distinctive clinical features
SCA1	6p22-23	*SCA1*	6-39	40-81	+	Ataxin-1	+	Ophtalmoplegia, dysphagia, dementia
SCA2	12q23-24.1	*SCA2*	15-29	35-59	++	Ataxin-2	+*	Marked reduction of saccade velocity
SCA3	14q24-qter	*SCA3/MJD*	12-41	55-84	+/-	Ataxin-3	+	Parkinsonism, diplopia, muscle atrophy
SCA4	16q22.1	Not identified						Sensory axonal neuropathy
SCA5	11p11-q11	Not identified						None
SCA6	19p13	*CACNA1A*	4-17	20-30	-	α_{1A}-PQ-VGCC		None
SCA7	3p12-21.1	*SCA7*	4-35	37-200	++	Ataxin-7	+	Retinal degeneration
SCA8	13q21	*SCA8*	16-21 CTA/CTG	91-200 CTA/CTG	+/-	Antisense RNA-transcript		Pyramidal and psychiatric features (still subject of discussion)
SCA10	22q13-qter	*SCA10*	10-22 ATTCT	920-4140 ATTCT	+	Ataxin-10		Epilepsy
SCA11	15q14-21.3	Not identified			-			None (apart form vertical nystagmus)
SCA12	5q31-33	*PPP2R2B*	7-28	66 - 78		'PP2'		Postural tremor
SCA13	19q13.3-13.4	Not identified						Mental retardation, developmental delay
SCA14	19q13.4-qter	*PRKCG*				PKCgamma		Dystonia in those with an early onset
SCA15	3p24.2-pter	Not identified						Postural and action tremor
SCA16	8q23	Not identified						Rotatory head tremor
SCA17	6q27	*TBP*	29-42 CAG/CAA	46-66	+/-	TBP	+	Psychiatric and extrapyramidal features, dementia
SCA18	7Q22-32	Not identified						Muscular atrophy, sensory neuropathy
SCA19	1p21-q21	Not identified						Myoclonus, cognitive impairment
SCA20	11 pericentromeric region	Not identified			-			Dysphonia, palatal myoclonus, dentate calcification
SCA21	7p21.3-15.1	Not identified						Akinesia, hyporeflexia, tremor, rigidity, cognitive impairment
SCA22	1p21-q23	Not identified						See SCA19
SCA23	20p13-12.2	Not identified						None
(SCA24)	Aut recessive inheritance!							Reading difficulties, myoclonic jerks, fasciculations, pes cavus
SCA-25	2p15-21	Not identified						Sensory neuropathy
FGF14	13q34	*FGF14*				FGF14		Early onset tremor, cognitive impairment

*NII, neuronal intranuclear inclusions; * NII have been found in SCA2 but they are considered rare. FGF, fibroblast growth factor; PKCgamma, isoform of protein kinase-C; 'PP2', brain-specific regulatory subunit of a protein phosphatase; α_{1A}-PQ-VGCC, α_{1A} subunit of the P/Q voltage gated calcium channel; TBP, TATA box-binding protein.*

Table 2.2 Clinical features, MRI morphology, and nerve conduction studies of the autosomal dominant spinocerebellar ataxias that are most frequently encountered in Western Europe.

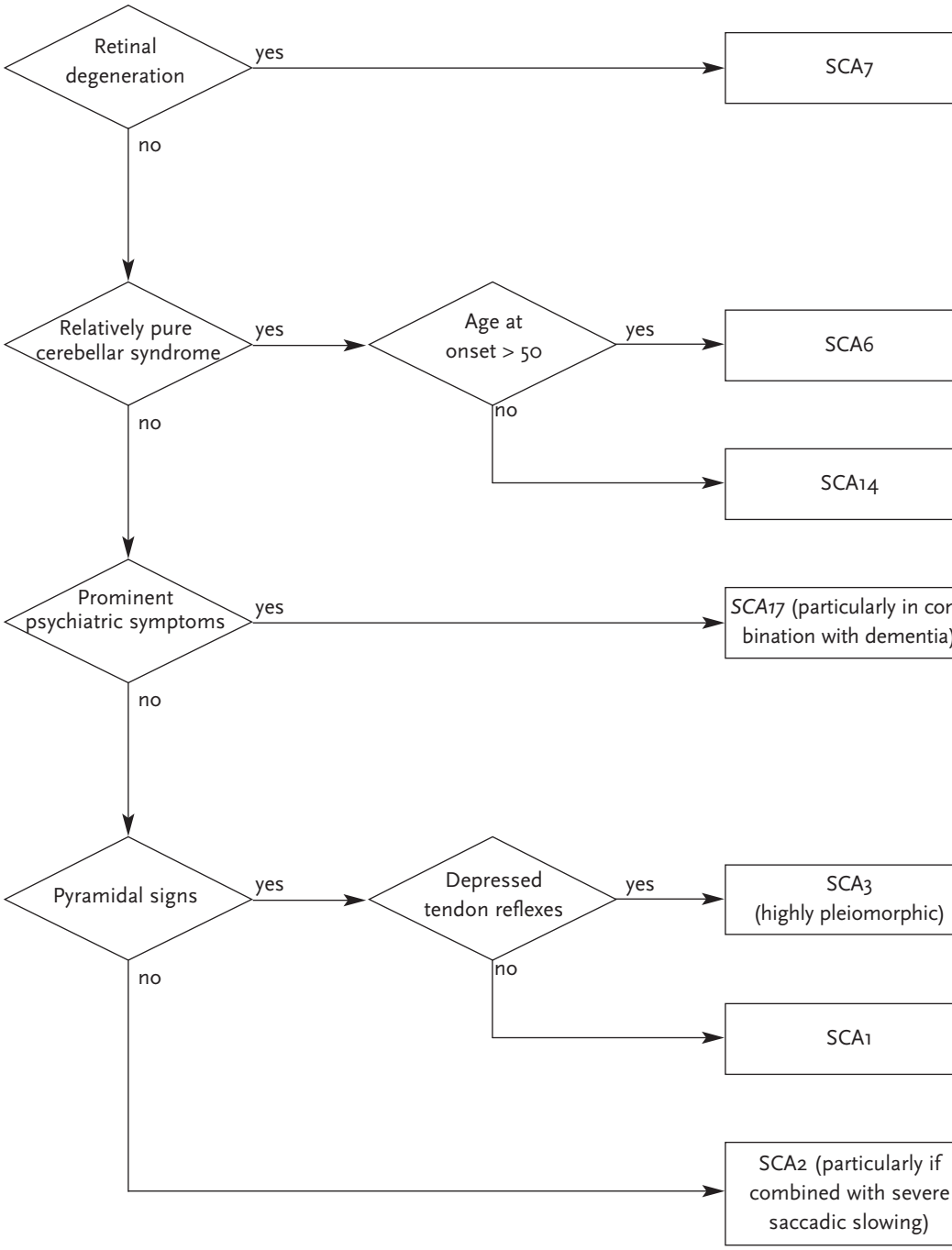
Disease	Associated clinical features	MRI studies	Nerve conduction studies
SCA1	Ophthalmoplegia, pyramidal and extrapyramidal signs, dementia, neuropathy, dysphagia, optic atrophy	Atrophy of cerebellum and pons	Reduced MCV of tibial and peroneal nerve
SCA2	As in SCA1 but more frequent reduction in saccade velocity, hyporeflexia	Like SCA1 but more pronounced	Reduction of SNAP mainly affecting the upper limbs
SCA3 /MJD	As in SCA1 but hardly ever saccade velocity reduction. More often extrapyramidal signs	Mild atrophy of cerebellum and brainstem	Reduction of SNAP and CMAP in both upper and lower limbs
SCA6	None	Pure cerebellar atrophy	Normal
SCA7	Retinal degeneration	Atrophy of the pons as an early sign. Cerebellar and brainstem atrophy	Conflicting results (see text)
SCA14	Pure cerebellar syndrome with extrapyramidal features in patients with an early onset	Marked atrophy of cerebellar vermis and hemispheres	Not reported
SCA17	Psychiatric symptoms and dementia	Most pronounced in cerebellum and occipital lobe. Atrophy of the caudate nucleus has been described	Not reported

MJD, Machado Joseph disease (SCA3); MCV, motor nerve conduction velocity; SNAP, sensory nerve action potential. CMAP, compound motor action potential.

If retinal degeneration is present in a patient presenting with a late onset, autosomal dominant cerebellar syndrome, then SCA7 is probable. At the beginning of disease most patients suffer from a predominantly cerebellar syndrome with less prominent involvement of non-cerebellar systems. However, if this is still the clinical picture many years after onset of symptoms and signs then SCA6 and SCA14 are most likely. Whereas in SCA6 clinical features frequently starts after the age of 50 (Sinke et al. 2001), the mean age at onset in SCA14 is around 40 (range 10-59). In SCA17, psychiatric symptoms and dementia are a hallmark of disease and might even be a presenting symptom. The distinction between SCA1, 2 and 3 can be very difficult. SCA3 is the SCA with the highest worldwide prevalence and its phenotype is extremely pleiomorphic. For this, SCA3 should always be considered. SCA2 is characterized by an extreme slowing of saccadic velocity (Wadia et al. 1998a). Furthermore, pyramidal features, usually present in SCA1 and SCA3, are usually absent in SCA2 (Ramesar et al. 1997; Schols et al. 1997a; Wadia et al. 1998). The presence (SCA1) or absence (SCA3) of tendon reflexes might help to distinguish between SCA1 and SCA3.

MRI of the brain in different SCAs is generally in accordance with the clinical findings (Table 2.2). Although it is known in clinical practice that the degree of atrophy is largely correlated to the age at onset and duration of disease, some generalizations might be considered. In SCA1 MRI usually shows mild olivopontocerebellar atrophy (OPCA), whereas in SCA2 there is severe OPCA. The involvement of the efferent dentatorubral system, with atrophy of the frontal and temporal lobes and globus pallidus distinct from OPCA, is characteristic of SCA3 (Murata et al. 1998). In SCA6 there is pure cerebellar atrophy with no evidence of brainstem involvement.

Figure 2.1 Algorithm for the work up of the spinocerebellar ataxias that are probably most frequently encountered in Western Europe. The algorithm should be dealt with restrain but can be used for executing DNA analysis in order of clinical likelihood.



In SCA7 pontine atrophy is a prominent and consistent finding regardless of the degree of cerebellar atrophy or duration of disease. Cerebellar atrophy is not found in those SCA7 patients with a short duration of disease or mild ataxia, but becomes prominent as the severity and duration of illness progresses (Jobsis et al. 1997; Bang et al. 2004). MRI studies of patients with SCA14 show marked atrophy of cerebellar vermis and hemispheres with no cortical atrophy, no basal ganglia abnormalities, and no white matter changes (van de Warrenburg et al. 2003). In SCA17, the atrophy is usually most pronounced in the cerebellum and occipital lobe (Rolfs et al. 2003), but subcortical atrophy of the caudate nucleus has also been described.

Nerve conduction studies support and extend the clinical findings of peripheral nerve involvement (Table 2.1). Both Schöls et al. (Schols et al. 1997a) and van de Warrenburg et al. (van de Warrenburg et al. 2002), found that in SCA1, the motor nerve conduction velocities of tibial and peroneal nerve, are significantly slower compared to SCA2, 3 and 6. Nerve conduction studies in SCA2 usually reveal a sensory neuronopathy, mainly affecting the upper limbs. In SCA3, nerve conduction studies are compatible with an axonal type of neuropathy, or neuronopathy. Until now, there is no evidence of peripheral nerve involvement in SCA6. There is some controversy concerning nerve conduction studies in SCA7. One study showed normal nerve conductions studies in five SCA7 patients. In contrast, another study found peripheral nerve involvement in all four SCA7 patients examined (van de Warrenburg et al. 2004; Kubis et al. 1999). Finally, nerve conduction studies of SCA14 and 17 have not been reported.

Trinucleotide repeats and neurodegenerative disorders

DNA is a long, threadlike, macromolecule made up of a large number of deoxyribonucleotides, each composed of a nitrogenous base, a sugar, and a phosphate group. The bases of DNA molecules carry genetic information, whereas their sugar and phosphate groups perform a structural role. The nitrogenous base is a derivate of purine or pyrimidine. The purines in DNA are adenine (A) and guanine (G), and the pyrimidines are thymine (T) and cytosine (C). Single copy DNA occur only once in the genome and make up approximately 75 percent of the genome. Repetitve DNA is primarily found in the non-coding regions of the genome and of the noncoding DNA, as many as 20 to 30 percent consist of repetitive DNA sequences. Trinucleotide repeats consist of a sequence of three base pairs repeated several times in the DNA. Trinucleotide repeats are polymorphic, which means that the number of repeats at one locus is variable in the population. In several neurodegenerative disorders there is difference between 'normal' and 'pathologically expanded' repeats. Normal repeats are stable; that is, the length of the repeat does not change from generation to generation. Pathological repeats are unstable and tend to expand during vertical transmission as well as in mitotic divisioning. To date, variable types of unstable trinucleotide repeat expansions have been identified (Figure 2.2, Table 2.3). The first type is represented by the fragile X syndrome. In these disorders CGG repeats are present in the 5' untranslated region (5' UTR; that part of DNA that is located before the start codon) of the gene. The CGG repeats are transcribed but not translated and current concepts suggest that the pathogenesis of these disorders is consistent with a loss-of-function mechanism in which the non-coding repeat expansion interfere with gene expression and cause a loss of protein production.
A second type, of which myotonic dystrophy, and SCA8 (although still questioned) are representatives, is caused by an expanded CTG repeat in the 3' untranslated region (3' UTR; that part of DNA that is located after the stop codon). Again, these CTG repeats are transcribed but not translated. Current evidence suggest that these mutations exert there deleterious effect at the RNA level (Ranum and Day 2004).
The third type, discovered in 1996, is caused by an intronic expanded GAA trinucleotide repeat expansion in intron 1 of the *frataxin* gene (Campuzano et al. 1996). In most control alleles the repeat length ranges from 6 to 9, while a small subset of so-called large-normal alleles (14-34 repeats) has been identified that may be the unique source of further expansions into an intermediate range of allele lengths (up to 90 repeats) or clearly disease causing (90 to 1700 repeats) alleles (Durr et al. 1996; Cossee et al. 1997). The pathogenesis of FRDA is consistent with the paradigma that recessive disorders are most often caused by a loss of

protein function as the intron expansion prevents the normal protein production. The fourth type, expanded GCG repeats in the *polyadenylate binding protein nuclear 1 gene (PAPBN1)*, encoding a polyalanine tract, is responsible for a late onset autosomal dominant muscle disorder called oculopharyngeal muscle dystrophy (OPMD). In this disorder, the accumulation of undegradable polyalanine rich molecules leads to the formation of nuclear filaments. How this accumulation leads to the symptoms of OPMD is still subject of research (Brais et al. 1998), but the pathophysiological concept of this disorder fits the classic concept that dominant disorders are caused by a gain or change of function of the mutant protein.
The fifth type, encoding expanded CAG repeat disorders, is responsible for SCA1, 2, 3, 6, 7 and 17. Other disorders caused by expansion of this triplet are Huntington disease and spinal and bulbar muscular atrophy (SBMA). Again, pathogenesis seems compatible with a change of function model.

Table 2.3 Disease-causing repeats differ in nucleotide sequence, length of the expansion, location within the respective disease gene and size of the repeated element (in this table only various types of trinucleotide repeat disorders are covered; see also figure 2.2).

Trinucleotide repeat expansions	Disease	Inheritance	Repeat size		Reference
			Normal	Disease	
CGG	Fragile X syndrome	X-linked	6-54 (55-200)*	>200	(Verkerk et al. 1991)
CTG	Myotonic dystrophy	Autosomal dominant	5-37	52->1000	(Buxton et al. 1992; Harley et al. 1992)
	SCA8	Autosomal dominant	25-44	107-127	(Koob et al. 1999)
	Huntington disease like-phenotype-2 (JPH3)	Autosomal dominant	10-27	51-57	(Koide et al. 1999; Bauer et al. 2002)
GCG	Oculopharyngeal muscle dystrophy	Autosomal dominant	6	9	(Brais et al. 1998)
GAA	Friedreich's ataxia	Autosomal recessive	< 90	100-1700	(Campuzano et al. 1996)
CAG	Huntington disease	Autosomal dominant	9-35 (36-39†)	39-121	(Read 1993; Rubinsztein et al. 1996)
	Spinal and bulbar muscular atrophy	X-linked	17-26	40-55	(La Spada et al. 1992)
	SCA1, 2, 3, 6, 7, 12, 17	Autosomal dominant	See table 2.1		
	DRPLA	Autosomal dominant	7-23	49-88	(Nagafuchi et al. 1994)

** 'Premutation', in this range the repeat expansion is mitotically unstable and responsible for the neurodegenerative disorder called fragile X associated tremor/ataxia syndrome (FXTAS) (Hagerman and Hagerman 2004).*
† intermediate range: the mutation is not fully penetrant in individuals with a borderline number of CAG repeats.
DRPLA, dentatorubropallidolusian atrophy.

CAG repeats in SCA: medical genetics

SCA's are usually characterized by a 'late onset', a unique pattern of neurodegeneration, a dominant inheritance and anticipation. However, there are juvenile forms of SCA, which are accompanied by a much more extensive neuropathology than the adult onset forms. These juvenile forms are genetically characterized by long CAG-repeats. CAG repeat expansion is observed particularly in paternal transmission and it is the genetic basis of anticipation.
SCA1, 2, 3, 6, 7, 12 and 17 are caused by an expanded CAG repeat (Table 2.3) (Banfi et al. 1994; Gispert et al. 1993; Kawaguchi et al. 1994; Zhuchenko et al. 1997; David et al. 1997; Nakamura et al. 2001). Unlike the other CAG repeat expansion syndromes, the CAG repeat expansions causing SCA6, and SCA17, are considered stable. However, certainly with respect to SCA17 the issue has not been completely settled (Fujigasaki et al. 2001a; Nakamura et al. 2001).

'Ataxins', the gene products in SCA

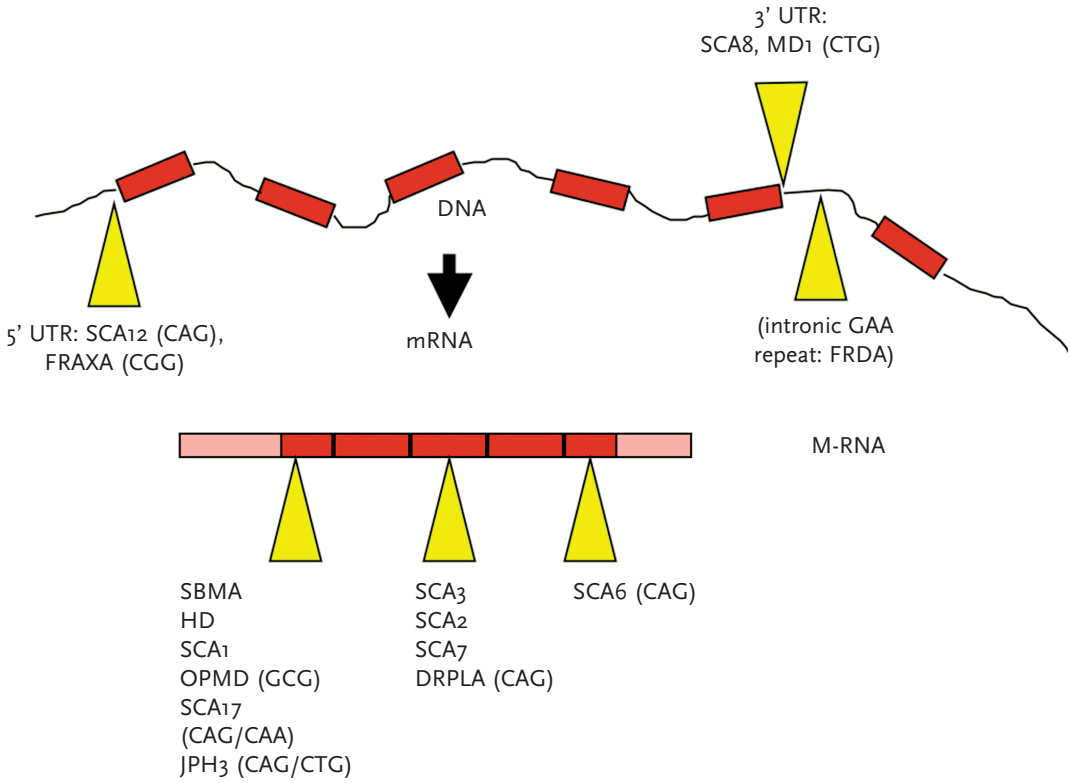
The gene products of *SCA1, 2, 3, 6, 7, 8, 10, 12, 14, 17* and *FGF14* have been identified (Table 2.1). Ataxin-1 (ATX1), a protein of 792-829 amino acids, has a nuclear localization in most neurons but in cerebellar Purkinje cells it is found in both the nucleus and the cytoplasm (Servadio et al. 1995). Current evidence suggests that it might be involved in RNA-processing pathways (Yue et al. 2001) Ataxin-2 (ATX2) is the gene product of SCA2. This 1313 amino acid protein is a basic protein with two domains implicated in RNA splicing and protein interaction. The 'wild type ataxin-2', is found in the cytoplasm (Huynh et al. 1999; Trottier et al. 1998).
Ataxin-3 (ATXN3), a nuclear and cytoplasmatic protein of 359 amino acids, is not only expressed in the brain but also in most, if not all, non-neuronal tissues. Recent evidence suggests that ataxin-3 is an ubiquitin-binding protein that functions in the ubiquitin-proteasome protein degradation pathway (Chai et al. 2004).
The gene product of SCA6 is the α_{1A}-subunit of a P/Q voltage gated calcium channel (P/Q-VGCC). The polyglutamine stretch is located within the intracellular carboxyl terminal of this PQ-VGCC (Zhuchenko et al. 1997).
Ataxin-7 is a subunit of the TATA-binding protein-free TAF-containing complex (TFTC) (Helmlinger et al. 2004). Although the normal function of this complex has, until now, not been completely elucidated, it certainly seemed to be involved in transcriptional regulation.
The gene product of SCA14, protein kinase Cgamma (PKCgamma), is a member of a family of protein kinases that plays an important role in fundamental neurobiological processes including neuronal development, plasticity, excitability, and survival. PKCgamma is highly expressed in Purkinje cells. Current evidence connect PKCgamma to ataxin-1 (Chen et al 2004) and this observation might suggest that a change of PKCgamma activity may be part of a common pathway in autosomal dominant spinocerebellar ataxia (for review see Pandolfo and van de Warrenburg 2005).
The gene product of SCA17, the TATA binding protein (TBP), is definitely a transcription factor (Nakamura et al. 2001).

Apart from the polyglutamine stretch, and in some, the involvement in transcriptional regulation and RNA metabolism, these proteins are completely dissimilar. As disease severity increases with the expansion of the polyglutamine stretch, it is likely that the polyglutamine expansion itself plays a crucial role in the mechanism of neurodegeneration.

From polyglutamine stretch to neurodegeneration: the missing link

Now that the initiating trigger, CAG repeats encoding polyglutamine, the gene product ataxin, and the clinical outcome are known research has focused on a common neuropathology. The wild type ataxins appear to be natively unfolded (Uversky 2003). However, when expanded, the ataxins are transported into the nucleus of

Figure 2.2 Disease-causing repeats differ in nucleotide sequence, size of the repeated element (not shown in this figure), length of the expansion, and location within the respective disease gene (here all placed in one schematic scheme). Some repeats reside within introns, others within exons containing untranslated regions, and still others within protein coding exons.



5' UTR, 5' untranslanted region (that part of DNA that is located before the start codon); 3' UTR, 3' untranslated region (that part of DNA that is located after the stop codon); mRNA, messenger RNA; FRAXA, fragile X syndrome; FRDA, Friedreich's ataxia; SCA, spinocerebellar ataxia; DRPLA, dentatorubropallidolusian atrophy; SBMA, spinobulbar muscular atrophy; *JPH3*, junctophilin-3 (mutated in Huntington disease like-phenotype 2).

neurons where they aggregate as insoluble, amyloid-like fibrils to form a neuropathological structure called 'neuronal intranuclear inclusions (NII)' (Davies et al. 1998). These NII have now been found in transgenic models, both mammalian (mice) and invertebrate (Drosophila) of SCA1, and in patients with SCA2, 3, 7, and 17 (Table 2.1) (Hardy and Gwinn-Hardy 1998; Davies et al. 1998). Aggregation of these ataxins can be increased by various mechanisms like increased concentration of the protein, proteolytic cleavage of the protein, phosphorylation of the ataxin, and oxidative protein modification (for review see Ross and Poirier 2004). However, although an increased propensity to form aggregates is a common feature of all polyglutamin disease proteins, the role of these NII is still far from clear. Current evidence suggests that for the pathogenesis the recruitment of other proteins into these polyglutamine aggregates might be essential. For example, for SCA1 the recruitment of the 14-3-3 protein seems crucial (Chen et al. 2003). This 14-3-3 protein, an important multifunctional regulatory molecule, mediates the neurotoxictiy of ataxin-1, by binding to and

stabilizing the protein, thereby slowing its normal degradation. The binding of ataxin-1 with 14-3-3 is regulated by Akt phosphorylation and is polyglutamine dependant. Current evidence suggests that a similar mechanism might also hold true for ataxin-2, 3, and 7 (personal communications).
The mechanism of neurodegeneration in SCA6 is probably of a different kind. Stable expanded CAG repeat and point mutation in the CACNA1A gene on chromosome 19p13 are translated in a mutant α_{1A}-subunit of the PQ-VGCC, lead to a loss of function of the channel resulting in an influx of calcium in the cell. The increase of intracellular calcium ultimately leads, by a cascade of deleterious events, to cell death and neurodegeneration (Zoghbi 1997).

Concluding remarks

The differential diagnostic problems of the autosomal dominant spinocerebellar ataxias are partly resolved with the use of a current available genetically based classification system. The 'completion' of this classification, is an important step in elucidating the process of neurodegeneration in these CAG/polyglutamine expansion disorders. A second step forward came with recognition of the NII, the pathological structures found in SCA1, 2, 3, 7 and 17. These two steps are essential for creating transgenic animal models that will enable research groups to study and 'plot' the pathway from genetic mutation to abnormal protein and cell death. Because expanding CAG triplet repeat disorders are known to be responsible for at least ten neurodegenerative diseases, a breakthrough in one of these diseases might mean a breakthrough for all of them.

The autosomal recessive cerebellar ataxias

Chapter 3

In contrast to the well-defined clinicogenetic classification of the autosomal dominant cerebellar disorders, much less is known about the autosomal recessive ataxias. Currently different forms have been identified. FRDA seems to be the most common form, with a prevalence of about 2 per 100,000 (Cossee et al. 1997). For a few other recessive Friedreich-like ataxias, such as ataxia with isolated vitamin E deficiency (AVED), autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) and ataxia with oculomotor apraxia type 1 and type 2, the molecular basis has also been elucidated (Table 3.1) (Ouahchi et al. 1995; Ben Hamida et al. 1993; Engert et al. 2000; Moreira et al. 2001b; Moreira et al. 2004). Still, these mutations explain only relatively few cases, and often occur in isolated populations only.
In the absence of such a commonly accepted clinicogenetic classification system, the overview presented in Table 3.1 might help investigators to categorize these patients. It is adapted from Harding's classification and has the advantage that it illuminates the progress that has been achieved in the unraveling of these disorders. The classification shows some inconsistencies ('secondary mitochondrial disorders' like FRDA and AVED, are not classified under the heading of metabolic disorders) and is certainly not very practical from a clinical perspective. A major challenge is to develop a classification that is based on relatively unmistakable clinical features and at the same time provide an optimal strategy for molecular diagnosis. However, the 'current state' is that such a classification is not available. In this chapter we will not address the metabolic disorders but instead, will refer the reader to chapter 13 of the excellent textbook edited by Klockgether (Bolthauser 2000). We will also not address Xeroderma pigmentosa and Cockayne's syndrome as these disorders usually manifest themselves not by cerebellar ataxia but by respectively, skin disease, and grow retardation with severe dementia (Harding 1984a).

Disorders characterized by secondary mitochondrial dysfunction

At least four different types of recessive ataxias are recognized as nuclear encoded disorders with secondary mitochondrial dysfunction.

Friedreich's ataxia
The most common and currently best know recessive ataxia is FRDA. Its estimated prevalence in the western world is about 2 in 100,000 individuals while the estimated gene carrier frequency is about 1:100 (Leone et al. 1990; Cossee et al. 1997; Durr 2002). In contrast, FRDA seems rare to absent in other ethnic groups, like black Africans and Japanese (Labuda et al. 2000). The causative mutation affects the *frataxin gene* on 9q13 and most commonly consists of an expanded GAA trinucleotide repeat expansion in intron 1 of the gene (Campuzano et al. 1996).

Initial clinical criteria as proposed by Geoffroy and Barbeau in 1976 were designed for research purposes and so strict that a diagnosis could not be made in many instances (Geoffroy et al. 1976). In 1981, Harding therefore, proposed less stringent clinical criteria (Harding 1981a). These included age at onset before 25 years, progressive ataxia of limbs and gait, absent knee and ankle jerks, and extensor plantar responses. The discovery of the pathogenic mutation has clearly shown that the clinical spectrum of FRDA is even wider than initially defined and currently includes onset ages over 30 years, a much milder disease course, spastic ataxia, and even extrapyramidal movement disorders such as myoclonus or chorea (Hanna et al. 1998; Klockgether et al. 1991). Cardiomyopathy is now known to be present in most cases, although its manifestations are variable and may often remain undetected (Dutka et al. 1999; Isnard et al. 1997).

Friedreich's ataxia is primarily a disease of dorsal root sensory neurons. The loss of these sensory neurons and the associated posterior column degeneration may in fact constitute the main cause of the initial ataxia. Nerve conduction studies reveal an early loss of sensory nerve action potentials. Those fibers that originate more caudally are more severely affected. In addition to degeneration of the sensory posterior columns, the spinocerebellar tracts and the corticospinal motor tracts degenerate as well. The cerebellar cortex shows

Table 3.1 Current state: the autosomal recessive spinocerebellar disorders.

I Ataxic disorders with known metabolic or other cause
A. Metabolic disorders
- 1. Progressive, unremitting ataxia:
  - Lysosomal disorders:
    - Hexosaminidase deficiency
    - pingolipidaemia
    - M. Refsum
    - Globoid cell leukodystrophy
    - Metachromatic leukodystrophy
    - Adrenoleukodystrophy
  - Lipid storage disorders
    - Cerebrotindinous xanthomatosis
    - Niemann-Pick type C
    - Copper-metabolic disorders
    - Wilson's disease
    - Ceruloplasmin deficiency hemosiderosis
  - Neuronal ceroid lipofuscinosis
  - Sialidosis
- 2. Intermittent ataxia:
  - Amino acid disorders
  - Organic aciduria
  - Pyruvate dehydrogenase deficiency
  - Urea cycle defects

B. Disorders characterized by (secondary) mitochondrial dysfunction
- Friedreich ataxia type 1
- Ataxia with isolated vitamin E deficiency (AVED)
- Abetalipoproteinemia
- X-linked sideroblastic anemia with ataxia

C. Disorders characterized by defective DNA repair
- Ataxia teleangiectasia (Louis Bar syndrome)
- Ataxia-teleangiectasia-like disorder (ATLD)
- Ataxia with oculomotor apraxia type I
- Ataxia with oculomotor apraxia type 2
- Spinocerebellar Ataxia with Axonal neuropathy (SCAN1)
- Xeroderma pigmentosum
- Cockayne syndrome

D. Disorders caused by a dysfunction in chaperone mediated protein folding
- Autosomal recessive spastic ataxia of Charlevoix-Saguenay

II Disorders for which the locus is well established but the mutation still unknown
- Friedreich ataxia type 2
- Infantile onset spinocerebellar ataxia and sensory neuropathy (IOSCA)
- Autosomal recessive ataxia with hearing impairment and optic atrophy (locus 6p21- 23)
- Autosomal recessive non-progressive infantile ataxia (locus 20q11-q13)

III Disorders of unknown metabolic or other cause
- 'EOCA'

only mild loss of Purkinje cells late in the disease course (Lamarche et al. 1984). Thus, cerebellar atrophy on MRI scanning is mild at most, and virtually absent in patients that have not yet progressed well into the disease (Klockgether et al. 1991).
FRDA is the sole example of an intronic expanded trinucleotide (GAA) repeat (Campuzano et al. 1996). In most control alleles the repeat length ranges from 6 to 9, while a small subset of so-called large-normal alleles (14-34 repeats) has been identified that may be the unique source of further expansions into an intermediate range of allele lengths (up to 90 repeats) or clearly disease causing (90 to 1700 repeats) alleles (Campuzano et al. 1996; Durr et al. 1996; Cossee et al. 1997; Epplen et al. 1997). Frataxin gene point mutations have been described, but such alleles may only be pathogenic in conjunction with an allele that carries a GAA expansion (Cossee et al. 1999).

The large expansions suppress frataxin gene expression, thus causing a classical recessive genotype with loss of function of the frataxin protein, a mitochondrial matrix protein (Campuzano et al. 1997; Koutnikova et al. 1997). In vitro, deletion of a yeast frataxin homologue (YFH1) resulted in a decrease in mitochondrial respiration; an increase in mitochondrial iron accumulation and oxidative stress; mitochondrial and nuclear DNA damage; and a strong reduction in the assembly of Fe/S protein dependent mitochondrial complex I, II and III subunits, as well as aconitase (Babcock et al. 1997; Wilson and Roof 1997; Adamec et al. 2000; Karthikeyan et al. 2002; Muhlenhoff et al. 2002). Results from these mechanistic studies in unicellular model systems have to a large extent been confirmed in fibroblasts, cardiomyocytes, cerebellar MRI and skeletal magnetic resonance spectroscopy studies of FRDA patients (for review see: Durr 2002). Therefore, an old hypothesis that was published in the eighties (Dijkstra et al. 1984) is currently mainstream: FRDA is a nuclear encoded mitochondrial disorder.

Hereditary ataxia with vitamin E deficiency (AVED)
This is a rare condition that clinically resembles Friedreich's ataxia; autosomal recessive early onset progressive spinocerebellar ataxia with marked proprioceptive loss and areflexia (Burck et al. 1981). Again, peripheral nerve pathology is prominent (Zouari et al. 1998). The crucial lab finding in this condition is the very low serum concentration of vitamin E/tocopherol. The underlying molecular defects in the various families described turned out to be mutations in the *alpha-tocopherol transfer protein gene (alpha-TTP)*, which is localized on chromosome 8q13 (Ouahchi et al. 1995). Most known patients come from Tunisia. Separate mutations have been described in Europe, North America and Japan.

Alpha-TTP is responsible for selective retention of alpha-tocopherol from dietary vitamin E, which is a mixture of alpha, beta, gamma, and delta-tocopherols and the corresponding tocotrienols. The alpha-TTP-mediated transfer of alpha-tocopherol into nascent VLDL constitutes the major determinant of plasma alpha-tocopherol levels in humans. AVED patients, therefore, have an impaired ability to incorporate alpha-tocopherol into lipoproteins secreted by the liver. The resulting loss of free radical scavenging capabilities and anti-oxidant capabilities may lead to mitochondrial dysfunction - similar to the situation in FRDA.

In these patients vitamin E should be supplemented, since trials have suggested a stabilization of neurological decline after the start of treatment (Gabsi et al. 2001).

Apart from AVED, other causes of low serum vitamin E concentration may lead to cerebellar ataxia, spinal cord degeneration and neuropathy. These causes include both hereditary diseases such as abetalipoproteinemia (Bassen-Kornzweig disease) and acquired disorders such as cystic fibrosis, chronic cholestatic liver disease, short bowel syndrome and celiac disease (Sitrin and Bengoa 1987; Harding et al. 1982b).

X-linked sideroblastic anemia with ataxia
This very rare disorder, described in a few families worldwide, consists phenotypically of juvenile onset non-

progressive ataxia, pyramidal tract signs and a siderblastic anemia in affected males. Allikmets et al. demonstrated a missense mutation in the *ABC7 gene*, a nuclear encoded mitochondrial ATP-binding cassette (ABC) transporter that localizes to the mitochondrial inner membrane and is involved in iron homeostasis (Allikmets et al. 1999). Thus, like in Friedreich's disease, mitochondrial iron homeostatic impairment may lead to cerebellar dysfunction.

Disorders characterized by defective DNA repair

Another group of ataxias is pathophysiologically characterized by defective DNA repair and clinically by early onset cerebellar ataxia, muscle wasting and prominent extrapyramidal features with a rather specific eye movement abnormality called oculomotor apraxia. This consists of initial saccadic slowing, head thrust on fast lateral gaze with gaze lag, difficulties in saccade initiation, and ultimately gaze paralysis. Ataxia telangiectasia (AT) is the best known of these disorders, but at least two novel forms have recently been identified.

Ataxia telangiectasia

The first clinical and pathological outline of AT was presented by Boder and Segwick in 1958 (Boder and Sedwick 1958). However, their description was preceded by reports from the Czechs Syllaba and Henner in 1926 (Syllaba and Henner 1926) and from the Belgian Denise Louis-Bar in 1941 (Louis-Bar 1941), whose name has become eponymic to the disease until recently. Although well known to many neurologists and pediatricians, this recessive disorder is in fact so rare that many may never have seen a case personally. Its incidence has been estimated at about 0.3-1.0 per 100.000 live births (Swift et al. 1986). The ATM mutated gene carrier frequencies have been estimated for western populations to be 0,5 - 1.0 percent, but percentages as high as 3,5 have been suggested (Swift et al. 1986).

The disease typically starts in early childhood with progressive cerebellar ataxia. Truncal ataxia precedes appendicular ataxia. Progressive oculomotor apraxia with absent optokinetic nystagmus is quite characteristic. Chorea, dystonia and related extrapyramidal features occur in the vast majority of patients. Deep tendon reflexes become diminished or absent by age eight and patients later develop diminished large-fiber sensation (Woods and Taylor 1992). Progressive spinal muscular atrophy that affects mostly hands and feet debuts at an older age. In combination with early-onset dystonic posturing this leads to striking combined flexion-extension contractures of the fingers. Memory problems may occur in elder patients but in general cognition is spared.

The gene responsible for AT is called *ATM* (AT mutated). ATM is a protein kinase that belongs to a highly conserved family of 'phosphatidyl-inositol 3-kinase (PI3K)-like protein kinases' (PIKKs) with serine/threonine-kinase activity (Savitsky et al. 1995). ATM may be functionally located at the apex of a response cascade that senses and responds to DNA damage and repair. Its role has been particularly well described in the very rapid response to double strand DNA breaks where it initiates a large number of various protein phosphorylation pathways (Shiloh 2000).

Apart from ataxia, the clinical manifestations in homozygotes consist of conjunctival telangiectasias, which typically become apparent between age three and five. Respiratory tract infections occur very frequently, but other infections have an increased incidence as well. Radiosensitivity and a predisposition to various forms of cancer complement the clinical problems of these patients. The most common malignancies associated with homozygous AT are T-CLL leukemia and B-cell lymphomas. Other malignancies include gastric adenocarcinoma, hepatocellular carcinoma, ovarian tumors, renal cell carcinoma and cerebellar glioma, medulloblastoma and breast cancer. Diagnostic laboratory features are an elevation of serum alpha-fetoprotein and carcinoembryonic antigen concentrations, dysgammaglobulinemias, and impairment of cellular immunity. Mutation detection at the ATM locus is difficult because of the large size of the gene (66 exons),

the fact that mutations are located throughout the gene with no hotspots, and the difficulty of distinguishing mutations from polymorphisms.

The neuropathology of AT seems not very specific. In advanced cases the cerebellum is severely atrophic, due to thinning of the molecular layer, granule cell depletion and Purkinje cell loss. Additional affected structures include the spinal cord, where dorsal column demyelination with neuroaxonal dystrophy, astrocytic proliferation, dorsal root ganglion and anterior horn cell degeneration may be observed.

Ataxia-telangiectasia like disorder (ATLD)
ATLD is a very rare disorder. The clinical features are similar to those of ataxia teleangiectasia with cerebellar ataxia and oculomotor apraxia. Some patients may present with chorea. Still, ATLD is characterized by a later onset and a slower progression. Affected patients do not show teleangiectasia. The disorder is the result of a deficiency of the human Mre 11 protein that is caused by a mutation in the *hMRE11 gene* (Meiotic Recombination). At the cellular level, the disorder exhibit hypersensitivity to ionizing radiation, radioresistant DNA synthesis and failure to induce stress activated protein kinases following exposure to ionizing radiation. For this, current evidence suggests that AT and ATLD act in the same DNA response pathway.

Ataxia with Oculomotor Apraxia type 1 (AOA1)
Since the early seventies many patients have been reported with an AT-like phenotype that could nevertheless be distinguished from true AT on clinical and biological grounds. The syndrome was recognized by its oculomotor apraxia, with added features of early onset cerebellar ataxia, choreo-athetosis, peripheral nerve involvement and, in a subset of patients, mental retardation (Barbot et al. 2001). However, telangiectasias or frequent infections were specifically absent, while hypoalbuminemia and hyperlipidemia were present in many patients, particularly those from Japan. In addition, normal immunoglobulin composition, and normal alpha-fetoprotein levels further contrasted these patients with classical AT.

The first mapping results, from five Portuguese families and two Japanese families, showed homozygosity and haplosharing over a region on 9p13 (Moreira et al. 2001a). The causative gene turned out to be a member of the histidine triad (HIT) superfamily and was named *aprataxin (APTX)* (Shimazaki et al. 2002). This allows the correct molecular classification of many patients with recessive cerebellar ataxia and has established the connection of hypoalbuminemia with the diseases. A long-form splice variant aprataxin has been found to interact with XRCC1 (x-ray repair cross-complementing group 1), and aprataxin and XRCC1 may constitute a multiprotein complex that is involved in single-strand DNA break repair (Sano et al. 2004). Thus, like AT, AOA1 seems primarily a DNA repair disorder.

Ataxia with Oculomotor Apraxia type 2 (AOA2)
A second locus on 9q34 was identified in two non-related families with five and four affected sibs, respectively (Bomont et al. 2000; Nemeth et al. 2000). Clinically these patients had late childhood or adolescent onset ataxia, inconsistent oculomotor apraxia and elevated levels of serum creatine kinase, gamma-globulin and alpha-fetoprotein (Nemeth et al. 2000; Watanabe et al. 1998b). The gene associated with this disorder has been designated *senataxin* and shares extensive homology to fungal Sen1p proteins that are involved in splicing and termination of tRNA, small nuclear RNA, and small nucleolar RNA. Senataxin may have RNA and DNA helicase activity and it may act in the DNA repair pathway, thus, again, linking AOA2 to a DNA repair defect (Moreira et al. 2004).

Spinocerebellar Ataxia with Axonal neuropathy (SCAN1)
This recessive ataxia has recently been described in a Saudi Arabian family and is clinically characterized by a mild cerebellar ataxia with mild cerebellar atrophy, and peripheral axonal motor and sensory neuropathy (Takashima et al. 2002). A homozygous A1478G transition mutation in the *Tyrosyl-DNA phosphodiesterase 1*

gene (TDP1), encoding a DNA repair enzyme was identified as the cause of this form of ataxia (Takashima et al. 2002).

Disorders caused by a dysfunction in chaperone mediated protein folding

Autosomal recessive ataxia of Charlevoix-Saguenay (ARSACS)

This disorder was first described in 1978 as a form of spastic ataxia in a group of families from the two eponymic Quebec regions (Bouchard et al. 1978). All related to a common founder who lived in Quebec around 1650. Clinical features of the original families included, apart from core ataxic features such as truncal and limb ataxia, dysarthria and oculomotor abnormalities, also lower limb spasticity, distal muscle wasting and foot deformities. Specific features appeared to include retinal striation reminiscent of early Leber's atrophy, mild mental retardation and the frequent presence of mitral valve prolapse. Disease onset was early: none of the patients ever walked normally. But after the age of 20, little disease progression was noted. Shared homozygosity analysis identified a locus on 13q11 and two distinct ancestral haplotypes were revealed (Engert et al. 2000). Based on these data the first families originating from outside Quebec could be identified in Turkey and Tunisia, while the cloning of the gene, called *SACs*, allowed additional families from Italy and Japan to be unambiguously diagnosed (Grieco et al. 2004; Mrissa et al. 2000; Criscuolo et al. 2004; Ogawa et al. 2004). Thus, the disease appears to occur in many more parts of the world than originally anticipated and concurrent with this notion, new mutations were found. It also appeared that neither retinal striation nor mitral valve prolapse were as specific as initially reported.

The mutated gene, *SACs*, encodes a novel protein, called sacsin. *SACs* is expressed in various tissues, including the central nervous system. The presence of heat-shock domains suggests a function for sacsin in chaperone-mediated protein folding.

Disorders for which the locus is well established but the mutation still unknown

FRDA2

A second locus, on 9p23-p11, was found to co segregate with a disorder that was phenotypically described as classic Friedreich's disease (Christodoulou et al. 2001). No progress has been reported on this entity.

Infantile onset spinocerebellar ataxia (IOSCA)

IOSCA is extremely rare, having been described exclusively in a small founder group of Finnish patients. Slowly progressive clinical symptoms manifested between one and two years of age in previously healthy infants, with as the first manifestation clumsiness and gait problems. Ataxia, athetosis and muscle hypotonia with loss of deep tendon reflexes could already be observed at that age. By school age ophthalmoplegia and hearing loss manifested while sensory neuropathy developed by adolescence. Female hypogonadism and epilepsy were late manifestations. Ancillary examinations showed marked decrease in sensory nerve condition velocities and a progressive loss of myelinated fibers in sural nerve specimen. Neuroradiological investigations revealed cerebellar atrophy (Koskinen et al. 1994). The IOSCA locus was mapped to 10q24 (Varilo et al. 1996). A gene has not been found yet.

Disorders of unknown metabolic or other cause

Early Onset Cerebellar Ataxia (with retained Tendon Reflexes)

The term EOCA with retained tendon reflexes was, again, introduced by Anita Harding in 1981, as a denominator for patients with a phenotype that is distinct from classical FRDA (Harding 1981a). This and later descriptions emphasized features that distinguish this entity from FRDA, such as a slower rate of progression, no cardiac abnormalities, the absence of scoliosis, and prominent cerebellar atrophy on MRI

(Klockgether et al. 1991; Filla et al. 1990). Although retained (lower limb) tendon reflexes have been considered part of the clinical definition, many patients appear to have lost them during the course of the disease.

The prevalence of EOCA is unknown; estimates yielded about half of the prevalence of FRDA. But, as most of the publications date from prior to the discovery of the FRDA gene, and given the clinical phenotypic heterogeneity associated with the FRDA mutation, inclusion of FRDA cases in these series must be suspected and prevalence data are not more than educated guesses. The current position of EOCA is that these cases represent a clinically and genetically heterogeneous group of recessive ataxias in which a definite genetic or biochemical diagnosis cannot be established, and with a slower disease course and a better prognosis than classical FRDA (Klockgether et al. 1998).

Part II

The pheno and genotype of a new dominant spinocerebellar syndrome (SCA19)

Clinical and genetic analysis of a four-generation family with a distinct autosomal dominant cerebellar ataxia

H.J. Schelhaas, P.F. Ippel, G. Hageman, R.J. Sinke,
E.N. van der Laan and F.A. Beemer

Chapter 4

Journal of Neurology 2001; 248: 113-120.

Abstract

The autosomal dominant cerebellar ataxias (ADCAs) are a heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar dysfunction in combination with a variety of other associative features. Since 1993, ADCAs have been increasingly characterized in terms of their genetic mutation and are now referred to as spinocerebellar ataxias (SCAs). Some families with an autosomal dominant spinocerebellar syndrome cannot be assigned to any of the known genotypes, which imply further genetic heterogeneity. We investigated the clinical symptoms of 12 patients of a four-generation family with an autosomal dominant cerebellar syndrome and carried out mutation and genetic linkage studies. The family showed a relatively mild cerebellar ataxic syndrome with cognitive impairment, poor performance on the Wisconsin Card Sorting Test (WCST), myoclonus, and a postural irregular tremor of slow frequency. Age at disease onset and severity of cerebellar signs and symptoms suggest anticipation. The genetic loci implicated in these disorders were excluded by mutation analyses (SCA1, 2, 3, 6, 7, 8, 12) and genetic linkage (SCA4, 5, 6, 10, 11). We conclude that this family represents a clinically and genetically distinct form of SCA.

Introduction

The autosomal dominant spinocerebellar ataxias are a heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar dysfunction in combination with a variety of other associated features (Harding 1983). The genetic loci implicated in these disorders have been labeled and numbered (*1-8, 10-25*) as SCA. Mutations have been identified for SCA1, 2, 3, 6, 7, 8 and 12 while the genes for SCA4, 5, 10 and 11, which have been linked to chromosomes 16, 11, 22 and 15, respectively, are yet to be isolated (http://www.gene.ucl.ac.uk/cgi-bin/nomenclature). Since not all families with an autosomal dominant spinocerebellar ataxia can be linked to one of the known loci, additional SCA loci are expected to be identified in the future. Here we describe a four-generation family with a genetically distinct form of SCA and a phenotype characterized by mild cerebellar ataxia, cognitive impairment, poor performance on the WCST, myoclonus, and a postural irregular tremor of slow frequency. Moreover, molecular analysis allowed classification of the disease in this family as a new type of SCA.

Patients and methods

Patients

The index patient (Fig. 1,V-31), a 56-year-old man of Dutch descent, presented with complaints of speech disturbance, walking difficulty and head tremor. The symptoms had started approximately at the age of 31 years, with the head tremor. There was no medical history of note. He had been unemployed for 10 years as a result of his poor coordination. The findings of the general physical examination were unremarkable. Upon neurological examination he showed a postural irregular tremor of slow frequency of his head, neck, and upper extremities that increased with changes in posture or standing in an upright position. Eye movements were intact in all directions, but there were fine saccadic movements and a first-grade nystagmus. Sensory examination showed impaired joint position and reduced vibration sense in both feet. Coordination was severely disturbed in all limbs, showing dysmetria and dysdiadochokinesis. His gait was ataxic. Case 2 was the mother of the index patient (IV-11), who had experience speech disturbance, jerky movements and poor coordination since the age of 45 years. On examination there was an evident cerebellar syndrome and some cognitive inconsistencies (Table 4.1). There were also intermittent, irregular brief muscle jerks in the neck. Twenty years previously electroencephalography (EEG) had shown paroxysmal rhythmic theta activity over the temporal and parietal lobes followed by intermittent brief muscle jerks in the neck. Case 3 was the eldest son of the index patient (VI-31), who had had mild dysarthria, fine saccadic eye movements and an intention tremor with hypermetric movements upon heel-shin test since the age of 10 years. He also had severe learning difficulty, because of which he had attended a special

Table 4.1. Clinical features in 12 patients.

	III-8	IV-4	IV-5	IV-9	IV-11	V-1	V-24	V-28	V-31	V-34	VI-28	VI-31
Sex	M	F	M	F	F	M	M	M	M	M	M	M
Age at onset (years)	40	30	n.d.	45	45	45	20	27	31	27	n.d.	n.d.
Age (years)[a]	78	87	5 1	8 3	80	5 5	48	5 7	5 6	5 1	2 8	11
Duration (years)	38	57	n.d.	38	35	10	28	30	25	26	n.d.	n.d.
Postural/stance disturbance[b]	n.d.	31/34	n.d.	27/34	28/34	6/34	16/34	12/34	13/34	7/34	0/34	0/34
Limb movement disturbance[b]	n.d.	40/52	n.d.	7/52	7/52	6/52	13/52	9/52	8/52	7/52	0/52	2/52
Speech disturbance[b]	n.d.	3/8	n.d.	2/8	2/8	2/8	4/8	2/8	2/8	2/8	0/8	2/8
Oculomotor disturbance[b]	n.d.	5/6	n.d.	1/6	5/6	4/6	4/6	3/6	3/6	2/6	2/6	2/6
Total ataxia score[b]	n.d.	79/100	n.d.	37/100	42/100	18/100	37/100	26/100	26/100	18/100	2/100	6/100
Babinski's sign	n.d.	-	n.d.	-	-	-	-	+	-	-	-	-
MMSE	n.d.	25/30	n.d.	25/30	26/30	27/30	26/30	26/30	27/30	26/30	27/29	26/29
Upper limb reflexes	n.d.	↓	n.d.	↓	↓	↓	↓	↓	↓	↑	N	↑
Knee reflexes	n.d.	↓	n.d.	↓	↓	↓	↓	N	↑	↑	N	↑
Ankle reflexes	n.d.	↓	n.d.	↓	↓	↓	↓	N	↓	↑	N	↑
Vibration sense	n.d.	↓	n.d.	↓	↓	↓	↓	N	↓	N	N	N
Joint position sense	n.d.	N	n.d.	N	N	↓	↓	↓	N	↓	↓	↓
Holmes tremor	n.d.	-	n.d.	-	-	-	+	-	+	-	-	+
Myoclonus	n.d.	-	n.d.	-	+	-	+	-	-	-	-	+

[a] *Age on 1 January 1999, except for III-8 and IV-5: age at death.*

[b] *According to the international cooperative rating scale, the total ataxia rating scale ranges from 0 (no ataxic features) to 100 (complete cerebellar syndrome).*

MMSE, Mini Mental State Examination.

+ = present; - = absent; ↓= decreased; ↑ = increased; Normal; n.d. = no reliable data available.

school. His brother (VI-32) did not show any ataxic features or learning difficulty. After identification of the index patient, his mother and his children, additional members of the family were invited to participate in the study. After they gave their informed consent, they were examined. Some information on patient III-8 was available because he and his eldest daughter IV-4 had attended a university clinic 40 years previously. The only information on patient IV-5, obtained by history from members of the family, was that he had suffered severe coordination problems and learning difficulty and had died at the age of 51 years. Patients IV-4, IV-9, IV-11, V-24, V-28, V-31 and V-34 had been investigated several times over a period of 20 years, which allowed us to assess the progression of the disorder. We used the international ataxia cooperative rating scale to quantify the severity of cerebellar symptoms (Trouillas et al. 1997). This scale involves a compartmentalized quantification of postural and stance disorders, limb ataxia, dysarthria and oculomotor abnormalities. Sub-scores for these symptoms can be studied separately. The total ataxia score ranges from 0 (no ataxic features) to 100 (complete cerebellar syndrome).

Neuropsychological methodology

Two patients of the fourth generation (IV-4 and IV-11), and three of the fifth generation (V-1, V-28, V-34) underwent neuropsychological testing. Global cognitive functions were assessed by the Mini Mental State Examination (MMSE). Premorbid IQ was estimated on the basis of socio-economic background and education (Luteyn 1983). Verbal and non-verbal intelligence was assessed with the Wechsler Adult Intelligence Scale-Revised (WAIS-R) and Raven Standard Progressive Matrices (RSPM) (Wechsler 1974; Raven 1960). Memory performance was tested with the WAIS digit span for immediate memory, with the 15-Words Test (a Dutch version of the Ray Auditory Verbal Learning Test) for immediate recall, delayed recall and recognition in the auditory domain, and with the Recognition Memory Test for Faces for memory in the visual domain (Heslinga et al. 1983; Warrington 1984). The tests for measuring executive function were the WCST and the Bobertag (Grant and Berg 1948) Cortical language functions were assessed with the SAN test (Deelman BG et al. 1983) Depression was rated with the Beck Depression Inventory (BDI) and the Zung Depression Scale (Beck et al. 1961; Zung 1965).

Electrophysiological studies

Nerve conduction studies were performed using the antidromic technique with surface electrodes for sensory and motor nerve testing. Concentric needle electrodes were used for electromyography. In this way the peroneal, sural, and median and/or ulnar nerves were investigated in six patients, with the H-reflex of the tibial nerve in five. Transcranial magnetic stimulation (TMS) was performed with a Cadwell MES 10 stimulator with a maximum magnetic field of 2T. Central conduction times were investigated in six patients with magnetic stimulation of the motor cortex and cervical spine. Motor evoked potentials were recorded with surface electrodes taped over the belly and tendon of the biceps and abductor digiti V muscles.

Cerebral magnetic resonance imaging

Seven patients were examined using 1.5-T magnetic resonance imaging (MRI). T1-weighted axial images (repetition time 550ms, echo time 20ms) and T2-weighted axial images (repetition time 4000ms, echo time 100ms) were recorded. Atrophy was qualitatively graded on a four-point scale (0=no atrophy, 1=mild, 2=moderate, 3=severe) by two experienced neuroradiologists who reported by consensus.

Molecular analysis

DNA was isolated from blood of 17 family members. Two of these samples were analyzed for CAG trinucleotide repeat expansions in the *SCA1, 2, 3, 6, 7* and *12* genes and the CTG trinucleotide repeat expansion, associated with *SCA8*, by polymerase chain reaction amplification and polyacrylamide gel electrophoresis. Furthermore, to exclude linkage to *SCA4, 5* and *10* loci we performed linkage analysis with markers specific for these loci, i. e. D16S3396 for the SCA4 locus, D11S1911, D11S1993 and D11S1978 for the SCA5 locus D22S274, D22S928 for the SCA10 locus and D15S659 for the SCA11 locus. LOD scores for the markers are shown in

Table 4.2 (selected from the Marshfield database; http://www.marshmed.org/genetics/ Map_Markers).

Table 4.2 LOD scores for various markers

Locus	Marker	θ=0	θ=0.1	θ=0.2	θ=0.3	θ=0.4	θ=0.5
SCA4	D16S3396	-7.23	-0.82	-0.32	-0.10	-0.02	0
SCA5 <?2>	D11S1911	-5.20	-0.86	-0.37	-0.14	-0.03	0
	D11S1993	-8.32	-1.56	-0.70	-0.27	-0.06	0
	D11S1978	-3.35	0.60	0.59	0.34	0.10	0
SCA6	D19S1150	-7.27	-1.65	-0.83	-0.39	0.13	0
SCA10 <?1>	D22S274	-5.40	-0.34	-0.05	0.03	0.02	0
	D22S928	-10.41	-1.06	-0.31	-0.03	0.04	0
SCA11	D15S659	-5.98	-0.39	0.02	0.12	0.08	0

Results

Clinical findings

Of the 12 affected members of the family 10 were available for neurological examination (Table 4.1). The mean age at onset in the fourth generation was 40 years (IV; n=3), in the fifth generation it was 30 years (V; n=5). In the sixth generation, patient VI-31 showed mild dysarthria, jerkiness of smooth pursuit eye movements and intention tremor before the age of 10 years. In contrast, patient VI-28 only showed ocular dysmetria at the age of 28. Cerebellar dysarthria was an early feature in all individuals. Patients V-24 and V-31 both presented with a postural and irregular tremor of slow frequency, which was consistent with Holmes tremor (Deuschl et al. 1998). Patient V-24 also had a segmental myoclonus (frequency 3 Hz) of the abdominal muscles, which in the absence of additional extrapyramidal symptoms and normal EEG findings (registered concurrently with electromyography at the time of the muscle jerks), was suggestive of spinal myoclonus. The irregular brief muscle jerks in patient IV-11 preceded by paroxysmal theta activity over the temporal and parietal lobes on EEG were considered indicative of cortical myoclonus. Walking difficulty with frequent falling was also an early symptom. Ataxia of the upper extremities was a relatively late symptom. All subjects showed fine saccadic eye movements, and in all but one a first-grade horizontal nystagmus was present. Only one patient complained of cognitive impairment (V-34).

Electrophysiological studies

Nerve conduction studies showed normal velocities in four patients but slightly decreased velocities in two (IV-4 and V-1). These two patients also had increased H-M intervals (35ms). Electromyography recorded fibrillation potentials in the leg muscles of patient IV-4. These findings were consistent with a mild axonal neuropathy. TMS showed slightly increased central conduction times in four patients (IV-11, V-1, V-28, V-31) with values of 9.1, 9.5, 9.6 and 10.2ms (normal values 7-8.5ms).

Neuropsychological test

Table 4.3 summarizes the test performance of the individuals investigated. Three patients showed evidence of severe or moderate depression and their estimated premorbid IQ was below 105 (education not completed). Despite this, the MMSE score was above the usual cut-off value of 24 in all five patients examined. Four of these five patients had scores at least 2 SD below the norm on the WCST and one a score more than 1SD below the norm.

Magnetic resonance imaging

Table 4.4 summarizes the results of the MRI studies. In six subjects there was mild to severe atrophy of the

cerebellar hemispheres, with moderate or mild atrophy of the vermis in four of them. In two patients there was mild cerebral atrophy.

Molecular analysis

Analysis of the CAG trinucleotide repeats in the *SCA1, 2, 3, 6, 7* and *12* genes and the CTG trinucleotide repeat at the *SCA8* locus did not reveal any abnormalities. Further linkage analysis also excluded the *SCA4, 5* and *10 loci* as candidate loci involved in the disease pathogenesis in this family. Since point mutations have been reported in the *SCA6 gene* in families with ataxia (Ducros et al. 1999; Yue et al. 1997), linkage to the *SCA6* locus was formally excluded by using the CAG trinucleotide repeat as a polymorphic marker.

Table 4.3 Neuropsychological tests in 5 patients.

	IV-4	IV-11	V-1	V-28	V-34
Age (years)[a]	87	80	55	57	51
Duration	57	35	10	30	26
MMSE	25/30	26/30	27/30	26/30	26/30
Estimated premorbid IQ	< 105	< 105	< 105	< 105	< 105
Intelligence					
Verbal IQ (WAIS)	n.d.	n.d.	103	83	80
Non-verbal (Raven SPM/CPM)	12/36	24/36	48/60	25/60	35/60
Memory					
Memory (MQ)	89	100	101	73	84
WMS Digit span (forward/backward)	4/2	5/4	5/3	4/2	4/3
15-Words/8-Words test (8 if > 70)					
Learning curve	2-4-2-3-4	3-4-5-6-5	2-4-6-5-7	3-5-4-6-4	2-2-5-5-5
Delayed recall	0/8	3/8	4/15	2/15	3/15
Recognition	13/16	14/16	28/30	21/30	21/30
Visual (RMT faces)	n.d.	n.d.	38	38	39
'Executive functioning'					
Bobertag	n.d.	A	A	N	N
WCST					
Categories	F	0	4	2	2
Correct	F	16	73	52	30
Perseverative errors	F	68	29	80	95
PE/TE 100 %	F	98.5	22.7	62.5	74.2
Visual-spatial perception					
Hooper VOT (impairment)	Severe	Moderate	Mild	Moderate	Mild
Visual-spatial construction					
WAIS-R (Block design)	n.d.	n.d.	6	5	7
Verbal fluency (SAN)	n.d.	48	18	48	48
Mood					
Zung (degree of depression)	n.d.	n.d.	N	Severe	Severe
BDI (degree of depression)	Moderate	n.d.	Mild	Severe	Moderate

[a] Age at 1 January 1999; Scores are raw scores, when not indicated otherwise; MMSE, Mini Mental State Examination; WAIS, Wechsler Adult Intelligence Scale; Raven SPM, Raven standard progressive matrices; Raven CPM, colored progressive matrices; WMS, Wechsler Memory Scale; RMT faces, Recognition Memory Test for Faces; WCST, Wisconsin Card Sorting Test; P/TE 100%, percentage of errors that are perseverative; SAN, cortical language test; BDI, Beck Depression Inventory; N, normal performance; A, abnormal performance; n.d., no reliable data available.

Table 4.4 MRI imaging features in 7 patients.

	IV-11	V-1	V-28	V-31	V-34	VI-28	VI-31
Age (years)[a]	80	55	57	56	51	28	11
Duration (years)	45	45	27	31	27	n.d.	n.d.
Atrophy							
Cerebellar hemispheres	+++	+	+	+	+	+	-
Vermis	++	-	-	+	+	+	-
Medulla	-	-	-	-	-	-	-
Pons	-	-	-	-	-	-	-
Cerebrum	+	-	-	-	-	+	-
White matter lesions	+	-	-	-	-	-	-

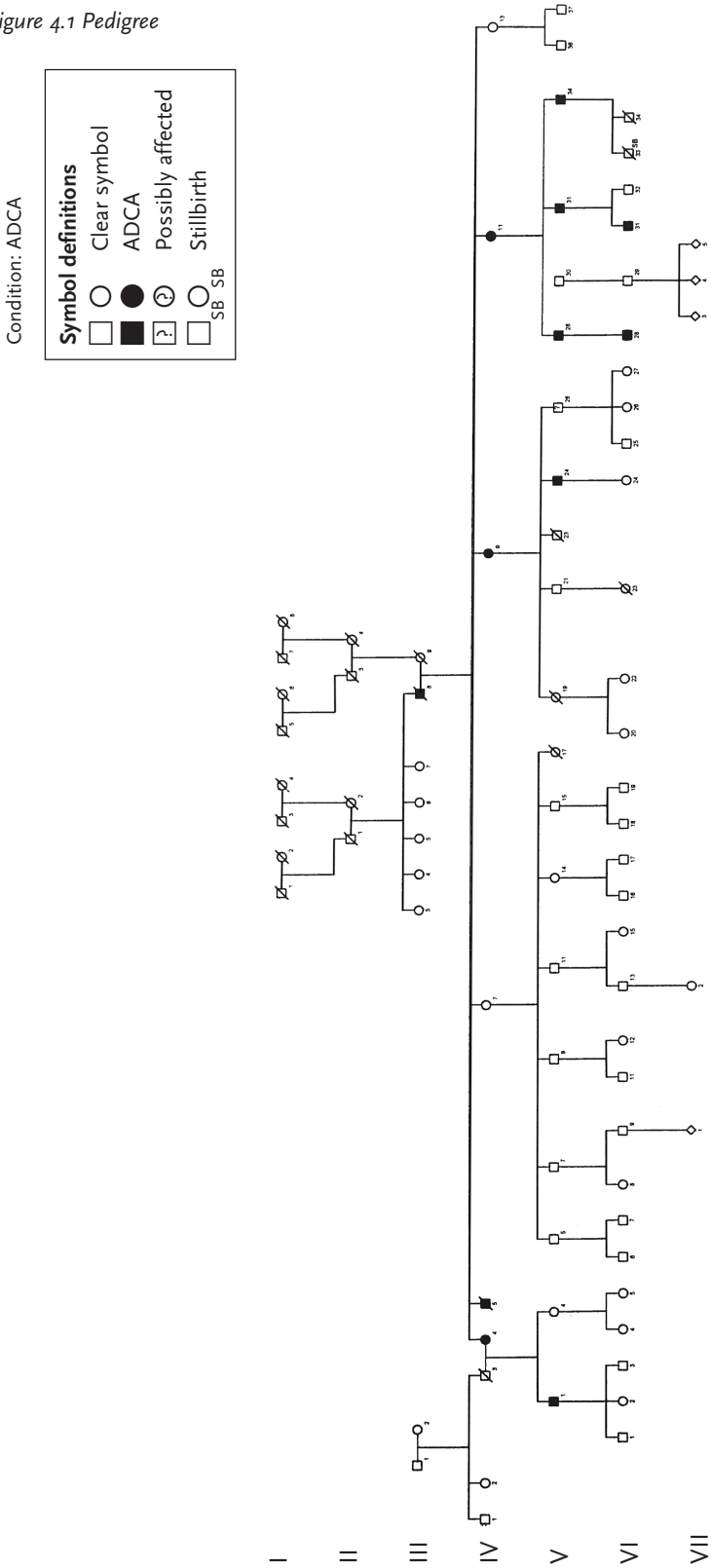
[a] *Age at 1 January 1999.*

- = no atrophy; + = mild atrophy; (++) moderate atrophy; (+++) severe atrophy; n.d., no reliable data available.

Discussion

In 1983 Harding proposed a useful clinical classification for 'late onset' ADCA by division of the ADCAs into type I (ophthalmoplegia, optic atrophy, dementia and pyramidal/extrapyramidal features), type II (pigmentary retinopathy) and type III ('pure' cerebellar ataxia) (Harding 1983). Since 1993, ADCAs have been increasingly characterized in terms of their genetic locus and are referred to as SCA. The mutations for SCA1, 2, 3, 6, 7 and 12 have been characterized as expansions of CAG trinucleotide repeats in the coding region of the respective genes. The mutations for SCA4, 5, 11, 13 and 16, which have been linked to chromosomes 16, 11, 19 and 8, respectively, are yet to be isolated, but the underlying gene defects remain to be identified. The relevance of the CTG trinucleotide repeat expansion, associated with SCA8, is not clear (Stevanin et al. 2000; Worth et al. 2000). In some families with ADCA, including the one reported here, there is no linkage to any of these loci. For these families Harding's classification could still be relevant. Given the evidence for cognitive impairment, pyramidal tract involvement, (Holmes) tremor, and peripheral neuropathy, members of this family would fall into the category of ADCA I. Since our family showed rather poor results on most neuropsychological tests and estimated premorbid IQ was below 105 in three of the five examined patients, careful follow-up should confirm whether the cognitive impairment is progressive. Nevertheless, the poor performance on the WCST was striking. Poor performance on the WCST, suggesting disturbance of abstract reasoning and the ability to shift cognitive sets to changing contingencies, is associated with damage to the dorsolateral prefrontal cortex or its connection to (sub)cortical structures (Nagahama et al. 1996). Poor performance on the WCST has been described in SCA1 and particularly SCA2 families (Kish et al. 1994; Burk et al. 1999; Gambardella et al. 1998; Storey et al. 1999). Similar cognitive defects have also been described in SCA3 (Maruff et al. 1996). In families with SCA4, 5, 6, 10, 11 and 12 no detailed neuropsychological studies have been performed (addendum 2004: for review see Schelhaas et al. 2003). One patient clinically presented with a spinal myoclonus and one patient with a cortical myoclonus. Unfortunately, it was not possible in either patient to reanalyze the myoclonus according to modern standards. For this, and as myoclonus has been described in SCA1, 2, 3, 7, 14 and even SCA6 families (Pareyson et al. 1999; Jobsis et al. 1997; Schols et al. 1997b; Watanabe et al. 1998a), we feel that the significance with regard to myoclonus as being characteristic of this family, is limited. Although there were too few affected individuals to be able to draw firm conclusions about anticipation, the available data on age at onset and the severity of additional features raise the possibility of anticipation. Such a phenomenon may be explained molecularly by expansion of unstable trinucleotide repeats during vertical transmission. Currently we are performing a genome-wide scan to determine the chromosomal localization of the disease gene in this family.

Figure 4.1 Pedigree



Identification of a novel SCA locus (SCA19) in a Dutch autosomal dominant cerebellar ataxia family on chromosome region 1p21-q21

D.S. Verbeek, H.J. Schelhaas, P.F. Ippel, F.A. Beemer,
P.L. Pearson and R.J. Sinke

Chapter 5

Human Genetics 2002; 111: 388-399.

Abstract

We present a linkage study in a four-generation autosomal dominant cerebellar ataxia (ADCA) family of Dutch ancestry. The family shows a clinically and genetically distinct form of ADCA. This neurodegenerative disorder manifests in the family as a relatively mild ataxia syndrome with some additional characteristic symptoms. We have identified a *SCA19* locus, approved by the Human Genome Nomenclature Committee that can be assigned to the chromosome region 1p21-q21. Our mutation analysis failed to identify any mutations in the known spinocerebellar ataxia (*SCA*) genes and linkage analysis excluded the remaining *SCA* loci. We therefore performed a genome-wide scan with 350 microsatellite markers to identify the location of the disease-causing gene in this family. Multi-point analysis was performed and exclusion maps were generated. Linkage and haplotype analysis revealed linkage to an interval located on chromosome 1. The estimated minimal prevalence of ADCA in the Netherlands is about 3:100,000. To date, sixteen different SCA loci have been identified in ADCA (*SCA1-8* and *SCA10-17*). However, mutation analysis has been commercially available only for the *SCA1, 2, 3, 6* and *7* genes. So far, a molecular analysis in these *SCA* genes cannot be made in about one-third of the ADCA families. Thus, the identification of this new, additional *SCA19* locus will contribute to expanding the DNA diagnostic possibilities.

Introduction

ADCA is one of the most frequently occurring subgroups of hereditary progressive neurodegenerative disorders. This serious and invalidating disorder is caused by a progressive neurodegeneration of the cerebellum, brain stem and spinal cord leading to a balance disturbance and loss of neurological functions. In addition to ataxia, various other symptoms such as tremor, epilepsy, retinal degeneration and mental retardation are seen. In general, the symptoms usually begin around the age of 40 years.

So far, a series of genes has been identified. Characterization of the first five spinocerebellar ataxia (SCA) genes, *SCA1, 2, 3, 6, 7* (Orr et al. 1993; Pulst et al. 1996; Kawaguchi et al. 1994; Zhuchenko et al. 1997; David et al. 1997), and the more recently identified *SCA17* gene (Koide et al. 1999; Nakamura et al. 2001), seem to point to a common underlying molecular defect, namely the involvement of a CAG repeat expansion in the coding region of the gene. However, these six genes do not show any homology in either structure or function, and the only common feature is the CAG repeat expansion. The CAG repeat expansions are translated into polyglutamine tracts leading to a so-called 'polyglutamine' disease (Paulson et al. 1997a). The initial CAG repeat number and the composition of the repeat (perfect or interrupted) seem to be related to the expanded CAG repeat numbers (Pearson et al. 1998). The repeat numbers of the expansion are generally associated with the severity of the disease and the age at onset, often reported as intra-familiar anticipation.

Based on testing of the *SCA1, 2, 3, 6* and *7* genes in all available Dutch ADCA families, we can conclude that mutations in these genes account for about 70% of the Dutch ADCA families. For the Netherlands, this indicates a minimal prevalence of the disorder of about 3:100,000, with the mutation in the SCA3 gene being found most frequently (44.1%), and followed by the *SCA6* gene (23.4%) (van de Warrenburg et al. 2002).

The idea that the only causative component in the pathogenesis of ADCA is expanded polyglutamine tracts becomes more unlikely (Richards 2001). The analyses of *SCA8, 10* and *12* genes, point to significant differences from the 'polyglutamine' hypothesis (Holmes et al. 1999; Koob et al. 1999; Matsuura et al. 2000; Zu et al. 1999). First, although the clinical characteristics between the different SCA genes show some overlap, there are a variety of additional neurological symptoms (Schols et al. 1997b), including tremor and mental retardation. Secondly, the mutations in these genes are also repeat expansions, but are non-coding CAG, CTG, and ATTCT repeats instead of coding CAG repeats (Holmes et al. 1999; Koob et al. 1999; Matsuura et al. 2000). How these non-coding repeat expansions contribute to the pathology of ADCA is not known. Thirdly, these mutations seem to be rare and have been described in only one or a few families. Some mutations seemed to be confined to specific populations (Fujigasaki et al. 2000; Fujigasaki et al. 2001a) and new

SCA genes still have to be identified to account for the remaining 30% of the Dutch ADCA families in which no mutation has been identified so far.
Here, we present a linkage analysis in a large Dutch ADCA family, in which no repeat expansion could be detected in the *SCA1, 2, 3, 6, 7, 8, 12* and *17* genes. The remaining SCA loci (*SCA4, 5, 10, 11, 13-14 and 16*), for which mutations still have to be identified (Flanigan et al. 1996; Herman-Bert et al. 2000; Matsuura et al. 1999; Miyoshi et al. 2001; Ranum et al. 1994; Worth et al. 1999; Zu et al. 1999), were excluded by 2-point linkage analysis (Schelhaas et al. 2001). We localized the disease gene involved in this family by performing a genome-wide screen. Significant evidence for linkage was found on chromosome region 1p21-1q21. The discovery of this novel locus, *SCA19*, adds to the extensive genetic heterogeneity exhibited by the ADCA phenotype.

Materials and methods

The patients

The family used in this study was described by Schelhaas et al (Schelhaas et al. 2001). Briefly, clinical investigation showed a relatively mild ataxic syndrome with some additional characteristic symptoms, including cognitive impairment, bad performance in the Wisconsin Card Sorting Test, myoclonus and postural irregular tremor of low frequency. Harding's classification characterizes the phenotype in this family as ADCA type I (Harding 1983). There were too few affected individuals in this family to draw firm conclusions about anticipation. Although, a decrease in age at onset is seen, this is not accompanied by an increase in severity of the disorder (Schelhaas et al. 2001). Sixteen family members were included in the clinical investigation and 11 were diagnosed as affected. There were no asymptomatic obligate carriers. There was also no indication for sex-limited transmission since both male-to-male and male-to-female transmissions were observed.

Mutation analysis of known SCA genes

High molecular weight genomic DNA was extracted from peripheral blood leukocytes using a routine salting-out method. Diagnostic analysis failed to identify any mutation in one of the known SCA genes (*SCA1-3, 6-8, 12 and 17*) (data not shown). Subsequently, linkage analysis with specific markers excluded the remaining known SCA loci (*SCA4, 5, 10, 11, 13, 14 and 16*) (Schelhaas et al. 2001). Since point mutations have been described in the SCA6 gene (Yue et al. 1997), this locus was also excluded by linkage analysis using the intragenic marker D19S1150. The markers used in this linkage study were selected from the Marshfield database: http://www.marshmed.org/genetics_Markers.

Genome-wide genotyping analysis

In an attempt to localize the disease-causing gene, a genome-wide genotyping analysis was performed after excluding all the known SCA genes and loci. The linkage mapping set consisted of 350 microsatellite markers, covering all autosomes, with an average spacing of 10-15 cM. Additional markers were selected to narrow down possible candidate regions. All markers were 5 end-labeled with fluorescent-dyes: 6-FAM, TET or HEX.
PCR reactions were performed in a total volume of 10 µl, containing 25 ng of genomic DNA, 1 µl PCR buffer II (Applied Biosystems, Foster City, Calif., USA), 25 mM $MgCl_2$, 2 mM dNTPs, 12.5 ng of each marker and 0.4 U of AmpliTaq Gold (Applied Biosystems). The PCR reaction started with a 10 min denaturation step at 94°C, followed by 33 cycles of 30 s denaturation (94°C), 30 s annealing (55°C) and 30 s extension (72°C), ending with a final extension step of 30 min at 72°C. The PCR was carried out on a GeneAmp PCR system 9600 or 9700 machine (Applied Biosystems). The PCR product (0.9 µl) was mixed with 1.6 µl loading buffer containing formamide, blue dextran, and GS500XL, the internal lane standard (Applied Biosystems) and analyzed on a 6% polyacrylamide gel (Longranger Single Packs, BioWhittaker Molecular Applications, Rockland, Me., USA) in an ABI 377 sequencer (Applied Biosystems). Fragment analysis was performed using Genescan (v 3.1) and Genotyper (v 2.1) software.

Linkage and haplotype analysis

In the initial screen, two-point lod scores were calculated for each microsatellite marker using MLINK of the FASTLINK (v 5.2) software package, assuming autosomal dominant inheritance, a disease frequency of 1:100,000, and 95% penetrance (Lathrop and Lalouel 1984). In the linkage analysis, equal allele frequencies were used for all markers. All affected individuals (n=11) and the five unaffected individuals older than 50 years were included in the initial linkage analysis. Two-point lod scores of 1.0 and higher were considered to indicate regions of interest. These candidate regions were tested by additional markers and haplotype analysis for further fine mapping. Finally, a multipoint analysis was performed for all autosomes using Genehunter v 2.0 (Kruglyak et al. 1996).

Results

Linkage analysis

Five potential candidate regions, with two-point lod scores of 1.0 or higher, were obtained after testing the whole linkage screening set, involving loci on chromosomes 1, 7, 9, 10 and 12. Significant linkage of the disease (lod score >3.0) was found only with the microsatellite marker D1S534 with a maximal two-point lod score (Z_{max}) of 3.82 at a recombination fraction of 0.00 (Table 5.1). In addition, a multipoint analysis was performed for each autosome. Figure 5.1 shows the multi-point analysis for chromosome 1. The other possible candidate regions located on chromosomes 7, 9, 10 and 12 were excluded by both multipoint and haplotype analysis (data not shown).

Haplotype analysis of chromosome 1

Flanking markers of D1S534 were tested and although high positive lod scores were obtained, none exceeded the Z_{max} of 3.82 (Table 5.1). The chromosome 1 candidate interval is defined by two recombination events in one affected person (III: 10) (Fig. 5.2). These two recombination events, proximal at D1S1588 and distal at marker D1S1595, occurred on opposite sides of the centromere. The marker order in this interval, based on the Marshfield database (October 2001), is: D1S1588 - 0 cM - D1S435 - 3.86 cM - GATA124C08 - 1.97 cM - GATA45B07 - 5.54 cM - D1S1631 - 12.32 cM - D1S1675 - 2.68 cM - D1S534 - 1.71 cM - D1S2696 - 1.15 cM - D1S442 - 6.31 cM - D1S1595. All the affected individuals share the same haplotype for the candidate interval flanked by D1S1588 and D1S1595, whereas none of the unaffected individuals showed this haplotype. The size of the interval, which harbors the disease gene in this family, is approximately 35 cM (Fig. 5.2).

Table 5.1 Two-point lodscores between the disease locus and 13 chromosome 1 markers.

	Lodscore at recombination rate (theta)						
Markers	0.00	0.10	0.20	0.30	0.40	Z_{max}	theta =
D1S551	-0.29	-0.10	0.07	0.09	0.04	0.09	0.28
D1S435	-6.04	-0.38	-0.01	0.11	0.11	0.13	0.35
D1S1588	-3.91	0.17	0.28	0.26	0.15	0.29	0.22
GATA124C08	2.34	1.89	1.39	0.84	0.31	2.34	0.00
GATA45B07	3.02	2.46	1.83	1.14	0.41	3.02	0.00
D1S1631	2.10	1.68	1.23	0.73	0.23	2.10	0.00
D1S1675	1.94	1.60	1.22	0.79	0.31	1.94	0.00
D1S534	3.83	3.15	2.40	1.55	0.63	3.83	0.00
D1S2696	3.23	2.94	1.99	1.26	0.49	3.23	0.00
D1S442	1.46	1.19	0.89	0.56	0.22	1.46	0.00
D1S1595	-3.43	1.16	1.02	0.67	0.24	1.16	0.11
D1S1679	0.60	0.69	0.54	0.32	0.10	0.71	0.06
D1S1677	-4.78	0.27	0.34	0.28	0.16	0.34	0.19

Discussion

We performed a genome-wide screen in a four-generation Dutch ADCA family. The disease in this family manifests as a relatively mild ataxia syndrome with some additional characteristic symptoms (Schelhaas et al. 2001). Given the presence of the main clinical characteristics, including pyramidal signs, peripheral neuropathy and cognitive impairment, the phenotype in this family is classified as ADCA type I (Harding 1983). Our marker analysis reveals significant linkage with marker D1S534 with a maximal two-point lod score (Z_{max}) of 3.82 at recombination fraction 0.00. The candidate interval is defined at the proximal boundary by marker D1S1588 and at the distal end by marker D1S1595. Multi-point and haplotype analysis of the chromosome 1 markers revealed a candidate interval of approximately 35 cM. This SCA19 locus, approved by the Human Genome Nomenclature Committee, can be assigned to the chromosome region 1p21-q21.

We used a haplotype sharing approach in an attempt to further narrow down the disease gene location of the *SCA19* locus, and we are currently searching for additional ADCA families exhibiting linkage with the same region. However, at this moment, we only have two additional large Dutch ADCA families available for linkage studies. In these families no linkage could be detected (data not shown), indicating the presence of at least one other SCA locus in the Dutch population. In addition, we are searching for possible candidate genes within the candidate interval, by first focusing on CAG repeat-containing genes. Mutation analysis has now (addendum 2003) excluded the CAG containing genes *KCNN3, TNRC4* and *KIAA0467* (data not shown). Secondly, genes encoding ion channels may be of particular interest, because of the presence of myoclonus and tremor in this family. Other genetic disorders exhibiting myoclonus and tremor also carry mutations in ion channels, including epilepsy (Escayg et al. 2000), episodic ataxia 2 (EA2) and SCA6 (Jodice et al. 1997). The *SCA6* gene, encoding the α_{1A}-subunit of a voltage-dependent calcium channel, CACNA1A, was originally identified as being involved in familial hemiplegic migraine (FHM) (Ophoff et al. 1994). Interestingly, distinct types of CACNA1A mutations have been identified in FHM and SCA6 (Denier et al. 1999). A second familial hemiplegic migraine locus has been localized to chromosome 1q21-q23 (Ducros et al. 1997). In parallel with the *CACNA1A* gene, candidate genes for FHM located on the long arm of chromosome 1 are also direct candidate genes for SCA19, although no migraine has been recorded in the affected individuals of the current family. Such candidates include the *KCNN3, KCNC4, KCNJ10, KCNA2* and *KCNA3* genes but now (addendum 2003) these genes have also been excluded (data not shown).

Following identification of SCA7, four novel SCA genes were defined (*SCA8, 10, 12* and *17*). Approximately 200 individual Dutch ataxia patients have been tested but revealed no mutations (data not shown) in the SCA10, 12 or 17 genes. An exception was the SCA8 gene in which a repeat expansion was detected in several cases (data not shown). However, non-pathogenic polymorphisms in the SCA8 gene have also been detected in normal controls, and secondly, additional SCA8 expansions have been identified in patients carrying mutations in one of the other *SCA* loci (Sobrido et al. 2001). The relation between *SCA8* mutations and the etiology of ADCA must still be established. The frequency of these novel identified genes seems to be so rare that none can be the main causative mutation for the other 30% of Dutch ADCA families. However, our identification of the mutation involved in the *SCA19* locus will improve the diagnostic possibilities and genetic counseling of some of the remaining ADCA families, and it will provide further insight into the controversy of whether expanded polyglutamine tracts are the main causation in the pathology of the ADCAs.

Acknowledgements. This work was supported by grant MAR00-107 from the Prinses Beatrix Fonds, the Netherlands.

Figure 5.1 Multipoint linkage analyses for 21 chromosome 1 markers, covering the entire chromosome. Marker D1S1588 and marker D1S1595 flank the candidate interval.

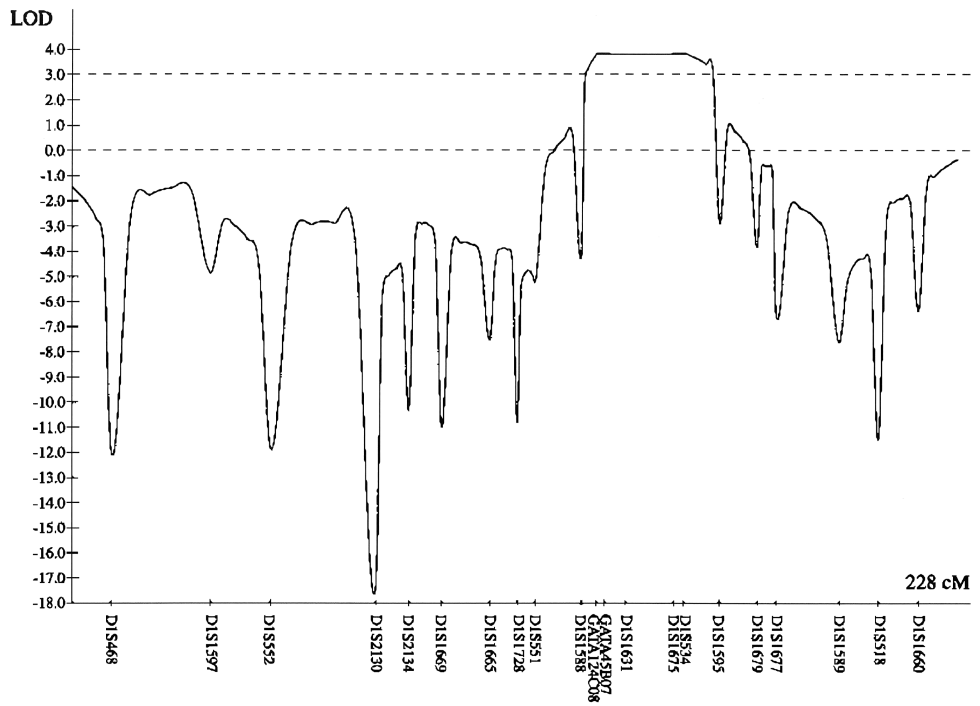
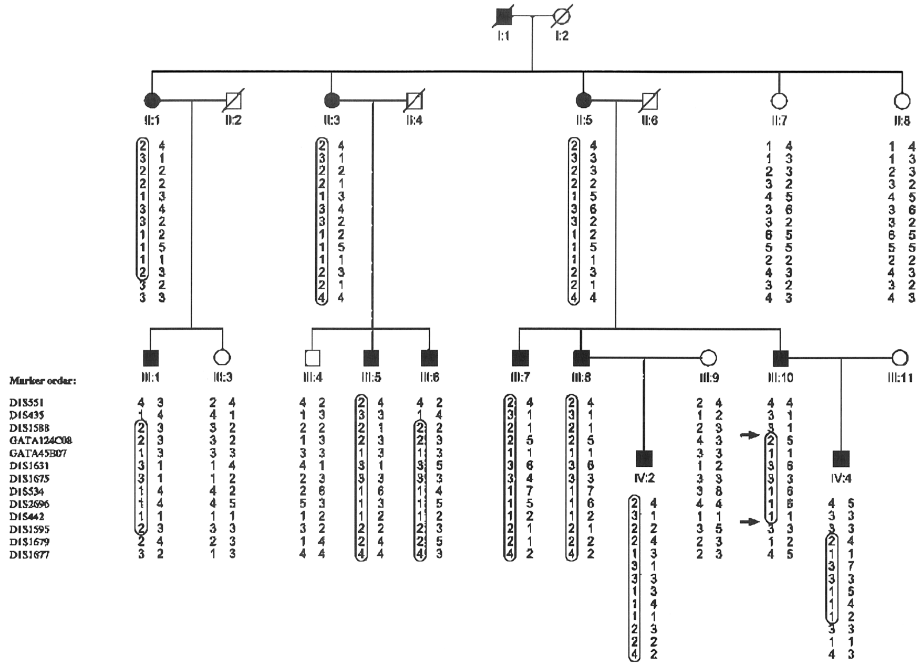


Figure 5.2 Haplotype analyses for 13 chromosome 1 markers. The putative disease haplotype is boxed. Arrows indicate the informative recombination events.



SCA19 and SCA22: evidence for one locus with a worldwide distribution

H.J. Schelhaas, D.S. Verbeek, B.P.C. Van de Warrenburg
and R.J. Sinke

Addendum to Chapter 5

Brain 2004 Jan;127 (Letter to the editor, electronic version).

We read with interest the paper by Ming-Yi Chung and colleagues (Chung et al. 2003). In this paper, the authors characterized a four-generation Chinese pedigree with an autosomal dominant phenotype for cerebellar ataxia (ADCA). Their genome-wide linkage study suggested linkage to a locus on chromosome 1p21-q23. The authors stated that the form of ADCA found in this family is distinct from other spinocerebellar ataxias (SCA) and designated this SCA as spinocerebellar ataxia type 22 (SCA22). However, this locus was previously assigned to SCA19 by the HUGO Gene Nomenclature Committee (http://www. gene.ucl.ac.uk/cgi-bin/nomenclature). Both the clinical features of the affected Dutch ADCA family and the identification of this locus have been extensively described (Schelhaas et al. 2001; Verbeek et al. 2002). The four-generation Dutch ADCA family has a relatively mild ataxia syndrome with slow progression and additional clinical features, including cognitive impairment, pyramidal signs and peripheral neuropathy (ADCA type I). Genome-wide screening revealed significant linkage with marker D1S534 (maximum lod score 3.82 with theta = 0,00). The candidate interval spans ~35 cM and is located between the markers D1S1588 and D1S1595. The SCA19 locus has been assigned to the chromosomal region 1p21-q21.

Chung and colleagues report that the candidate SCA22 gene is located between the markers D1S206 and D1S2878. There is significant overlap between the two regions: ~26.9 cM between the markers D1S206 and D1S1595. Although it cannot be excluded that the genes lie in close approximation at this locus, it is more likely that the Dutch and Chinese families suffer from a mutation in the same gene, and that SCA19 and SCA22 represent an identical condition, which should be designated SCA19 as this was the first linkage assigned. By first focusing on CAG repeat-containing genes, both research groups indicated that *KCNN3, TNRC4* and *KIAA0467* were the most likely candidate genes. However, CAG expansions in these genes were not found in affected individuals of the Chinese family, and therefore *KCNN3, TNRC4* and *KIAA0467* can be excluded as candidate genes. Potassium channel genes (e.g. *KCNJ10*, potassium channel, inwardly rectifying, subfamily J, member ten) are also mapped to this region and were mentioned as serious candidate genes for both SCA19 and SCA22. These genes are of particular interest, because of the presence of myoclonus and tremor in the Dutch SCA19 family.

Unfortunately, Dr Chung and colleagues must have overlooked this earlier assignment. Their excellent work has narrowed the genetic region of interest and suggests that SCA19 is an SCA with worldwide occurrence. The correct assignment of these two families will help clarify the evolving chaos in the genetic classification of these disorders.

Part III

Expanding the phenotype of SCA3 and SCA6

The 'split hand' phenomenon: evidence of a spinal origin

H.J. Schelhaas, B.P.C. van de Warrenburg, H.P.H. Kremer and M.J. Zwarts

Chapter 6

Neurology 2003; 61: 1619-1620.

Abstract

The clinical phenomenon of a split hand, dominant muscle atrophy in the thenar as compared to the hypothenar complex, has been used to support the theory of primary cortical degeneration in ALS. However, the same phenomenon, both clinically and electrophysiologically, was observed in three diseases with a second but not first motor neuron affection: autosomal dominant spinal muscular atrophy, spinocerebellar ataxia type 3, and juvenile muscular atrophy. Therefore, we conclude that neurogenic loss according to a split hand distribution points to a spinal instead of cortical origin.

Introduction

The term 'split hand', first coined by Willbourn et al. refers to a type of hand muscle atrophy in which the muscles of the lateral aspect of the hand (first dorsal interosseus and thenar) are more affected than those of the medial aspect (hypothenar) (Wilbourn 1994). Willbourn described the split hand in the context of amyotrophic lateral sclerosis (ALS) and stated that, although not limited to ALS, this phenomenon seems to be specific for anterior horn disease (Wilbourn 2000). However, as the thenar and hypothenar complex have the same segmental supply and a different cortical presentation, others suggested that this pattern of muscle wasting is of cortical origin (Weber et al. 2000). As such, the dissociation of hand muscle wasting is considered to confirm the theory of cortical dysfunction with anterograde degeneration as the initial step in the pathogenesis of ALS. We wondered whether the split hand is also observed in disorders that are known to be characterized by lower motor neuron degeneration in the absence of cortical degeneration. Therefore we performed nerve conduction studies in patients with autosomal dominant distal spinal muscular atrophy (DSMA), spinocerebellar ataxia type 3 (SCA3), and juvenile muscular atrophy (JMA).

Patients and methods

Patients

All five affected members of a three-generation family with DSMA, five consecutive SCA3 patients, and three patients with JMA participated in the study. Each patient was subjected to a thorough neurological and electrophysiological examination.

Nerve conduction studies

Nerve conduction studies were recorded with a Medelec Synergy EMG system (Oxford Medical Instruments). Skin temperature was maintained at 34°C and limbs were warmed if necessary. CMAPs of the thenar and hypothenar complexes were evoked by supramaximal peripheral stimulation of the median and ulnar nerves, respectively. One SCA3 subject (data not shown) with neurophysiological evidence of a carpal tunnel syndrome was excluded.

Statistics

Analysis was done using binomial distribution statistics.

Results

The five members of the DSMA family showed a mild disease severity, slow progression, with lower limb predominance. Dissociated hand muscle wasting was evident in four of five member of the DSMA family.
The phenotype of the five SCA3 patients was characterized by a late onset (40-60 years), slow progression, cerebellar ataxia, cramps, fasciculations, and muscle atrophy. A split hand pattern of muscle atrophy was present in four of five patients with SCA3.
The clinical course of the three (male) JMA patients was characterized by an insidious onset, slow progression and, ultimately, stabilization of disease. Dominant muscle atrophy in the thenar as compared to

the hypothenar complex was present in two patients with JMA. One JMA patient showed a reverse pattern of muscle wasting, with sparing of the thenar and prominent involvement of the hypothenar muscles. Intriguingly, this patient showed lower focal cervical cord atrophy on cervical MRI.
In four of five subjects with DSMA, in all SCA3 patients, and in two patients with JMA, the electrically evoked hypothenar CMAP was larger than the thenar CMAP (Table 6.1).
Concerning a total population of 13 patients, a binomial distribution and an 'a prior probability' of 0.5 (no significant difference in the thenar as compared to the hypothenar CMAP in normal controls), with 13 trials and two failures: p = 0.0111 (one-tailed sign test).

Table 6.1 Clinical features and nerve conduction studies in 5 patients of a three-generation family with autosomal dominant DSMA, in 5 patients with SCA3, and in 3 patients with JMA.

		Thenar		Hypothenar	
Diagnosis	Age y/sex	Muscle weakness (MRC)	CMAP (mV) †	Muscle weakness (MRC)	CMAP (mV)‡
DSMA	82/f	2	0	5	11.4
DSMA	59/f	4	7.8	5	12.6
DSMA	56/m	4-	0.5	5	13.4
DSMA	50/m	4+	11.7	4	9.8
DSMA	27/m	4	5	4	9
SCA3*	61/f	4	5.0	4	8.5
SCA3	62/f	4	6.1	5	11.7
SCA3	66/f	4-	2.0	4	10.4
SCA3	51/m	4	6.1	5	18.3
SCA3	52/m	4	6.7	5	12.3
JMA	34/m	4-	2.0	4	10.8
JMA	38/m	4-	4.9	4	9.5
JMA	29/m	5	15.7	4-	3.1

** Clinically this patient showed generalized, not dissociated, small hand muscle atrophy.*
† thenar CMAP reference value (mean, SD): 13.4 ± 3.4
‡ hypothenar CMAP reference value (mean, SD) 12.3 ± 1.9
CMAP, compound muscle action potential; DSMA, distal spinal muscular atrophy; SCA3, spinocerebellar ataxia type 3; JMA, juvenile muscular atrophy of distal upper extremity; MRC, Medical Research Counsel examination.

Discussion

Our results support the theory that a dissociation of hand muscle atrophy, in which the thenar muscles are more affected than the hypothenar muscles, is an important clinical feature that localizes the lesion to the anterior horn. Greater thenar than hypothenar involvement is also seen in patients with T1 root involvement and in patients with lower trunk plexus involvement. However, the indefinite onset with slow progression over years in the absence of pain or sensory symptoms makes the possibility of additional radicular or plexus involvement unlikely.
DSMA, SCA3, and JMA are clinically and genetically very different disorders. Still, distal muscle atrophy and motor neuron degeneration is a common feature in all. Autosomal dominant DSMA is a rare, genetically heterogeneous, lower motor neuron disorder that may be difficult to distinguish clinically from type II Charcot-Marie-Tooth disease (De Angelis et al. 2002). SCA3 or Machado-Joseph disease (MJD) is an autosomal dominant cerebellar ataxia caused by CAG trinucleotide repeat expansions in the MJD/SCA3 gene

(Kawaguchi et al. 1994). The phenotype of the five SCA3 patients studied here is compatible with a SCA3 subtype (subtype III) (Lopes-Cendes et al. 1996), in which late onset, slow progression, cramps, fasciculations, and muscle atrophy are typical features and degeneration of anterior horn cells a common neuropathological finding (Kinoshita et al. 1995). JMA is a focal amyotrophy with unilateral or asymmetrical bilateral wasting of C7 - Th1 innervated muscles (Hirayama et al. 1963). Whereas some studies suggest that JMA is an intrinsic motor neuron disease (Schroder et al. 1999), others favor the view that the disorder result from mechanical distortion of the cervical spinal cord due to neck flexion during growth (Hirayama and Tokumaru 2000).

Since the pathophysiological mechanisms of DSMA, SCA3, and JMA are presumably very different, the observation of a 'split hand' in these diseases weakens the theory of the cortical origin of degeneration in ALS and supports the theory of an intrinsic vulnerability of spinal motor neurons subserving the thenar complex.

Neuronal intranuclear inclusions, dysregulation of cytokine expression and cell death in SCA3

B.O. Evert, H.J. Schelhaas, H. Fleischer, R.A.I. de Vos,
E.R. Brunt, L. Ozimek, K. Tolksdorf, W. Stenzel,
T. Klockgether and U. Wüllner

Chapter 7

Submitted for publication.

Abstract

We previously identified the inflammatory mediators IL-1ß, IL-1ra, IL-6 and the transcription factors IRF-1 and C/EBPdelta in a cell model of spinocerebellar ataxia type 3 (SCA3) by gene expression profiling. Here, we analyzed the expression of the respective proteins in the central nervous system of SCA3 patients in relation to neuronal cell loss and ataxin-3 positive macroscopic neuronal intranuclear inclusions (NII) using light- and electron microscopy immunohistochemistry. NII were found almost exclusively in brain regions that also showed neuronal cell loss, i.e. in pons and dentate nucleus neurons, rarely in putamen and thalamus, but not in cerebral or cerebellar cortex. NII were more readily labeled with C-terminal than with N-terminal antibodies, suggesting that C-terminal ataxin-3 fragments are the major components of NII. Quantitative analysis of NII in the pons showed that more severe neuronal cell loss was accompanied by reduced frequencies of NII. Only a higher probability of NII bearing pontine neurons to degenerate can account for this phenomenon that renders a protective role for NII in SCA3 unlikely.
The expression of IL-1ß, IL-1ra, IL-6 and C/EBPdelta was altered only in pons and dentate nucleus neurons, but was not restricted to neurons with NII, suggesting that although NII and transcriptional changes coincide, macroscopic NII are not a prerequisite for transcriptional changes. No generalized upregulation of cytokines or microglia reaction was observed in SCA3 brains, ruling out the possibility that enhanced cytokine expression was an early and generalized event mediating neuronal dysfunction in SCA3.

Introduction

Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease is the most common dominantly inherited ataxia. Clinically, SCA3 is characterized by progressive ataxia in combination with various non-cerebellar symptoms including oculomotor abnormalities, spasticity, basal ganglia symptoms, peripheral neuropathy and cognitive impairment (Burk et al. 1996; Durr et al. 1996). The SCA3 gene encodes ataxin-3, a protein of yet unknown functions (Albrecht et al. 2003; Scheel et al. 2003). Although ataxin-3 is ubiquitously expressed, neuronal death preferentially occurs in distinct brain regions including the pontine nuclei and the dentate nucleus (Paulson et al. 1997a; Schmidt et al. 1998). Like Huntington's disease (HD), dentatorubropallidoluysian atrophy (DRPLA), spinobulbar muscular atrophy (SBMA) and other spinocerebellar ataxias, SCA3 is caused by a translated unstable CAG trinucleotide repeat expansion, resulting in a polyglutamine expansion in the respective proteins (Cummings and Zoghbi 2000). The expansion of the polyglutamine stretch leads to conformational changes of the protein with subsequent oligomerisation and formation of amyloid-like fibrils (Paulson et al. 1997b; Scherzinger et al. 1997). In SCA3 and many other polyglutamine disorders, neuronal intranuclear inclusions (NII) containing aggregated mutated polyglutamine, ubiquitin and various other proteins have been found (Paulson et al. 1997b; Cummings et al. 1999). While initially NII were believed to be a prerequisite of neurodegeneration in all polyglutamine disorders, recent data suggest a more complex picture. In HD, NII were more frequent in the cortex than in the striatum, although the striatum is the most severely affected brain region in HD (Gutekunst et al. 1999). Similarly in SCA1 and SCA2, NII were absent from Purkinje cells despite prominent involvement of Purkinje cells in these disorder (Koyano et al. 2002). Also, in a number of experimental studies in cellular and animal models of HD, SCA1 and SCA3, a dissociation of polyglutamine toxicity from macroscopic aggregate formation was found (Saudou et al. 1998; Cummings et al. 1999; Warrick et al. 1999). Thus although increased propensity to form aggregates represents a common feature of all polyglutamine disease proteins, the role of NII is far from clear. A systematic study of the distribution of NII in relation to neuronal cell death in SCA3 brains has not yet been performed.
In addition to a toxic gain of function, specific protein-protein interactions and possible impaired physiological functions of the respective proteins appear to contribute to the pathogenesis of most polyglutamine disorders (Yue et al. 2001; Zuccato et al. 2003). In SCA3, sequestration of ataxin-3 and loss of the physiological function as a potential transcriptional co-repressor may cause transcriptional dysregulation

involving both up- and down regulation of a variety of genes (Li et al. 2002). We recently found increased expression of the inflammatory cytokines IL-1ß, IL-1ra, IL-6 and the transcription factors IRF-1 and C/EBPdelta together with several other genes involved in extracellular matrix remodeling and transcriptional regulation in a cell model of SCA3 (Evert et al. 2001; Evert et al. 2003). Irregular expression of these genes could constitute an early event in the disease process and might contribute to neurological deficits prior to actual cell death (Li et al. 2002; Luthi-Carter et al. 2000; Tait et al. 1998). Alternatively, transcriptional dysregulation might be restricted to neurons committed to death. To clarify the functional significance of these findings, we examined the expression of IL-1ß, IL-1ra, IL-6, IRF-1 and C/EBPdelta in relation to cell loss, microglia infiltration and the occurrence of NII in SCA3. For our study we selected a number of brain regions including pons, dentate nucleus and pallidum that are known to be severely involved in the disease process and compared these regions with others such as the cerebellar cortex and the cerebral cortex which are believed to be neuropathologically spared in SCA3.

Materials and methods

SCA3 tissues were derived from five patients from unrelated families with genetically confirmed diagnosis (m:f = 4:1, age 54±9 years, disease duration 19±5 years, p.m. delay 15±3 hours); CAG repeat lengths in SCA3 patients were very similar, ranging from 70 to 75 (Tab. 7.1). Six individuals without a history of neurological or inflammatory disease served as control (m:f = 4:2; age 68±8 years, p.m. delay 31±14 hours). All brains were sampled at autopsy by one of us (RdV). Tissue samples of the same patients had been thoroughly studied previously (Rüb et al. 2002). For the present experiments, serial 6 µm sections were cut from formalin fixed tissue blocks of cervical and thoracic spinal cord, upper pons, oculomotor nucleus, substantia nigra, putamen, thalamus (ventral anterior, ventral posterolateral, parafascicular and mediodorsal nuclei), cerebellar cortex, dentate nucleus, frontal, occipital and insular cortex. Regularly spaced consecutive sections were analyzed using standard neuropathological staining techniques and the antibodies indicated below; in each case at least three sections per region per antigen were used for semi-quantitative densitometric and morphological measurements without counterstain using a Nikon Eclipse E800 microscope. For the cell counts in the pons, we used sections at the level of the abducens nerve, which were available from the four male cases, spaced by 30µm. At least 250 neurons were sampled for each case (290 ± 52). Digitised images at 20x magnification were collected with a Sony 3CCD digital camera and the Nikon LUCIA imaging software package was used to analyze the number, size (area) and staining intensity (mean optical density) of cytoplasm and nucleus of all neurons per given section.

Table 7.1 Patients characteristics.

Age at onset (yr.) / gender	Time to wheel-chair (yr.)	Time to death (yr.)	Repeat length	Accompanying symptoms: ataxia *plus*
30 / m	14	32	70 (23)	Bradykinesia
35 / m	20	30	70 (23)	-
35 / f	13	24	72 (20)	Neuropathy, dystonia, amyotrophy
30 / m	12	21	72 (27)	Spasticity, dystonia
23 / m	5	11	75 (20)	Bradykinesia

Light microscopy

For immunohistochemistry formalin fixed and paraffin embedded tissue was used. Regularly spaced 6 µm sections (Histobond) were hydrated in Xylol (10 min, 3x5 min), downgraded alcohols (100% 2x5 min, 96% 2x5 min, 70% 3 min) and aqua dest (2 min). For antigen retrieval sections were microwave-heated in citrate-buffer (pH 6,2) for 3x5 min at 750 W and subsequently incubated in H_2O_2 (1,5 % in methanol) for 15 min

to reduce peroxidase activity, in phosphate-buffered saline (PBS) containing 20% normal serum (species dependent of origin of secondary antibody) and 0,4% Triton X for 60 min for 1H9, IRF-1, IL-1ra, IL-6 and C/EBPdelta at room temperature; for IL-1ß and CD68 'DAKO Blocking Solution Serum Free' (DAKO Cytomation, Gastrup, Denmark) was used according to the instructions of the manufacturer. The following primary antibodies and dilutions were used: 1H9 mouse monoclonal anti- ataxin-3 antibody (1:1500; detects a 20 amino acid polypeptide at the C-terminus from E213 to L233 close to the glutamine tract (kindly provided by Y. Trottier (Trottier et al. 1998b)); mouse monoclonal anti-human CD68 (PG/M1) antibody (1:50; Dako); rabbit polyclonal anti-human IRF-1(C20) antibody (1:250; Santa Cruz Biotechnology, CA); rabbit polyclonal anti-human IL-6 (H-183) antibody (1:50; Santa Cruz); goat polyclonal anti-human IL-1ra antibody (1:15; R&D Systems, Minneapolis, MN); goat polyclonal anti-human IL-1ß antibody (1:10; R&D Systems); rabbit polyclonal anti human C/CEBPdelta antibody (1:2000; Santa Cruz).
Secondary antibodies were biotinylated anti-mouse, -rabbit or -goat dependent of origin) made in rabbit or goat (1:200; Linaris, Wertheim, Germany). Incubation with primary antibodies was performed overnight at 4°C in PBS containing 5% normal serum and 0,4% Triton X for 1H9, IRF-1, IL-1ra, IL-6 and C/EBPdelta; in 'DAKO Antibody Dilutent with background reducing components' for IL-1ß and CD68. After washing in PBS and incubation with secondary antibodies for 40 min at room temperature containing 5% normal serum and 0,4% Triton X for 1H9, IRF-1, IL-1ra, IL-6 and C/EBPdelta and in 'DAKO Antibody Dilutent with background reducing components' for IL-1ß and CD68, respectively, visualization was performed with Avidin-Biotin-Complex (Vectastain Elite Standard ABC-Kit, Linaris) for 40 min at room temperature and DAB staining for 2 min (Sigma-Aldrich, St. Louis, MO, USA).

Table 7.2 Cell loss, nuclear inclusions (NII) and staining intensity in SCA3 neurons.

	Cell loss	NII	AT-3	CD68	IRF-1	IL-1ra	Il-1ß	Il-6	C/EBPdelta
Pons	Moderate	+++	n.c.	++	n.c.	++	++	++	++
Oculomotor nuclei	Severe	+	n.c.	++	n.c.	(+)	(+)	n.c.	(+)
Substantia nigra	Moderate	(+) #	n.c.	+	n.c.	n.c.	n.c.	n.c.	n.c.
Dentate nucleus	Severe	++	n.c.	+++	n.c.	+	(+)	n.c.	+
Purkinje cells	Mild	o	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Granule cells	None	o	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Thalamus	Mild	+	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Subthalamic nucl.	Severe	o	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Putamen	Moderate	+	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	*
Pallidum (internal)	Severe	+	+	+	n.c.	*	n.c.	n.c.	n.c.
Pallidum (external)	Mild	o	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Insular cortex	None	o	n.c.	n.c.	n.c.	*	n.c.	n.c.	*
Cerebral cortex	None	o	n.c.	n.c.	n.c.	*	n.c.	n.c.	n.c.
Basal nucl. (Meynert)	None	o	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Spinal cord	Moderate	o	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.

Nuclear inclusions (NII) in more than 20 % of neurons per investigated region (+++), approximately 5 % (++), approximately 1 % (+); ataxin-3 positive Marinesco bodies were identified in the substantia nigra of SCA3 patients and controls (#).
Ataxin-3 (AT-3, IRF-1, IL-1ra, Il-1ß, Il-6, C/EBPdelta) immunoreactivity not changed (n.c.), increased (+), strongly increased (++) compared to control; (*) single strongly labeled neurons in the internal pallidum and layer V of the insular (and occipital) cerebral cortex.

Electron microscopy

For the EM studies SCA3 pontine tissue was available from two of the SCA3 patients. Pons sections of two healthy individuals without a history of neurological illness served as control. In addition to the antibodies described above, we used another C-terminal (CT1) and a N-terminal (NT1) antibody (both antibodies kindly provided by E. Wanker). NT1 is directed against aminoacid 1 to 178; CT1 against aminoacid 179 and 360; CT1 was used for double-immunogold labeling experiments with anti-ubiquitin (Tait et al. 1998b). Secondary antibodies were goat anti-rabbit- (for CT1 and NT1) or goat anti-mouse (for 1H9) gold conjugates (10 nm, 1:20; British BioCell, Cardiff, UK).

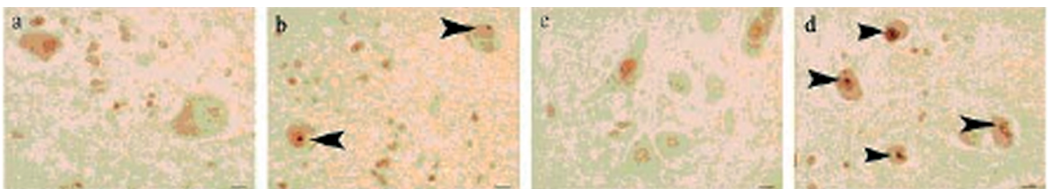
For immunogold electron microscopy the tissue was fixed overnight in 4% paraformaldehyde and 0,1% glutaraldehyde in PBS, washed 3x10 min in PBS and dehydrated in graded alcohols for 15 min each. Tissues were incubated in a 1:2 mixture of 90% alcohol and LR White acrylic (LRW) for 1 hour and embedded in pure LRW for 1 hour at room temperature and in fresh LRW overnight at 4°C. Tissues were transferred into gelatine-capsules filled with LRW for polymerization under UV-light at 4°C. Regularly spaced ultrathin sections (60 nm) were cut on a ZEISS Ultracut-Microtome and fixed on 300 mesh nickel grids. Consecutive sections were stained with the C- and N-terminal antibodies (20 sections per antibody). Ultrathin sections were rinsed on drops of 0,1% glycerine in aqua dest. for 15 min to block paraformaldehyde-structures. Unspecific binding-sites were blocked with 5% bovine serum albumin, 0,1% cold water fish skin gelatine washing buffer (CWFS) and 5% normal goat serum in PBS for 25 min. Sections were again washed in PBS containing 0,5% BSA and 0,1% CWFS and incubated with the respective primary antibodies at 4°C in wet chambers overnight. After 6x5 min washing in PBS, sections were incubated with the secondary antibody for 2 hours at room temperature, washed 6x5 min with washing buffer and 3x5 min with PBS, postfixed with 2% glutaraldehyde for 10 min, washed in PBS for 5 min and contrasted with uranyl acetate for 1 min. Examinations were performed with a Zeiss EM 900 electron microscope.

Results

Neuronal cell loss and nuclear inclusions

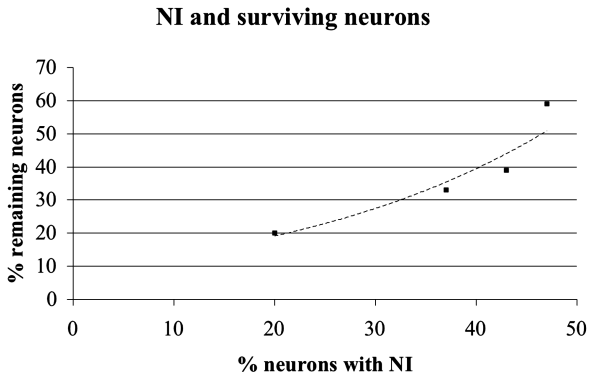
A significant loss of neurons was evident in pons and dentate nucleus and also the pallidum, the anterior horn of the spinal cord, the oculomotor nucleus and the substantia nigra of SCA3 patients (Tab. 7.2). Compared to healthy controls, the number of pontine neurons was reduced to 38 ± 14 % in SCA3. Ataxin-3-positive NII were present only in SCA3 brains and were confined to pons, dentate nucleus, putamen, thalamus and the oculomotor nucleus (Tab. 7.2, Fig. 7.1). By far the most NII were found in the pons where 37 ± 9 % of the neurons per field of view contained NII, approximately 5 % more than one. In contrast, only 4 ± 2 % of the neurons in the dentate nucleus, and less than 1 % of the neurons in the oculomotor nucleus, putamen and thalamus contained NII. In the pons, SCA3 patients with severe neuronal loss showed less

Figure 7.1 Distribution of ataxin-3-immunoreactivity.



Ataxin-3-immunoreactivity in dentate nucleus (a,b) and pontine neurons (c,d) in SCA3 patients (b, d) and controls (a, c); no significant differences were detected although we observed a tendency towards increased nuclear and decreased cytoplasmic ataxin-3 staining intensity in SCA3 patients. Neurons with NII (arrowheads) displayed both strong or weak nuclear staining intensities and similar strong or weak nuclear staining was also observed in neurons without NII. Scale bar: 10 µm.

Figure 7.2 Correlation of nuclear inclusions and surviving neurons in SCA3 pons.



x-axis: percentage of surviving neurons with NII; y-axis: percentage of surviving neurons compared to control; (—-) polynomic trendline. The percentage of NII bearing neurons was higher in patients with less severe cell loss, a finding that can be explained only if the probability of neurons with NII to die is higher.

NII-positive neurons whereas patients with less severe cell loss displayed a higher frequency of NII-positive neurons (Fig. 7.2). Ataxin-3-positive NII were not detected in any other brain region, but both SCA3 patients and controls showed ataxin-3 positive structures, most likely Marinesco bodies, in many pigmented neurons of the substantia nigra. Glial inclusions positive for ataxin-3 or cytoplasmic aggregates were not found.

We detected no consistent morphological differences between ataxin-3 positive neurons in SCA3 and control except a tendency towards smaller neuron area, which reached significance only in dentate nucleus neurons (data not shown). Similarly, we observed a tendency to increased nuclear and decreased cytoplasmic ataxin-3 staining intensity in SCA3 patients in dentate nucleus and to a lesser extent also in pontine neurons (Fig. 7.1). Interestingly, the staining intensity in the neuropil of SCA3 patients appeared also to express less ataxin-3. Analysis of the intracellular distribution of ataxin-3 in pontine neurons in SCA3 with and without NII showed that within a given patient the average area of nuclei with NII was reduced to 83 ± 6 % and the mean nuclear ataxin-3 staining intensity was increased to 124 ± 6 % compared to nuclei without NII. In the other regions however, cytoplasmic and nuclear staining intensity varied greatly, both in SCA3 patients and controls suggesting that the intracellular localization of ataxin-3 might rather reflect the functional state of individual cells but is not altered as a consequence of expression of the mutated protein.

We further examined pontine sections of SCA3 and healthy controls using immunogold electron microscopy with antibodies directed against the N-terminal (NT1) or the C-terminal (CT1, 1H9) regions of ataxin-3. With all antibodies, ataxin-3 was found at similar levels in the cytoplasm and nuclei of neurons and showed no particular association with specific subcellular structures i.e. mitochondria, ER, lysosomes or the nuclear membrane neither in control or SCA3 pons (Fig. 7.3a). However, in SCA3, only the C-terminal antibodies strongly labeled NII in pontine neurons. In adjacent sections, containing essentially the same cellular structures, the C-terminal antibody CT1 detected 12 NII in 35 neuronal cell nuclei while the N-terminal antibody NT1 labeled only 2 NII, suggesting that C-terminal fragments are the major component of NII. The frequency of NII-bearing neurons corresponded well with the lightmicroscopic exams of the same patients. Two inclusions within the same nucleus were found in 5-10 % of NII-bearing neurons. The NII displayed two distinct morphologies: the majority showed an irregular surface and appeared to bind chromatin structures or were attached to the nucleolus (Fig 7.3e). Approximately 10 % of NII were clearly separated from the nucleolus and were surrounded by a membrane- or ring like structure (Fig 7.3b,c,d). When we performed ubiquitin single- and double-labeling immunogold electron microscopy with anti-ataxin-3 antibodies (Fig. 7.3e,f), all NII were double-labeled but no distinct regional pattern of ubiquitin within the NII became evident. In contrast, promyelocytic leukemia protein (PML) was found exclusively in the outer perimeter of the inclusion (Fig 7.3d). In addition to the preferential neuronal staining pattern, the C-terminal antibodies also labeled astrocytic (GFAP-positive) fibers in SCA3 and control pons (Fig. 7.3g,h).

Expression of IL-1ß, IL-1ra, IL-6, IRF-1 and C/EBPdelta

IL-1ß is one of the most prominent cytokines in the brain and is expressed in neurons, microglia and astrocytes (Boutin et al. 2003). Constitutive expression of IL-1ß in healthy neurons is extremely low and aberrant synthesis of IL-1ß is thought to contribute to the development of acute and chronic CNS pathologies (Rothwell 2003). In healthy human controls only very weak immunostaining for IL-1ß was detected in all regions examined. In contrast, IL-1ß was strongly increased in the cytoplasm of pontine neurons in SCA3 (Fig. 7.4) while neurons of the dentate nucleus displayed a heterogeneous, less distinct staining pattern. Similarly, in the oculomotor nucleus, substantia nigra, dentate nucleus, cerebellar and occipital cortex both SCA3 patients and controls showed variable nuclear or cytoplasmic staining intensity. The known actions of IL-1ß are blocked by the selective, competitive interleukin-1 receptor antagonist (IL-1ra). No agonist action has been described for IL-1ra so far and no information on the expression of IL-1ra in the human CNS has yet been published. Complementary to IL-1ß, we found increased IL-1ra expression only in the cytoplasm of pontine and dentate nucleus neurons of SCA3 patients (Fig. 7.4). Double-labeling experiments showed no differences of IL-1ß or IL-1ra staining between neurons with or without NII and IL-1ß and IL-1ra were not sequestered into or co-localized to NII. Interestingly, we frequently found intensely IL-1ra positive neurons also in the insular and deeper layers of the occipital cortex in SCA3 patients, which were only occasionally observed in controls.

Interleukin 6 (IL-6) is another member of the IL-family and may function as a mediator of IL-1ra production to induce both neuronal survival and differentiation (Taga and Kishimoto 1997), IL-6 is expressed in neurons and increased levels of IL-6 are consistently detected in the brains of Alzheimer's disease patients (Hull et al. 1996). IL-6 was increased in the cytoplasm of pontine neurons in SCA3 (Fig. 7.4), but not in any other brain region. Again, no differences between neurons with or without NII were detected. Interferon regulatory factors (IRF's) are involved in immune regulation, cell growth and hematopoetic development; IRF-1 can contribute to induction of growth arrest and programmed cell death (Taniguchi et al. 2001). In our experiments IRF-1 showed a heterogeneous staining pattern both in SCA3 and control tissues. In SCA3 pons many neurons displayed decreased nuclear and increased cytoplasmic staining intensity; this trend, however, did not reach statistical significance. No consistent difference in the glial expression pattern was observed.

CCAAT/enhancer-binding-proteins (C/EBPs) comprise a family of transcription factors that act as pivotal regulators of cellular differentiation; in addition C/EBPs have been implicated in the regulation of IL-6-expression (Vales and Friedl 2002). The predominantly cytoplasmic immunoreactivity of C/EBPdelta was strongly increased in SCA3 pontine neurons and to a lesser extent also in the dentate nucleus (Fig.7.4). Similar to IL-1ra, single strongly labeled cells where found in the deeper layers of the insular cortex, but no such cells appeared in the occipital cortex. No sequestration into or co-localization to NII was observed.

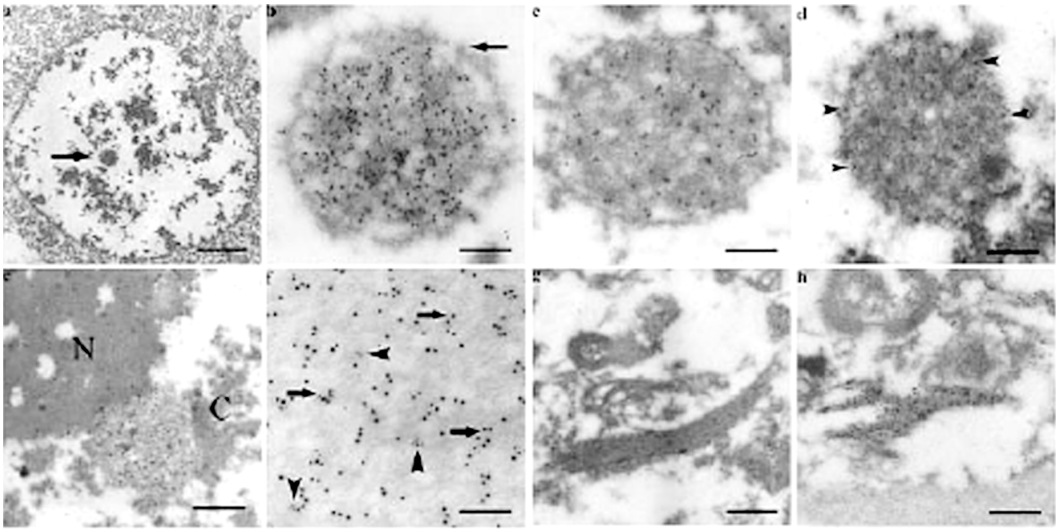
Presence of CD68-positve microglia

Increased numbers of microglia cells were present in pons and dentate nucleus and also the pallidum, and the oculomotor nucleus and the substantia nigra of SCA3 patients, i.e. in areas of moderate or severe cell loss (Tab. 7.2). Compared to healthy controls, the number of microglia cells was not altered elsewhere.

Discussion

The principal findings of this study can be summarized as follows: (1) In SCA3 brains, NII were found almost exclusively in brain regions that also showed neuronal cell loss; the by far greatest frequency of NII was found in the pons. (2) In the pons, NII were less frequent in SCA3 brains with severe neuronal cell loss, suggesting that NII-bearing neurons have a higher probability to die. (3) Ataxin-3 was found in the cytoplasm and nucleus of neurons without association with specific cellular organelles. NII were more readily labeled with C-terminal than with N-terminal antibodies suggesting that C-terminal ataxin-3 fragments are the major components of NII. (4) Increased expression of IL-1ß and related proteins was confined to the pons and dentate nucleus, two brain regions heavily involved in neurodegeneration. Within the pons,

Figure 7.3 Electronmicroscopy of SCA3 pons.



(a) pontine SCA3 neuronal nucleus with NII (arrow); bar: 3,75 µm. (b) NII from (a) at higher magnification, labeled with anti-ataxin (CT1); no immunoreactivity is found at the surrounding membrane-like structure (arrow); bar: 400 nm. (c) NII labeled with anti-ubiquitin; no immunoreactivity is found at the membrane-like structure; bar: 400 nm. (d) NII labeled with anti-PML, immunoreactivity is found only at the membrane-like structure (arrow-heads); bar: 400 nm. (e) NII with irregular surface, in contact with the nucleolus (N) and chromatin (C); double-labeling with ataxin and ubiquitin; bar: 900nm. (f) higher magnification of (e): ataxin (arrows), ubiquitin (arrowheads); bar: 200nm. (g) ataxin-labeled glial fiber in SCA3 pons, bar: 750 nm. (h) GFAP-labeled glial fiber in SCA3 pons, scale bar: 750 nm.

increased IL-1ß expression occurred both in neurons with and without NII. There was no generalized up regulation of cytokines or increased microglia in SCA3 brains, and in some brain regions undergoing severe neurodegeneration such as the pallidum, cytokine expression was not increased. These observations rule out the possibility that enhanced cytokine expression is an early and generalized event mediating neuronal dysfunction in SCA3.

NII in SCA3 brains are associated with neuronal cell death and neurodegeneration.

The frequency of NII-bearing neurons found in the present study is in good agreement with previously published data (Paulson et al. 1997b; Schmidt et al. 1998). Although the limited number of cases calls for caution, our quantitative analysis indicates that the relative proportion of NII-positive neurons in the pons decreases with progressing cell loss (Fig. 7.2). Assuming that more neurons develop NII during the disease and that the equilibrium of newly formed and (possibly) dissolved NII results in a gain in NII, a decrease of the relative proportion of NII-positive neurons with progressing cell loss fits only with a greater probability of NII-positive neurons to degenerate, suggesting that in SCA3 neurons with NII indeed have a greater likelihood to degenerate. On the contrary, if NII were protective, the relative proportion of NII-positive neurons would increase over time, the faster the higher the likelihood that neurons develop NII (a three state Markov model is available at http://www.meb.uni-bonn.de/neurologie/SCA to illustrate the process). This conclusion fits well with the observation that NII were present only in brain regions that also showed neuronal cell death. A protective role of NII in SCA3, as suggested for HD, is thus unlikely. However, it cannot be deduced from the present data whether NII in SCA3 only 'indicate' neurons that are more likely to die or are somehow implicated in the pathogenesis itself.

Ataxin-3 expression and NII

We initially hypothesized that altered localization of ataxin-3 might be responsible for the observed transcriptional changes and therefore investigated whether altered subcellular distribution of ataxin-3 in SCA3 might correlate with the observed transcriptional changes. The intracellular localization of ataxin-3 initially was reported with a predominant cytoplasmic ataxin-3 staining in various subsets of neurons and intense nuclear staining only in Purkinje cells (Trottier et al. 1998). Subsequently, various investigators found both cytoplasmic and nuclear staining of various cell types (Paulson et al. 1997a; Tait et al. 1998). Schmidt and co-workers suggested that non-expanded ataxin-3 might be present preferentially in the cytoplasm whereas expanded ataxin-3 or C-terminal fragments localized to the nucleus (Schmidt et al. 1998). Consistent with this hypothesis, neurons with NII displayed increased nuclear staining intensity, which could point to a nuclear sequestration of ataxin-3. However, using electron microscopy we found ataxin-3 in the nuclei and cytoplasm of human pontine neurons with different C- and N-terminal antibodies. While both C- and N-terminal antibodies were bound to structures in the nucleus and cytoplasm, only the C-terminal antibody consistently labeled NII. Our finding that C-terminal ataxin-3 fragments are the major components of NII is in good agreement with recent *in vitro* data showing that ataxin-3 fragments with an N-terminal deletion of 257 amino acids have the greatest tendency to aggregate (Kobayashi and Kakizuka 2003). No association of expanded ataxin-3 with specific cellular organelles was found in SCA3 pontine neurons. While the majority of NII displayed an irregular surface and appeared attached to the nucleolus, approximately 10 % were clearly separated from the nucleolus and were surrounded by a membrane- or ring like structure. An association with the nuclear matrix, as suggested earlier, was not found (Tait et al. 1998; Perez et al. 1999). Also, we found no evidence for a particular pattern of ataxin-3 and ubiquitin at the ultrastructural level within NII in SCA3. At the ultrastructural level all NII in pontine neurons appeared to include both ataxin-3 and ubiquitin (Hayashi et al. 2003). While Fujigasaki and co-workers postulated a ring-like structure of ubiquitin surrounding the NII, double-labeling immunogold electron microscopy revealed no distinct pattern of ubiquitin within the two types of NII while PML-immunoreactivity was indeed concentrated in the outer membrane-like structures as described earlier (Fujigasaki et al. 2000; Yamada et al. 2001).

Increased expression of cytokines is a specific phenomenon in SCA3 pons and dentate nucleus neurons.

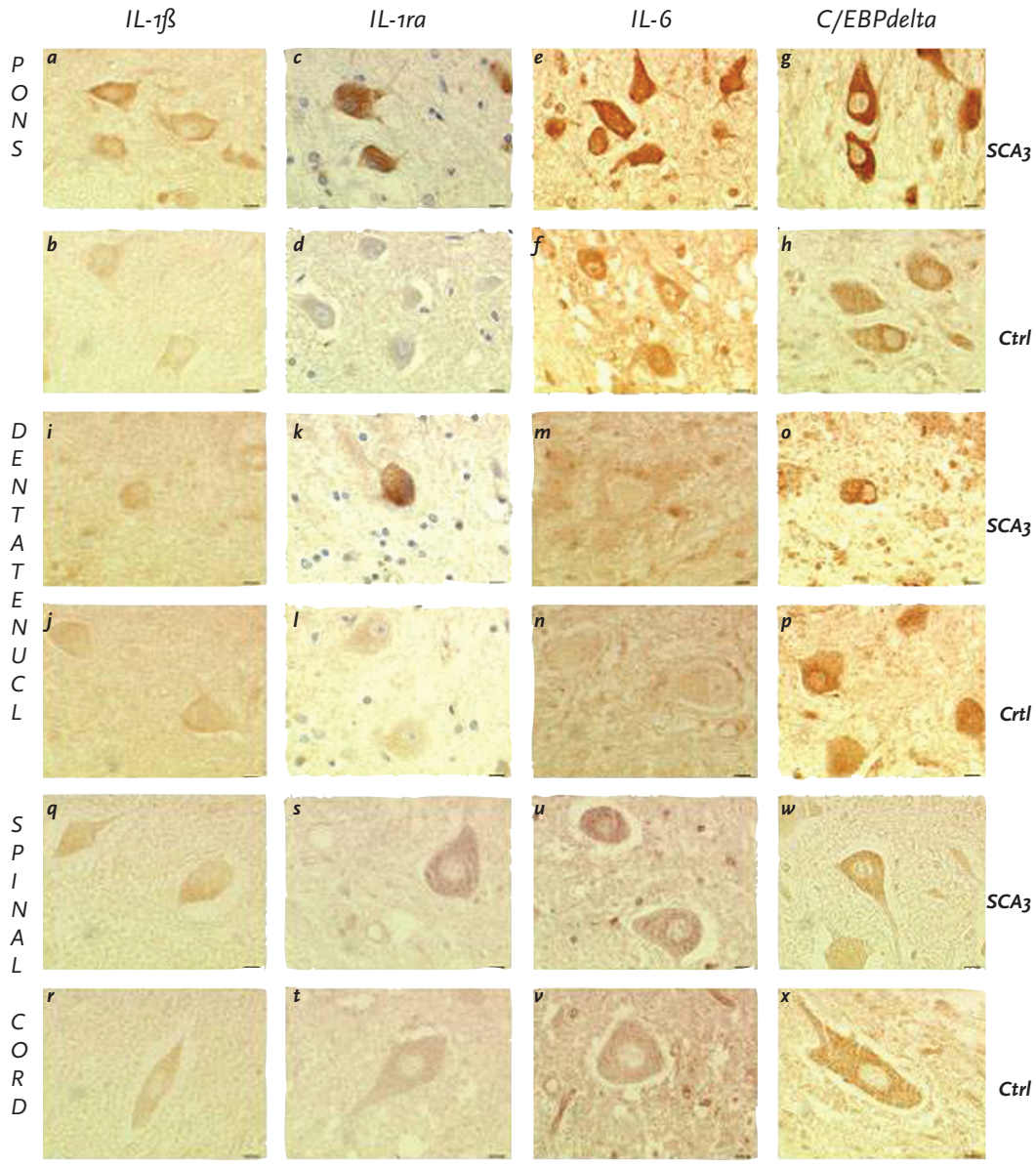
Upon identification of several differentially expressed genes related to inflammation in a cell model of SCA3, we investigated whether the expression of the respective proteins might constitute a general mechanism in SCA3. Enhanced expression of the cytokines IL-1ß, IL-1ra and IL-6 and the cytokine-inducible transcription factor C/EBPdelta could be functionally interconnected. IL-1ß, IL-1ra and IL-6, like many other genes, contain CCAAT enhancer elements in the respective promoter regions and are transcriptionally regulated by C/EBPs in response to inflammatory stimuli. Vice versa, the IL-1ß and other pro-inflammatory cytokines have been shown to induce the synthesis of the transcription factors C/EBPdelta and C/EBPdelta in astrocytes (Cardinaux et al. 2000). It was conceivable, that in vulnerable neuronal populations a vicious cycle was initiated early in the disease process. Consistent with the hypothesis that transcriptional dysregulation is involved in the pathogenesis of SCA3, we found increased cytoplasmic expression of Il-1ß, IL-1ra, IL-6 and C/EBPdelta - but confined to two brain regions severely affected by the disease process, pons and dentate nucleus and most pronounced in the pons. No changes in the expression of Il-1ß, IL-1ra, IL-6 and C/EBPdelta were found in cortical, putaminal, pallidal or nigral neurons, although the clinical presentation of the SCA3 patients examined included dystonia and parkinsonian symptoms. The lack of expression changes in areas implicated in clinically compromised functions argues against a hypothesis which implies transcriptional changes in the functional neurological deficits of SCA3 patients but rather supports the notion that an inflammatory cascade is involved in the cell death process in specific cells in SCA3. It is intriguing, that transcriptional changes and the occurrence of NII-bearing neurons was most pronounced in the pons, although comparable cell loss is also found in the pallidum or the oculomotor nucleus. Within the surviving pontine nucleus neurons no difference was detected between neurons with or without NII, suggesting that transcriptional dysregulation constitutes an event that occurs in a specific vulnerable neuronal phenotype independent or even prior to macroscopic NII.

Taken together, the present study identified occurrence of NII and enhanced cytokine expression as cellular events associated with neurodegeneration in specific affected brain regions in SCA3. Neither of the two, however, appeared to be a necessary or sufficient condition of neuronal cell death. The essential mechanisms mediating neurodegeneration in SCA3 remain to be identified.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (DFG, FOR401), and University of Bonn Grant (BONFOR). We thank Y. Trottier and E. Wanker for generous supply of antibodies and U. Rüb for critical evaluation of the manuscript.

Figure 7.4 Expression of cytokines in SCA3.



Immunoreactivity of pontine (row 1,2; a-h), dentate nucleus (row 3,4; i-p) and spinal cord neurons (row 5,6; q-x) for IL-1ß (column 1), IL-1ra (column 2), IL-6 (column 3) and C/EBPdelta (column 4); note the increased staining intensity especially in pontine SCA3 neurons (top row) compared to control (second row). Bar: 10 µm.

Neuromuscular transmission in SCA6

H.J. Schelhaas, B.P.C Van de Warrenburg,
H.P.H. Kremer and M.J. Zwarts

Chapter 8

Annals of Neurology 2004; 55: 451-452.

Spinocerebellar ataxia type 6 (SCA6) is caused by a small CAG expansion in the *CACNA1A* gene encoding the α_{1A}-subunit of the neuronal voltage-dependent P/Q-type Ca^{2+}-channel (P/Q-VGCC) (Zhuchenko et al. 1997). P/Q-VGCCs are widely expressed, not only in the central nervous system (CNS), but also at the neuromuscular junction (NMJ) (Uchitel et al. 1992). The presence of P/Q-VGCCs at motor nerve terminals, together with the demonstration that SCA6 related polyglutamine expansions may influence channel function (Piedras-Renteria et al. 2001), suggests that neuromuscular transmission might be compromised in SCA6. However, muscle weakness is not a clinical feature of SCA6. Therefore, a possible neuromuscular defect is likely to be sub-clinical and might appear as an increased jitter without blockings in single fiber electromyography (SFEMG).

Ten SCA6 patients from eight different families participated in the study. In all patients, genetic analysis revealed a CAG expansion in the *SCA6* gene of 22 repeats. In order to study both proximal and distal muscles, the variability in latency (jitter) of single-fiber action potentials was assessed in both the extensor digitorum communis muscle (4 patients) and in the orbicularis oculi muscle (6 patients). The data are shown and the results were all normal (Table 8.1). Our results confirm a preliminary observation that neuromuscular dysfunction is not a feature of SCA6 (Jen et al. 2001). These findings appear to be inconsistent with the results in patients with episodic ataxia type 2 (EA-2) (Jen et al. 2001), a disorder allelic with SCA6, but usually caused by more damaging frameshift mutations or aberrant splicing that both result in truncated proteins. Presently, the question whether SCA6 is a channelopathy due to an endogenous dysfunction of the calcium channel involved or results from a toxic gain of function attributable to the expanded polyglutamine tract, as seen in other CAG expansion disorders, remains unanswered. Either way, our study indicates that SCA6 results from CNS-specific disease pathways. Two possible mechanisms might account for the CNS-selectivity. First, the presence of pathogenic splice forms of the CACNA1A gene products may be confined to the CNS compartment. Second, interaction with tissue-specific subunits of the channel seems to be important, as a previous study suggested that interaction with the $ß_4$-subunit, which is primarily found in Purkinje cells and not in the NMJ, is crucial for the development of neuronal dysfunction (Restituito et al. 2000).

Although some believe that SCA6, EA-2, and familial hemiplegic migraine are representatives of the same disease with a large phenotypical variability, the nature of the mutation apparently results in a distinctive phenotype at the level of neuromuscular transmission.

Table 8.1 Single-fiber EMG in ten patients with spinocerebellar ataxia type 6.

	M. Ext. Dig. Com.			M. Orbicularis Oculi		
Age(y)/sex	Mean MCD (µs) (N < 25)	MCD range (µs) (N < 40)	% of pulses blocked (N = 0)	Mean MCD (µs) (N < 20)	MCD range (µs) (N < 31)	% of pulses blocked (N = 0)
62/F	24	12-38	0			
39/F	18	8-32	0			
73/F	23	12-35	0			
34/F	22	13-30	0			
27/M				17	6-29	0
33/M				20	13-26	0
56/F				18	10-29	0
58/M				16	10-23	0
49/M				16	10-27	0
69/M				18	9-29	0

M. Ext. Dig. Com., musculus extensor digitorum communis; MCD, mean consecutive difference.

Part IV

Expanding the phenotype and classification system

of the early onset cerebellar syndromes

Neurophysiological studies in the non-Friedreich early onset cerebellar ataxias (NF-EOCA)

H.J. Schelhaas, B.P.C. van de Warrenburg, M.M. Bos, C.J. Houtman,
H. Scheffer, A. Gabreëls-Festen, H.P.H. Kremer and M.J. Zwarts

Chapter 9

Submitted for publication.

Abstract

The discovery of the gene for Friedreich's ataxia (FRDA) has not only broadened the FRDA phenotype, but has also identified a group of patients with early onset cerebellar ataxia, who resemble FRDA clinically, but who do not carry a mutation in the *frataxin* gene. In order to identify subgroups that may represent a uniform underlying disorder, we performed nerve conduction studies and electromyography (EMG) in 15 patients with an early-onset, slowly progressive, non-dominant, unexplained ataxia. Fifteen patients, classified as 'non-Friedreich' early-onset cerebellar ataxia (NF-EOCA), participated in the neurophysiological studies. In addition, transcranial magnetic stimulation (TMS) studies were conducted in nine of these patients and sural nerve biopsy data were available in four patients. The neurophysiological data identified three distinctive groups of NF-EOCA patients. Three patients with normal conduction studies (group 1); three patients with a mild motor axonal neuropathy (group 2); and nine patients with a severe sensory and motor axonal neuropathy (group 3). We conclude that neurophysiological studies are useful in splitting the heterogeneous group of NF-EOCA and that, based on these studies, at least three distinctive entities can be delineated.

Introduction

Harding was the first to recognize a clinical syndrome characterized by progressive cerebellar ataxia, retained tendon reflexes, and onset before the age of 25 years (Harding 1981a). The syndrome was called early-onset cerebellar ataxia (EOCA) and was distinguished from FRDA mainly by the preservation of tendon reflexes and a much slower disease progression. Nowadays, EOCA is considered a heterogeneous syndrome that shares clinical features and, indeed, overlaps clinically with classic FRDA, atypical FRDA and other slowly progressive, early-onset, non-dominant, cerebellar syndromes in which no definite genetic or biochemical diagnosis can yet be established (Lynch et al. 2002). To prevent confusion with the original description of EOCA we prefer to use the term non-Friedreich early-onset cerebellar ataxia (NF-EOCA) to describe this heterogeneous group of patients. For FRDA, current data has shown that peripheral nerves do express the underlying pathophysiological mechanisms (Santoro et al. 1999). We hypothesized that the extent and nature of involvement of the peripheral nervous system might also allow identification of specific NF-EOCA subtypes. To test this hypothesis we performed neurophysiological studies in 15 patients with NF-EOCA.

Material and methods

Patients

After approval of this study by the ethical committee of our hospital, the medical records of all patients referred to our department and classified as 'unexplained slowly progressive cerebellar ataxia' were reviewed and 109 patients were identified. In this study the diagnosis 'NF-EOCA' was based on the following criteria: (1) progressive, unexplained cerebellar ataxia with a disease-onset before the age of 25 years, (2) family history compatible with autosomal recessive inheritance, including sporadic patients, (3) absence of mental retardation, pigmentary retinal degeneration, oculomotor apraxia, and myoclonus, (4) MRI evidence of cerebellar atrophy, (5) absence of a GAA expansion in the frataxin gene. Of the 109 patients, patients were excluded with an age at onset > 25 years, possibly indicating multi-system atrophy or spinocerebellar ataxia type 1, 2, 3, 6, 7, 14 (n = 72), mental retardation (n = 4), pigmentary retinal degeneration (n =4), and myoclonus (n = 6). Of the remaining 23 patients fulfilling our criteria of NF-EOCA, 15 patients were willing to participate in the neurophysiological studies. Nine of these patients also underwent transcranial magnetic stimulation (TMS) studies. Sural nerve biopsy data were available of four patients plus an affected brother of one patient. In all these patients, the various differential diagnostic conditions were excluded by extensive ancillary tests (vitamin E, alpha-fetoprotein, hexosaminidase A, very long-chain fatty

acids, serum albumin, ceruloplasmin, serum lactate and, on indication, muscle biopsy for histology and mitochondrial DNA analysis). We used the international ataxia co-operative rating scale (ICARS) to quantify the severity of cerebellar symptoms. The scale (rating from 0 [normal] to 100 [severe]), involves a compartmentalized quantification of postural and stance disorders, limb ataxia, dysarthria, and oculomotor abnormalities (Trouillas et al. 1997).

Nerve conduction studies and EMG recording

A Synergy EMG machine (Oxford Medical Instruments) was used to record nerve conduction and concentric needle EMG studies and these studies were performed according to standard techniques (Dumitru et al. 2002). Eleven patients underwent a predesigned protocol. In four other patients, recent previous EMG studies were re-evaluated (patient 3, 4, 7 and 10). The predesigned protocol covered motor nerve conduction studies of the median, peroneal and tibial nerve, antidromic sensory nerve conduction studies of the ulnar, radial, and sural nerve, H-reflexes and M-response recording the soleus-gastrocnemius muscle, and needle EMG of one proximal (rectus femoris), and one distal lower limb muscle (tibialis anterior or extensor hallucis). The amplitudes of the sensory nerve action potential (SNAP) and compound muscle action potential (CMAP) were measured from peak to peak.

Transcranial magnetic stimulation

In nine patients, motor evoked potentials (MEPs) were evoked by a Magstim 200 (2.0 Tesla) magnetic stimulator and recorded from the abductor digiti minimi muscle (ADM) of the left hand and from the vastus medialis muscle (VM) of the left leg. The stimulator was fitted by a circular 70mm coil and stimulation intensity was set at 100%. Cervical and lumbar motor roots were stimulated over the seventh cervical spinal level or lumbar region, respectively. Two subsequent stimuli were given at each stimulation site. During cortex stimulation the target muscle was contracted slightly. The central motor conduction time (CMCT) was calculated by subtracting the shortest latency of the MEP in response to root stimulation from the shortest latency of the MEP in response to cortical stimulation. Signals were amplified and filtered between 10 Hz and 1 KHz. Two pulses were delivered in order to check their reproducibility. Both the absence of a MEP to cortical stimulation in one of more muscles and a CMCT 2 SD beyond the mean of our normal values were considered abnormal.

Nerve Biopsy

Sural nerve biopsy had been performed in four patients, 19 to 29 years before the present electrophysiological study, and in an affected brother of one patient. Midcalf sural nerve biopsy was prepared for light and electron microscopic examination, including teased fiber studies, using standard techniques (Gabreëls-Festen et al. 1992). The specimen for electron microscopy was fixed in buffered 2% glutaraldehyde at pH 7.4, postfixed in buffered 2% osmium tetroxide, dehydrated and embedded in epoxy resin. Semi-thin, 1 µm sections were stained with 1% toluidin blue. Ultra thin sections were stained with uranyl acetate and lead citrate. Eight to ten randomly chosen electron microscopical prints (x 1700) were used for morphometrical analysis. Enlargements were checked with the aid of a rating grid. Density and diameter distribution of myelinated fibers were determined using a Zeiss TGZ particle size analyzer.

Molecular analysis

DNA was isolated from peripheral blood using the high salt/ chloroform extraction method. Mutation analysis of the gene responsible for autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS or *SACs*), covering mutations 6594delT and 5254C→T, was carried out as previously described by automated direct sequencing (Richter et al. 1999). The *APTX* gene, encoding aprataxin and responsible for ataxia with oculomotor apraxia type 1 (AOA1), was analyzed as described previously (Moreira et al. 2001b).

Results

Patients

The clinical features are summarized in Table 9.1. Eight male and seven female patients participated in the study (mean age 39.5, standard deviation 10.0, range 23-53). In seven subjects of five different families, family history strongly suggested an autosomal recessive inheritance (consanguinity or two affected sibs with unaffected parents). The other eight patients were the only affected members of their family. The age at onset varied between 0 and 19 years (mean 10.1 ± 6.4 years) and the duration of symptoms varied between 12 and 52 years (29.3 ± 10.5). Vibration sense at the ankles was impaired in 10 patients (patients 1, 4, 6, 8-11, 13-15). In three patients lower limb reflexes were absent while in 12 patients these were retained (patients 1-10, 12 and 14). Foot deformity was present in seven (patients 1, 7-9, 12-14), sphincter dysfunction in five (patients 7, 9, 10, 14 and 15) and mitral valve prolapse in one (patient 11). Only one patient showed a clinically relevant spastic paraparesis. The ataxia severity varied between 24 and 74 (49.1 ±15.3).

Nerve conduction and EMG

The electrophysiological data are shown in Table 9.2. Abnormal motor or sensory conduction or both were present in 12 of the 15 patients. In seven patients (7-9 and 11-14) both no CMAP's and SNAP's could be evoked in the lower extremities. In seven patients (8-12 and 14, 15) no SNAP's could be evoked in both lower and upper extremities. If elicited, the conduction velocities were sometimes slowed but were compatible with an axonal type of neuropathy. Abnormalities in both motor and sensory nerve conduction studies and EMG showed a distal-to-proximal gradient. Nerve conduction studies and EMG clearly differentiated three groups. Group one (patient 1-3) with normal conduction studies, group two (patients 4-6) consisted of patients with a relatively mild motor axonal neuropathy and group three (patients 7-15) included those with a severe sensory and motor axonal neuropathy.

Table 9.1 Clinical features in 15 patients with NF-EOCA.

Patient	Sex	Age,y	Age at onset,y	Ataxia severity (../100)*	Vibration sense	Babinski sign	Lower limb muscle atrophy	Lower limb tendon reflexes	Foot deformity	Sphincter dysfunction
1	M	23	3	57/100	-	+	-	+	+	-
2	F	34	14	30/100	+	-	-	+	-	-
3	M	44	14	49/100	+	-	-	+	-	-
4	F	36	18	48/100	-	+	+	+	-	-
5	M	53	17	31/100	+	-	+	+	-	-
6	F	31	19	24/100	-	+	+	+	-	-
7	F	26	3	48/100	+	+	+	+	+	+
8	M	42	12	54/100	-	+	-	+	+	-
9	M	49	8	54/100	-	+	-	+	+	+
10	F	32	3	56/100	-	+	+	+	-	+
11	F	46	10	57/100	-	+	+	-	-	-
12	F	30	3	26/100	+	+	-	+	+	-
13	M	41	16	58/100	-	+	+	-	+	-
14	M	53	12	71/100	-	+	+	+	+	+
15	M	52	0	74/100	-	+	+	-	-	+

* *Ataxia severity, as measured by the Ataxia Rating Scale.*

(+) = clinical feature present; (-) = clinical feature absent.

Table 9.2 Nerve conduction studies and needle EMG in 15 patients with NF-EOCA.

Patient*	CMAP (mV) ;ncv (m/s)			SNAP ((V)/ncv(m/s)				Needle EMG					
								M. Rectus femoris			M. Tibialis / M.extensor hall.		
	Median nerve	Peroneal nerve	Tibial nerve	Median n (dig. IV)	Ulnar n (dig V)	Radial nerve	Sural nerve	Dur (ms)	Amp (mV)	Sp	Dur (ms)	Amp (mV)	Sp
Normal	>10;>50	>5;>40	>10;>40	>10;>50	>10;>50	>13;>50	>5;>40	10-15	<2	-	10-15	<2	-
1	12.5;57	6.0;52	16.4;46	12.5;52	19.4;52	27.6;61	6.6;65	10-15	0.5-2	-	5-15	0.5-2	-
2	17.2;60	6.7;49	n.p	23.2;48	24.4;49	n.p	6.5;42	10-15	0.5-2	-	5-15	0.5-2	-
3	n.p	11.7;42	13,7;48	n.p	n.p	n.p	7.0;55	10-15	0.5-2	-	5-15	0.5-2	-
4	12.9;55	2.1;43	5.9;45	n.p	n.p	24.6;51	8.6;45	10-20	3-4	-	10-20	4-6	-
5	8.3;50	4.8;49	3.0;40	14.7;47	10.0;34	17.5;53	7.5;50	10-20	3-5	-	10-20	4-6	-
6	9.0;56	0.2;40	0.8;37	18.5;48	15.1;46	19.8;57	6.0;44	10-20	2-4	-	10-20	5-7	-
7	11.9;44	n.r	n.r	4.6;39	6.1;35	3.2	n.r	10-20	2-4	-	10-20	4-7	-
8	6.3;45	n.r	n.r	n.r	n.r	n.r.	n.r	10-20	2-4	-	10-20	4-7	+
9	7.8;37	n.r	n.r	n.r	n.r	n.r	n.r	10-20	2-4	-	n.v.c	n.v.c	++
10	8.7;45	1.8;37	n.r	n.p	n.r	n.r	n.r	n.v.c	n.v.c	-	10-20	4-7	+
11	0.2;32	n.r	n.r	n.r	n.r	n.r	n.r	n.p	n.p	-	n.v.c	n.v.c	++
12	17.2;43	n.r	n.r	n.r	n.r	n.r	n.r	n.p	n.p	-	10-20	4-7	+
13	0.5;36	n.r	n.r	n.r	n.r	2.8;30	n.r	n.v.c	n.v.c	-	10-20	4-7	+
14	7.8;37	n.r	n.r	n.r	n.r	n.r	n.r	10-20	4-6	-	10-20	4-7	++
15	12.5;41	0.6;31	0.7;32	n.r	n.r	n.r	n.r	10-20	4-6	+	10-20	4-7	+

**The number of patients from table 9.2 align with those of table 9.1.*
CMAP, compound muscle action potential; SNAP, sensory nerve action potential; SP, fibrillations and positive sharp waves; n.r, no response; n.p, not performed; n.v.c, no voluntary contraction.
- = absent; + = mild; ++ = moderate; +++ = severe.

Transcranial magnetic stimulation

MEPs in response to cervical radicular stimulation in nine patients revealed abnormal latencies in eight (Table 9.3) and MEPs in response to lumbar radicular stimulation (data not shown) were delayed in two. MEPs elicited by cortical stimulation in both ADM and VM were abnormal in all patients examined. The CMCT elicited from the ADM was abnormal in eight of nine patients examined (normal in patient 5) and the CMCT elicited from the VM were abnormal in all.

Table 9.3 Transcranial magnetic stimulation.

Patient *	Abductor digiti minimi latency (ms)			Vastus medialis latency (ms)		
	Root	Cortical	CMCT	Root	Cortical	CMCT
Normal **	< 16	< 23.5	< 9	< 13.5	< 26	< 15.5
1	14.8	n.r	n.r	8.7	n.r	n.r
5	17.7	26.1	8.4	11.7	31.4	19.7
7	17.5	36.9	19.4	11.0	n.r	n.r
8	22.8	37.1	14.3	14.2	n.r	n.r
9	19.2	32.5	13.3	11.7	n.r	n.r
11	22.3	34.1	11.9	13.5	32.8	20
12	17.2	38.5	21.3	8.7	n.r	n.r
14	18.4	n.r	n.r	12.4	n.r	n.r
15	16.9	41.5	25.5	12.2	n.r	n.r

**The number of patients from table 9.3 align with those of table 9.1.*
***mean + 2 SD*
CMCT, central motor conduction time; n.r, no response.

Sural nerve biopsy

In four patients a sural nerve biopsy was performed 19 to 29 years before the present electrophysiological study (Table 9.4). A fifth sample had been obtained in a five years younger brother of patient 11, who showed an identical clinical syndrome. Myelinated fiber density was in the normal range or slightly decreased, except in patient 13, who showed a severe loss of myelinated fibers. The percentage of large diameter fibers (< 8µm) was decreased, except in patient 14. In three patients some active axonal degeneration was observed, especially in the biopsy of patient 11. Few clusters of small regenerated fibers were present in patient 13.

Table 9.4 Sural nerve biopsy.

Patient *	Age at biopsy	MF density/mm^2	% MF > 8µm	Details
9	20 y	8800	2.4	-
11	19 y	8180	9.9	AxD ++
brother of pat. 11	14 y	12040	8.1	AxD +
13	20 y	2370	2.4	AxD ±, clusters ±
15	23 y	8740	24.1	-

**The number of patients from table 9.4 align with those of table 9.1.*
MF density, myelinated fiber density, normal for age 11-31 year: 10060 fibers/mm^2.
% myelinated fibers > 8µm, normal for age 11-31 year; 23.4 %.
AxD, active axonal degeneration, ± incidental, + mild, ++ moderate.

Molecular analysis

Molecular analysis excluded a 6594delT and 5254C→T mutation in the SACs gene in all patients. Sequencing of exons 5, 6 and 7 of the *APTX* gene, responsible for AOA1, did not reveal a mutation in any of the patients with abnormal nerve conduction studies.

Discussion

In the present study we performed nerve conduction and EMG studies in 15 patients with NF-EOCA. The clinical and neurophysiological data clearly differentiated three phenotypes. Group one, with retained tendon reflexes and normal conduction studies, group two, with an onset in the second decade, absence of foot deformities, and a mild motor axonal neuropathy, and group three, with pyramidal features and a severe sensory and motor axonal neuropathy. None of our patient displayed a pure sensory neuropathy as encountered in classical FRDA, infantile-onset spinocerebellar ataxia, and ataxia with isolated vitamin E deficiency. The patients in groups 1, 2 and 3 did not differ significantly in age, disease duration, or ICARS score which suggests that the neurophysiological differences did not, purely, reflect differences in disease progression. The presence and type of neuropathy has differential diagnostic consequences and, therefore, might guide additional tests (see discussion and the end of this thesis). The nerve conduction studies of the three patients from group two, that consist of a reduction of the CMAPs, with a distal to proximal gradient, suggests motor neuropathy rather than involvement of spinal motor neurons. In these patients, the differential diagnostic possibilities include ataxia teleangiectasia and hexosaminidase deficiency (Navon et al. 1997; Larnaout et al. 1998), but additional studies ruled out these disorders. In nine patients (group three), a severe sensory and motor axonal neuropathy was demonstrated. In four of the nine patients, sural nerve biopsy was performed which showed a mild to marked loss of large myelinated fibers and several fibers in a stage of active axonal degeneration. TMS, in seven of these nine patients, confirmed the clinical suggestion of pyramidal involvement by showing a complete absence of motor evoked potentials to the musculus vastus medianus in all. In patients with both an early onset cerebellar ataxia and a severe sensory and motor axonal neuropathy, various diagnostic possibilities like, Bassen Kornzweig disease (Wichman et al. 1985), congenital disorders of glycosylation (different types) (Marquardt and Denecke 2003), cerebellar ataxia with oculomotor apraxia type 1 (AOA1) (Le, Ber et al. 2003), and type 2 (AOA2) (Le, Ber et al. 2004), should be considered. However, in our patients, clinical features, metabolic studies and DNA analysis (AOA1) made these diagnoses extremely unlikely. The combination of early onset ataxia with pyramidal features, MRI data (data not shown), nerve conduction studies and sural nerve findings (loss of large myelinated fibers) in patients of group three were not incompatible with autosomal recessive spastic ataxia of the Charlevoix-Saguenay type (ARSACS) (Peyronnaud JM et al. 1979; Bouchard et al. 1978). Unlike ARSACS however, none of our patients showed a clinical relevant spastic syndrome and non showed prominent myelinated fibers in the fundus. Although current evidence suggests that ARSACS might be much more common than originally anticipated (Gomez 2004; Criscuolo et al. 2004; Grieco et al. 2004), these data, in combination with an absence of mutations in the first two described SACs'hotspots', suggests that our patients do not suffer from ARSACS. Therefore, these patients might be classified as spinocerebellar ataxia with axonal neuropathy (SCAN). SCAN1, recently described, shares some of the clinical features of our patients, but is distinguished by the presence of pyramidal features and the absence of hypoalbuminemia-, and hypercholesterolemia (Takashima et al. 2002). Apparently, SCAN is a heterogeneous group of disorders, which might be split by the presence, or absence of pyramidal tract involvement. For now, we conclude that, on the basis of nerve conduction studies and EMG, at least three distinctive groups of patients with NF-EOCA can be delineated and that differentiation between these groups can be useful for differential diagnostic consideration. Whether this splitting also reflects a fundamental phenotypic difference and, therefore, may direct future DNA studies, remains to be established.

The clinical subtypes of degenerative early-onset cerebellar ataxia

M.M. Bos, H.J. Schelhaas, B.P.C. van de Warrenburg,
E. Sistermans, T. Kleefstra, H. Scheffer,
M.J. Zwarts and H.P.H. Kremer

Chapter 10

Submitted for publication.

Abstract

Harding was the first to recognize a clinical syndrome characterized by progressive cerebellar ataxia, retained tendon reflexes and an onset before the age of 25 years. The syndrome was called early-onset cerebellar ataxia (EOCA) and was distinguished from Friedreich's ataxia (FRDA) mainly by the preservation of tendon reflexes, a slower disease course and a better prognosis. Nowadays, EOCA is considered a heterogeneous syndrome that overlaps clinically with classic FRDA, atypical FRDA and a group of other slowly progressive early-onset hereditary cerebellar syndromes that consists of well-known clinical entities as well as a group of disorders for which a definite genetic or biochemical diagnosis cannot be established yet. To further characterize this last group, we re-evaluated the clinical features, progression, and neurophysiological findings of 191 patients that visited our outpatient clinic with a slowly progressive cerebellar ataxia over a period of nearly 10 years. In 51 patients no known metabolic or genetic disorder could be diagnosed. These patients could then be subdivided in four groups, based on their clinical phenotypic features.
Patients with an uncomplicated cerebellar syndrome (group I) or uncomplicated spinocerebellar syndrome (group II) appeared to have a slow disease progression and a good clinical outcome. In contrast, most patients with a spinocerebellar syndrome and neuropathy (group III) were wheelchair bound. The handicap of the patients with a 'complicated' spinocerebellar syndrome with additional auditory, ophthalmologic or mental impairment (group IV) was determined by both their ataxia and the other clinical features. We conclude that further differentiation within this group of EOCA patients is important for clinical counseling.

Introduction

The degenerative ataxias are a clinically and pathophysiologically heterogeneous group of neurological disorders which share the clinical feature of slowly progressive ataxia due to degeneration of the cerebellum, the spinal tracts, the peripheral nervous system and various other nervous system structures (Harding 1984; Klockgether et al. 1998). The most basic classification of this group of disorders distinguishes sporadic forms, such as multiple systems atrophy (MSA), from hereditary ataxias.
The hereditary ataxias are further subdivided into autosomal dominant cerebellar ataxias (ADCAs) and autosomal recessive forms. Since 1993, when the SCA1 CAG repeat expansion was first described (Orr et al. 1993), molecular methods have identified a large number of ADCA genotypes that now include the spinocerebellar ataxia loci SCA 1-8, and 10-26. Mutations have been identified for SCA1, 2, 3, 6, 7, 8, 10, 12, 14, 17 and fibroblast growth factor type 14 (FGF14) (http://www.gene.ucl.ac.uk/cgi-bin/nomenclature) (Schols et al. 2004). Striking differences between genotype frequencies in different countries and populations have been reported (vd Warrenburg et al. 2002; Brusco et al. 2004).
Much less is known about the genetic background of autosomal recessive ataxias. In western countries, Friedreich's ataxia (FRDA) seems to be the most common type with an estimated prevalence of about 2 per 100,000. Linkage to chromosome 9 was described in 1988 and the gene with a pathogenic GAA trinucleotide repeat expansion in intron 1 was described in 1996 (Chamberlain et al. 1988; Campuzano et al. 1996). Since then, the molecular basis has been elucidated for additional recessive Friedreich-like, as well as non-Friedreich-like slowly progressive ataxias, such as ataxia with isolated Vitamin E deficiency (AVED), autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), ataxia with oculomotor apraxia type 1 and type 2 (AOA1 and 2), infantile-onset spinocerebellar ataxia (IOSCA), spinocerebellar ataxia with axonal neuropathy (SCAN1) and ataxia teleangiectasia (See also Table 10.1) (Ouahchi et al. 1995; Richter et al. 1999; Engert et al. 2000; Moreira et al. 2001; Németh et al. 2000; Nikali et al. 1995). However, these mutations probably explain only a minority of cases in large populations, and the range of genotypes that cause recessive ataxias may be as broad as that for the dominant ataxias.
In clinical practice, many patients present with a very slowly progressive (spino) cerebellar ataxia that is not a dominant disorder or FRDA. The ataxia usually starts at an early age, deteriorates over the years, and leads to severe disability later in life. Before the *frataxin* gene was identified, many of these cases were classified

according to Harding's definition as EOCA (Harding 1981a). EOCA was distinguished from FRDA mainly by the preservation of tendon reflexes and a better prognosis (Geoffroy et al. 1976; Harding 1981a). However, the discovery of the *frataxin* gene revealed that the phenotype was broader than originally anticipated. In contrast to the original clinical criteria, mutation analysis made it possible to unambiguously diagnose Friedreich's ataxia in patients with a disease onset older than 25 years, with retained tendon reflexes, a much slower disease course and even atypical neurological features, e.g. extrapyramidal signs (Durr et al. 1996). Moreover, it is now possible to exclude a diagnosis of Friedreich's ataxia in patients presenting with a clinical phenocopy of the disorder.

Table 10.1 Current state: the autosomal recessive spinocerebellar ataxias with the gene(locus) and gene product (see also chapter 3; table 3.1).

Recessive ataxia	Gene product	Gene/locus
Ataxia teleangiectasia	ATM protein kinase	*ATM*
Ataxia teleangiectasia like disorder	Human Mre11 protein	*MRE11*
Ataxia with axonal neuropathy (SCAN 1)	TDP1	*TDP1*
Ataxia with oculomotor apraxia type 1	Aprataxin	*APTX*
Ataxia with oculomotor apraxia type 2	Senataxin	*SETX*
Ataxia with vitamin E def. (AVED)	TTP1	alpha-TTP
Charlevoix-Saquenay (ARSACS)	Sacsin	*SACs*
Early onset recessive ataxia with hearing impairment and optic atrophy	Unknown	*6p21-23*
Friedreich's ataxia type 1 (FRDA)	Frataxin	*Frataxin*
Friedreich's ataxia type 2 (FRDA 2)	Unknown	*9p23-p11*
Infantile onset spinocerebellar ataxia and sensory neuropathy (IOSCA)	Unknown	*10q23.3-q24.1*
Non-progressive infantile ataxia	Unknown	*20q11-q13*
X-linked sideroblastic anemia with ataxia	ATP-binding cassette transporter	*ABC7*

For abbreviations, see last page of this thesis.

The current clinical situation is that many slowly progressive, early-onset spinocerebellar ataxias cannot yet be classified in detail. Patients with sporadic disease may or may not have recessive disease. Many recessive genes have not yet been found, and even in the case of known genes, the complete genomic sequence that is required to identify mutations often exceeds the capacity of available DNA diagnostic services. Phenotypic recognition and classification may help to elucidate the molecular genetics of the early onset progressive cerebellar ataxias and generate adequate and targeted diagnostic strategies.
We undertook the clinical analysis and classification of clinical variability in a cohort of patients with early-onset slowly progressive degenerative cerebellar ataxias, without a known metabolic or genetic defect. We attempted to define certain subgroups that might share a common pathogenesis; a common prognosis; and to obtain evidence that would support recessive inheritance in the various subgroups. To this end we investigated a large cohort of patients with ataxia that had visited our clinic for hereditary brain disease from 1994 until 2003.

Patients and methods

We conducted a retrospective chart review of all patients with a slowly progressive cerebellar ataxia who had visited our out patient clinic, a tertiary referral centre for the Netherlands, and in whom non-degenerative causes such as multiple sclerosis, alcohol abuse or a slowly growing posterior fossa tumor had been exclu-

ded. The sampling period was from 1 January 1994 to 31 December 2002. All patients were older than 16 years when they first visited our clinic. Cases were grouped according to clinical features as obtained from clinical examination or ancillary (primarily neurophysiological) examination.

Patients

All patients with a slowly progressive cerebellar ataxia with onset after the age of 45 were excluded, as many may ultimately be diagnosed with the cerebellar subtype of multiple system atrophy (MSA) (Wenning et al. 1994; Gilman et al. 1999), a dominant ataxia, e.g. SCA6, or with idiopathic late-onset cerebellar ataxia. Whereas 'early onset' is usually 'arbitrary' defined as an age at onset before 25, we now know that autosomal recessive inheritance can also present many years after the age of 25. For this, and as little is known about the phenotype and pathogenesis of patients presenting with a slowly progressive ataxia starting after 25 and before 45 we decided not to exclude these patients. All patients were examined by one of the authors (HPHK), and underwent a comprehensive neurological examination. Pedigrees were obtained from the patients themselves and their accompanying family members, but no additional genealogical studies were performed. Ancillary tests were performed to exclude other differential diagnoses. These tests were either performed prior to referral or at our clinic, and included standard clinical chemical and hematological evaluation, creatine kinase, thyroid-stimulating hormone, total thyroxin or free thyroxin, lues serology, serum vitamin E concentration, anti-gliadin IgM serology, serum very long-chain fatty acids, serum lactate, serum albumin, alpha-fetoprotein, cholesterol and urine organic acid analysis. All patients were screened for mutations in the SCA1, 2, 3, 6 and 7 and *frataxin* genes. If oculomotor apraxia or elevated levels of alpha-fetoprotein were present, patients were screened for the AOA1 mutation. Patients with spastic ataxia and neuropathy were screened for mutations in the SACs gene, covering mutations 6594delT and 5254C→T, but the entire gene was not sequenced. In all cases, except one, 1.0 or 1.5-T magnetic resonance imaging of the brain (standard T_1 and T_2 sequences) was performed. Those patients with leuco-encephalopathy underwent additional lysosomal enzyme analysis to investigate possible metabolic storage disorders. Needle muscle biopsy was performed for histology and mitochondrial DNA analysis only in those patients suspected to have mitochondrial disease. Ophthalmological, cardiological, and otological investigations were performed as necessary. Nerve conduction studies and electromyography were performed in all but five patients.

Chart review

Clinical details were extracted from the patient files. Onset age was defined as the age at which the first motor signs became apparent, as revealed by either the patient's own recollection or the history provided by an accompanying family member. Patients were followed up for several years, at 1- or 2-year intervals.
A cerebellar syndrome was defined as the presence of cerebellar ataxia, dysarthria and eye movement disorders such as nystagmus or saccadic pursuit eye movements. Peripheral nerve disease was considered present if nerve conduction studies were abnormal, or if typical signs were found on clinical examination. Pyramidal tract lesion was defined as the presence of a Babinski's sign or, in case of a severe neuropathy with paresis of the lower leg muscles, a delayed central motor conduction time.

Results

From 1 January 1994 to 31 December 2002 we examined 191 patients with a slowly progressive cerebellar syndrome with an age at onset younger than 45 years. Of these, 129 were excluded after being diagnosed with ADCA (n=105) or FRDA (n=24). Of the remaining 62 patients, 11 had other metabolic or characterized genetic diseases: 2 Mohr-Tranebjaerg syndrome, 2 Niemann Pick type C, 1 episodic ataxia (with negative family history), 1 mevalonate kinase-deficiency, 2 mitochondrial myopathy and ataxia, 1 mitochondrial complex I deficiency, 1 Klinefelter Syndrome, and 1 multiple sclerosis (despite a referral after initial exclusion).

Sample description

The remaining 51 patients (20 women, 31 men) formed our study population. The mean age at onset was 16.5 years (median 14.0; range 0-44); mean disease duration at time of the study was 21.4 years (range 4-48). Females had an earlier disease onset (median 11.0 years, range 0-41 vs. median 16 years, range 0-44, respectively). Tables 10.2.A and 10.2.B provide the clinical details of the study population.

The presenting symptom was gait impairment in 26 patients (50.9%), speech impairment in 4 patients (7.8%), tremor in 5 patients (9.8%), dizziness in 3 patients (5.9%), hearing loss in 1 patient (2.0%) and visual impairment in 1 patient (2.0%). Delayed motor milestones were present in 8 patients (15.7%), 7 of whom had a disease onset before the age of 2 (range 9 months to 2 years). In 3 patients (5.9 %) the first signs could not be retrospectively assessed. Fourteen patients were older than 25 years at disease onset and this group presented with walking impediments (11 patients; 78.6%) or speech problems (3 patients; 21.4%).

The 51 patients came from 45 families. Twenty patients (39.2%) from 14 different families had one or more similarly affected siblings. In 5 of these 14 families we could investigate all affected family members: 4 families consisted of two affected sibs, and 1 family consisted of three affected sibs. In the remaining 9 families (and patients) only the affected index sibling was investigated; the other affected sibs with similar signs and disease course were not available for examination. Parental consanguinity was reported in two families only.

At initial examination, 15 patients were wheelchair bound (mean duration after onset till wheelchair: 14.6 years; range: from 'never been able to walk' until 34 years), while 36 were still ambulant. The mean duration from onset till examination was in the wheelchair-bound group 23.6 years, in the wheelchair-independent group 20.7 years. Only one patient died while under observation.

Table 10.2 Clinical characteristics study population/baseline details.

	Study population	%
Number	51	
Men vs. women	31 vs 20	
Mean age at onset (yr)	16.5	
Range age at onset (yr)	0-44	
Mean follow up (yr)	21.4	
With affected family member	20	39.2
Wheelchair bound	15	29.0
Presenting symptoms:		
Gait impediment	26	50.9
Tremor	5	9.8
Vision impairment	1	2.0
Dizziness	3	5.9
Motor milistone delay	8	15.7
Speech impairment	4	7.8
Hearing loss	1	2.0
Unknown	3	5.9

Clinical subtypes

The predominant features, apart from ataxia (the inclusion criterion), were peripheral nerve disease and pyramidal tract lesion. The 51 patients were grouped according to these predominant signs, culminating into four subtypes. The first group consisted of patients with an uncomplicated, pure cerebellar syndrome. Group II consisted of patients with both cerebellar ataxia and pyramidal features (uncomplicated spinocerebellar syndrome). Group III consisted of patients with both cerebellar ataxia and peripheral nerve disea-

se. This group was further subdivided into those without pyramidal tract dysfunction (group IIIA) and those with pyramidal tract dysfunction (group IIIB). Group IV consisted of patients with additional clinically distinct features (other than cerebellar ataxia, pyramidal tract dysfunction or peripheral nerve disease).

Group I: Uncomplicated cerebellar syndrome (Table 10.3).
Seven patients from seven different families showed a pure cerebellar syndrome that consisted of dysarthria, ataxia, and ocular movement disturbances, without pyramidal tract lesions, neuropathy, or other clinical signs. Gait ataxia or dysarthria were the presenting signs. Median age at onset was 19 years (range 14-41 years). None of the patients was wheelchair bound, even though the long mean disease duration of 15.1 years. None of these patients had an affected sib. No parental consanguinity was reported in any of the families.

Table 10.3 Clinical characteristics of the patients with an uncomplicated cerebellar syndrome (group I).

	Sex	Age at onset (Years)	Time from onset till study (Years)	Affected sibs	Presenting signs	Tendon reflexes	Wheelchair bound
1	M	14	26	1/7	Dysarthria, ataxia	Normal	-
2	F	41	10	1/3	Dysarthria	Absent	-
3	F	36	5	1/1	Ataxia	Vivid	-
4	F	18	18	1/3	Ataxia	Vivid	-
5	F	38	6	1/2	Ataxia	Vivid	-
6	F	14	20	1/3	Ataxia	Normal	-
7	M	19	21	1/8	Ataxia	Vivid	-

Group II: Uncomplicated spinocerebellar ataxia (Table 10.4).
Eight patients had cerebellar ataxia and a pyramidal tract lesion without clinical or neurophysiological signs of peripheral nerve disease. Median age at onset was 36.5 years (range 3-42 years). Mean disease duration at the time of the study was 13.1 years. There was one outlier in this group (nr. 2 in Table 10.4). This patient was wheelchair bound and had a very early disease onset but disease duration was not significantly different. Apart from this patient, clinical characteristics were similar. Age at onset was well over 25 years, all patients were male, and none was wheelchair bound. One patient refused an EMG, but there were no clinical signs of neuropathy (nr.1). One patient (nr. 3) had an affected family member that was not seen by us. No parental consanguinity was reported.

Table 10.4 Clinical characteristics of the patients with an uncomplicated spinocerebellar (cerebellar ataxia and pyramidal features) syndrome (group II).

	Sex	Age at onset (Years)	Time from onset till study (Years)	Affected sibs	Presenting signs	Sensory disturbance	Wheelchair bound
1	M	37	15	1/4	Ataxia	-	-
2	M	3	17	1/2	Hypertonia	+	+
3	M	41	8	2/12	Ataxia	-	-
4	M	36	4	1/3	Spasticity	-	-
5	M	40	15	1/3	Dysarthria	-	-
6	M	42	15	1/3	Ataxia	+	-
7	M	30	22	1/5	Spastic ataxia	+	-
8	M	29	9	1/2	Dysarthria, ataxia	-	-

Group III (spino) cerebellar syndrome with neuropathy (Tables 10.5A and 10.5B).
Nineteen patients had additional peripheral nerve disease. Four patients (two males and two females) suffered from cerebellar ataxia and neuropathy without pyramidal features (group IIIA). Mean age at onset was 21 years (range 17-28 years). Mean disease duration was 17.3 years. None of the patients had an affected family member and consanguinity was not reported (Table 10.5A). Although the age at onset and prognosis (none was wheelchair bound) might initially suggest a homogenous group, nerve conduction studies clearly distinguished between these patients. One patient showed a pure but severe sensory axonal neuropathy, a second patient showed a pure motor axonal neuropathy, and two patients had a fairly mild, mixed motor and sensory axonal neuropathy.

Table 10.5A Clinical characteristics of patients with cerebellar ataxia and neuropathy, no pyramidal tract lesion (subgroup IIIA).

	Sex	Age at onset (Years)	Time from onset till study (Years)	Affected sibs	Presenting signs	Neuropathy	Wheelchair bound
1	M	17	32	1/3	Ataxia	Axonal, motor	-
2	F	17	13	1/4	Ataxia	Axonal, mixed*	-
3	M	22	15	1/5	Ataxia	Axonal, sensory	-
4	F	28	9	1/2	Ataxia	Axonal, mixed*	-

* *Mixed, sensory and motor neuropathy.*

Fifteen patients suffered from a spinocerebellar syndrome (ataxia and pyramidal features) and neuropathy (group IIIB; Table 10.5B). The group consisted of seven males and eight females. Median age at onset was 3 years (range 1-16 years). Mean disease duration was 29.3 years. Nine patients were wheelchair bound (60%); mean disease duration from onset to being wheelchair bound was 17.4 years (range, 7 to 23 years) (see Table 10.5B). Nerve conduction studies, performed in all patients in this group, revealed a highly uniform picture with absent sensory nerve action potentials (SNAPs) and compound muscle action potentials (CMAPs) in the lower extremities and reduced SNAPs and CMAPs in the upper extremities. Conduction velocities were slowed, but were still compatible with an axonal type of neuropathy. Only one patient displayed a pure sensory neuropathy. In one patient (nr. 13) asymptomatic mitral valve prolapse was discovered during cardiological evaluation. The fifteen patients came from 10 separate families and 10 of the patients had an affected family member. In one case (nr. 13), the affected sibling was not seen by us. One family had three affected members (nr. 1, 2 and 3); the other families had two affected members. Consanguinity was present in 4 patients (nr. 8 and in the three affected siblings, nr. 1, 2, 3). Thus, recessive inheritance was proven in 11 of these 15 patients.

Group IV: (Spino) cerebellar syndrome with complex features
The 17 patients who had other features besides (spino) cerebellar ataxia or neuropathy were clustered in a clinically heterogeneous group, group IV. The additional features were both neurological and non-neurological (see Table 10.6). Five male and one female patients suffered from a progressive cerebellar ataxia together with myoclonus ('Ramsay-Hunt syndrome') (nr. 5, 12, 13, 14, 15 and 16). The 11 other patients in group IV could not be placed into known clinical entities. Five patients were mentally retarded and one patient suffered from hypogonadism. In 5 patients various forms of retinal degeneration were found, In 1 patient there was hyperphoria and 3 patients suffered from cataract.
Various patients showed extrapyramidal signs: blepharospasm, dystonia, torticollis (and myoclonus). Four patients suffered from epilepsy. Hearing loss was noted in 5 patients. Most patients in this group showed more than one other feature. One patient (nr. 10) died of renal failure during follow up; the renal problems were not considered to be associated with the cerebellar syndrome.

Table 10.5B Clinical characteristics of patients with a spinocerebellar (cerebellar ataxia and pyramidal features) syndrome and neuropathy (group IIIB).

	Sex	Age at onset (Years)	Time from onset till study (Years)	Affected sibs	Presenting signs	Neuropathy	Wheelchair bound
1	F	1	21	3/3	Ataxia	Axonal, mixed*	+
2	M	1	21	3/3	Ataxia	Axonal, mixed*	+
3	F	2	24	3/3	Ataxia	Axonal, mixed*	+
4	F	3	23	2/2	Ataxia		-
5	M	12	30	2/9	Ataxia	Axonal, sensory	+
6	F	3	25	1/2	Ataxia	Axonal, mixed*	+
7	M	6	33	2/9	Ataxia	Axonal, mixed*	-
8	F	3	35	1/2	Ataxia	Axonal, mixed*	-
9	M	12	37	2/3	Spastic ataxia	Axonal, mixed*	+
10	F	1	24	2/2	Spastic ataxia	Axonal, mixed*	-
11	F	1	33	1/3	Ataxia	Axonal, mixed*	+
12	M	1	45	2/3	Ataxia	Axonal, mixed*	-
13	F	10	30	2/2	Eye movement-disorders	Axonal, mixed*	+
14	M	16	20	1/3	Ataxia	Axonal, mixed*	+
15	M	8	38	1/5	Ataxia	Axonal with demyelinating features, mixed*	-

** Mixed: sensory and motor neuropathy.*

Discussion

An early onset, degenerative ataxia that is slowly progressive over many years presents a formidable challenge to clinicians in terms of diagnoses to be considered. Most, if not all, patients may suffer from hereditary disease, but once Friedreich's ataxia has been excluded (together with dominant forms) it becomes difficult to make a correct diagnosis. A number of additional recessive genotypes have been established in the recent years (Table 10.1) but the molecular diagnoses and work-up still present many practical problems. Complete sequencing, including exon / intron boundaries, exceeds the capacity of individual routine DNA diagnostic labs. We analyzed a sample of patients with early onset, over many years slowly progressive degenerative cerebellar ataxia, without or with associated neurological features. Whereas 'early onset' is usually arbitrarily defined as an age at onset before 25 (Harding 1984), we now know from Friedreich's ataxia that autosomal recessive ataxia can also present many years after the age of 25. For this reason, and in order to be as comprehensive as possible in our clinical grouping, we decided to include patients with onset up to age 45. We assumed that later onset would yield many patients with multiple systems atrophy, as well as cases with possible dominant disease.

It may be assumed that almost all patients with such early onset ataxia suffer from hereditary recessive disease, once dominant disease has been excluded. We grouped sporadic together with familial cases. Twenty out of the 51 cases had at least one affected sib, while in 1 sporadic patient parental consanguinity made recessive disease very likely. Thus, in our sample, 40 % of cases represented proven recessive cases.

Contrary to FRDA, the clinical progression and outcome of our cases appears to be relatively mild. Only one out of our 51 patients died (of a non-neurological cause), and 36 were still ambulant after many years of disease. The presence of a severe sensory and motor axonal neuropathy that was found in 15 patients had a clearly negative influence on mobility. This relatively benign course of the disease has been documented previously (Harding, 1981; Klockgether et al 1998).

Table 10.6 Clinical characteristics of the patients with cerebellar ataxia, and additional neurological and non-neurological features (group IV).

	Sex	Age at onset (Yrs)	Time from onset to study (Yrs)	Affected sibs	Presenting signs	Neuro-pathy	Babinski sign	Additional features	Wheel -chair bound
1	M	27	27	1/2	Ataxia	-	+	Retinal degeneration	-
2	M	44	25	2/5	Ataxia	Motor axonal	-	Blepharospasm	-
3	M	25	22	2/7	Eye movement disorder	n.p.	-	Hypogonadism, cataract	-
4	M	18	17	1/2	Ataxia and deafness	-	-	Hearing loss, hyperforie	-
5	M	14	4	1/3	Myoclonus mental retardation	Mixed axonal	-	Mental retardation, myoclonus, epilepsy, retinal degeneration	+
6	F	15	10	2/2	Visual loss, retardation	n.p.	+	Hearing loss, visual loss, mental retardation	-
7	F	3	29	2/2	Ataxia	Mixed axonal	+	Cataract	-
8	M	11	19	1/2	Visual loss	n.p.	-	Retinal degeneration, Hearing loss, epilepsy	+
9	M	2	26	2/2	Spastic ataxia	Mixed axonal mixed	+	Cataract, mental retardation	-
10	F	31	11	3/6	Ataxia	Mixed axonal	-	Retinitis pigmentosa, epilepsy	-
11	M	7	44	2/11	Ataxia	-	-	Retinal degeneration,	+
12	M	0	48	1/12	Mental retardation	-	-	Mental retardation, myoclonus, opticopathy	-
13	M	15	27	1/2	Myoclonus	-	-	Myoclonus, hearing loss, epilepsy, dystonia	+
14	M	16	16	2/2	Myoclonus	Mixed axonal	+	Myoclonus	-
15	F	12	10	2/2	Myoclonus	n.p.	+	Torticollis, myoclonus	-
16	M	1	18	1/2	Ataxia	-	-	Mental retardation, myoclonus	
17	F	0	25	1/3	Hypertonia	-	+	Hearing loss	

** Mixed: sensory and motor neuropathy.*

Table 10.7A tentative new classification for autosomal recessive ataxia.

I	Uncomplicated cerebellar syndrome
II	Uncomplicated spinocerebellar syndrome
III	(Spino)cerebellar syndrome with neuropathy
IIIA	Without pyramidal features
IIIB	With pyramidal features
IV	Complicated (spino) cerebellar syndrome, with or without neuropathy, and with prominent additional non-ataxic features

We grouped our patients into four subgroups, on the basis of relatively simple clinical features as well as neurophysiological studies (Table 10.7): uncomplicated cerebellar ataxia, uncomplicated spinocerebellar ataxia, (spino)cerebellar ataxia with neuropathy, and complicated forms of (spino)cerebellar ataxia. In patients with an uncomplicated, or pure, cerebellar syndrome (group I) the age at onset was relatively late, between 14 and 41 years. Progression was slow and clinical outcome remained good. None of these patients died, and all were still ambulant at the time of the first clinical examination. No family members were known to be affected. Thus, patients with an isolated and uncomplicated cerebellar ataxia and an age at onset in the second, third, or fourth decade, may represent a non-hereditary disease, akin to what has been termed Idiopathic Late Onset Cerebellar Ataxia (ILOCA; Harding 1981(c)). In patients with uncomplicated spinocerebellar syndrome (group II), the clinical characteristics were again quite distinct, apart from one outlier. As in patients with an isolated cerebellar syndrome (group I), disease progression also seemed to be slow and to lead to a relatively benign disease. Age at onset, again, was after 25 years, and none was wheelchair bound. The fact that all patients were male may represent an X-linked inheritance, although the total number of 8 patients is small and it can also be a statistical anomaly. X-linked ataxia with sideroblastic anemia should be considered in the differential diagnosis (Pagon et al. 1985), but the age at onset and the absence of anemia and mental retardation excludes this possibility.

In the patients with cerebellar ataxia and neuropathy (group III), a further differentiation was made between those without (group IIIA) and those with (group IIIB) pyramidal features. Group IIIA consisted of only four patients with highly variable nerve conduction findings. Age at disease onset ranged from 17 to 28 years. None of the patients was wheelchair bound. Group IIIB was fairly large and strikingly homogenous. Age at onset in this group was infancy to 16 years. The mean disease duration was 19 years while 60 percent of patients were wheelchair bound. This suggests that progression to disability is faster than in the other groups. Nine patients showed unambiguous recessive inheritance. Clinical features, including the severe sensory axonal neuropathy, could be compatible with autosomal recessive spastic ataxia of the Charlevoix-Saguenay type (ARSACS; Bouchard et al 1998). Unlike ARSACS, however, none of these patients showed a clinically relevant spastic paraparesis and none showed prominent retinal myelinated fibers. Subclinical mitral valve prolaps was showed in only one patient. None of the patients carried the ARSACS mutations (6594delT and 5254C→T), had hypo-albuminemia or elevated alpha-fetoprotein, or AOA1 mutations. It was not possible to sequence the entire sacs-gene, but the phenotype seemed different from the patients described with other mutations in the sacs gene (no dementia or oculomotor symptoms). AOA2 mutations were also not assessed, but none of the patients had the characteristic oculomotor disorder nor an elevated alpha-fetoprotein level. The absence of pyramidal features and the type of neuropathy clearly distinguished group IIIA from group IIIB, and this distinction may be important in the assessment of the prognosis.

The clinical features of group IV were highly diverse. The impairment of these patients was caused as much by the additional features such as loss of vision, hearing loss or mental retardation, as by the ataxia.

We conclude that four clinically different phenotypes of slowly progressive cerebellar ataxia can be distinguished. Because phenotypic characteristics such as disease onset, progression and clinical characteristics are fairly compatible at least for group I, IIB, and III it may be assumed that the patients in these subgroups share a similar pathogenesis. We cannot exclude the possibility that at least some of the patients within these groups represent cases of ARSACS, AOA1 or AOA2, but we strongly suspect that yet unknown genotypes are represented as well. The clinical differentiation between these subgroups is important for patient counseling regarding prognosis and might also be important for research. Those with an uncomplicated cerebellar syndrome that starts in the second, third or fourth decade, as well as those with a spinocerebellar ataxia without peripheral nerve disease fare definitely better than those with neuropathy or auditory, ophthalmological or mental impairment.

Acknowledgements: AOA1 mutation analysis was performed by Dr. D. Dooijer, Dept. of Clinical Genetics, Erasmus University Medical Center, Rotterdam.

Summary, discussion and conclusions

Chapter 11

Summary

This thesis presents the results of clinical and genetic research into various autosomal dominant and autosomal recessive cerebellar syndromes. The thesis starts with the recognition that 'inherited cerebellar ataxia' always has been (**Section I**; *chapter 1*) and still is a bewildering complex neurological syndrome. The aim of the study was to unravel several aspects of this complex syndrome and defined as 'expanding the knowledge regarding the pheno and genotype of the autosomal dominant and autosomal recessive spinocerebellar syndromes'. Before being able to tackle the issue of expanding knowledge a review of the literature concerning the current state of the autosomal dominant (*chapter 2*) and autosomal recessive cerebellar disorders (*chapter 3*) is necessary, and provided. Then different aspects, covering the genotype of the dominant spinocerebellar syndromes (section II), the pathofysiology of the dominant spinocerebellar syndromes (section III), and phenotype recognition of the recessive cerebellar syndromes (section III), are covered in three sections.
In **Section II**, we focused on the issue that in about thirty percent of dominant spinocerebellar syndromes a mutation cannot be found. These patients cannot be classified according to the SCA classification and both their diagnosis and prognosis remain surrounded with uncertainty. We tried to tackle this issue by searching for a new locus in a Dutch ADCA pedigree that could not be assigned to any of the known loci.
In the first chapter of this section (*chapter 4*) the clinical, neuropsychological, MRI, and neurophysiologic data of this family are described. The clinical features are dominated by a relatively mild cerebellar syndrome, frontal cognitive dysfunction and myoclonus.
In *chapter 5* we documented the linkage analysis for the above-mentioned Dutch ADCA family. No repeat expansion could be detected in the *SCA1, 2, 3, 6, 7, 8, 12, 14* and *17* genes. The remaining *SCA* loci, for which mutations still have to be identified, were excluded by 2-point linkage analysis. By further linkage studies evidence for a new locus was found on chromosome region 1p21-1q21. The new locus was assigned *SCA19*, and current evidence suggests that it may be a new SCA with a worldwide distribution (Schelhaas et al. 2004).

In **Section III**, we addressed some pathophysiological aspects regarding the dominant spinocerebellar syndromes. For this, we focused our attention on SCA3 and SCA6. SCA3 and SCA6 are the two SCAs with the highest prevalence in the Netherlands and current evidence suggests that their pathogenesis might be different. Although the neuropathology of SCA3 shows considerable variation, some generalizations can be made (*chapter 6*). For example, while the cerebral cortex is typically spared, the anterior horn is usually affected. This pattern of neurodegeneration made it possible to determine whether the clinical feature of a 'split hand' has localizing value. The 'split hand' refers to a type of atrophy by which the muscles of the lateral aspect of the hand are affected more than those of the medial aspect. Initially, the phenomenon was considered to be specific for anterior horn disease but, as the thenar and hypothenar muscles have the same segmental supply and a different cortical presentation, it could also be of cortical origin. We observed this phenomenon, both clinically and electrophysiological, in five consecutive patients with SCA3. As we were able to confirm our observation in patients with two other disorders that are characterized by a 'comparable pattern' of neurodegeneration, we concluded that muscle atrophy in a split hand distribution points to a spinal, instead of cortical, origin.

Whereas in chapter six the 'general' pathology of SCA3 was sufficient to explain an interesting clinical aspect of the disorder, in *chapter 7* the neuropathology is addressed in far more detail. The central issue in this chapter is the relation between celdeath, the presence of neuronal intranuclear inclusions (NII) and the expression of cytokines and transcription factors.
Like various other SCAs, SCA3 is caused by a translated unstable CAG trinucleotide repeat expansion. The translation of the CAG repeat in an expanded polyglutamine stretch leads to conformational changes of the protein (ataxin-3), with subsequent formation of NII. While initially, NII was considered a prerequisite for

neurodegeneration in all CAG repeat disorders, recent data suggest a more complex picture. Additional factors, such as transcriptional dysregulation, appear to contribute to the pathogenesis of these disorders. To clarify the functional significance of transcriptional dysregulation in relation to cell loss and the occurrence of NII, we investigated the expression of these factors in several regions of normal, and SCA3, human brain and spinal cord. In SCA3 brains, NII were found almost exclusively in brain regions that also showed neuronal cell loss. In the pons, NII were less frequent in SCA3 brains with severe neuronal cell loss, suggesting that NII-bearing neurons have a higher probability to die. Increased expression of IL-1ß and related proteins was confined to the pons and dentate nucleus, two brain regions heavily involved in neurodegeneration. Within the pons, increased IL-1ß expression occurred both in neurons with and without NII. There was no generalized up regulation of cytokines or increased microglia in SCA3 brains, and in some brain regions undergoing severe neurodegeneration such as the pallidum, cytokine expression was not increased. Taken together, we concluded that the occurrence of NII and enhanced cytokine expression are cellular events associated with neurodegeneration in specific affected brain regions of SCA3. Neither of the two however, appeared to be a necessary or sufficient condition for neuronal cell death.

In *chapter 8*, we focused on SCA6. SCA6 is caused by a small CAG expansion in the *CACNA1A* gene encoding the α_{1A}-subunit of the P/Q-VGCC. P/Q-VGCCs are widely expressed, not only in the CNS, but also at the neuromuscular junction. The presence of P/Q-VGCCs at motor nerve terminals suggests that neuromuscular transmission might be compromised in SCA6. If so, this could have major clinical and scientific consequences. For this, we investigated neuromuscular function in ten patients with SCA6. Abnormalities in neuromuscular transmission were not detected and thus our study indicates that SCA6 results from CNS-specific disease pathways.

In **Section IV**, we studied the phenotype of the autosomal recessive spinocerebellar syndromes.

The discovery of the *frataxin* gene and the suggestion of a worldwide occurrence of the genes for *AOA1, AOA2* and *ARSACS* suggest that a genetic classification for the autosomal recessive cerebellar syndromes is only a matter of time. Until then some guidance for a realistic clinical work up (*chapter 9*), and a clinical classification system seems essential for patient care (*chapter 10*).

In *chapter 9* we performed nerve conduction and EMG studies in 15 patients with NF-EOCA. The clinical and neurophysiological data clearly differentiated three phenotypes: group 1 with retained tendon reflexes and normal conduction studies; group 2 with an onset in the second decade, absence of foot deformities, and a mild motor axonal neuropathy; and group 3 with pyramidal and ataxic features and a severe sensory and motor axonal neuropathy. The presence and type of neuropathy has differential diagnostic consequences and, therefore, might guide additional tests. Whether this distinction also reflects a fundamental phenotypic difference and thus can be used to guide future DNA studies remains to be established.

In order to develop a useful clinical classification system for EOCA patients we re-evaluated the clinical features, progression, and neurophysiological findings of 51 EOCA patients that visited our outpatient clinic over a period of nearly 10 years (*chapter 10*). Patients with an uncomplicated cerebellar syndrome appeared to have a slow disease progression and a good clinical outcome. Most patients with cerebellar ataxia and neuropathy are wheelchair-bound and only a few are still ambulant. We concluded that in those patients with autosomal recessive ataxia, in whom extensive testing has excluded metabolic or genetic disorders, a clinical classification system is important for counseling concerning the expected prognosis.

Discussion (clinical approach)

In the last phrase of the section 'aims of the study' we promised that we would try to provide a clinical algorithm for both the autosomal dominant and autosomal recessive cerebellar disorders. Whereas the algorithms that are described in this chapter are based on acquired experience and available literature, the usefulness of the approach has not been confirmed by a prospective study. For this, the clinician should realize that these algorithms can be used for the clinical work-up but should not be looked upon as a fixed set of knowledge.

Anatomy

The afferent and efferent connections of the cerebellar cortex are topographically organized, resulting in functional specialization of different parts of the cerebellum. For example, dysfunction of the lower vermis (vestibulocerebellum) leads to truncal ataxia; spinocerebellar lesions (upper vermis and anterior parts of hemispheres) lead to unsteadiness of gait and stance; and neocerebellar damage (cerebellar hemispheres) leads to ataxia of intended limb movements. A logical first step in the management of patients with a cerebellar ataxic syndrome would be to localize the lesion. However, while knowledge of the topographical organization of the cerebellum might be helpful in localizing the lesion in focal cerebellar disease, it is only of limited value in the differential diagnosis of non-focal cerebellar disease, such as cerebellar degeneration.

Therefore, and generally accepted, the first step in the work up of patients with a hereditary cerebellar syndrome is the determination of the age at onset. While the autosomal recessive cerebellar ataxias usually start before the age of 25, the age at onset in patients with ADCAs is usually after the age of 35 (Klockgether et al. 1998). In the following clinical approach we arbitrarily choose 30 years to be the cut-off between early- and late-onset ataxia.

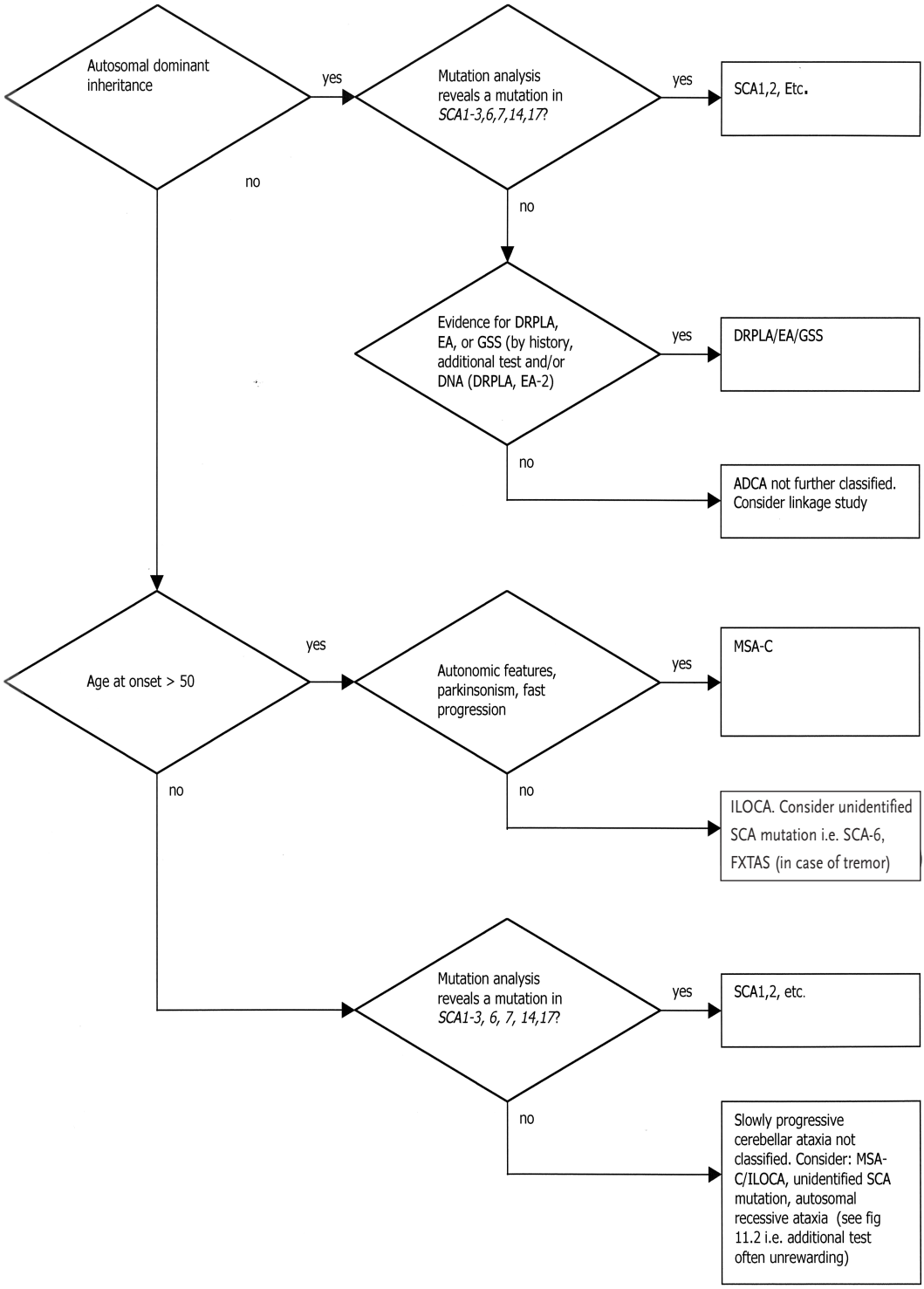
Late-onset cerebellar ataxia

In those patients with a late onset of symptoms, the first step is to establish the family history (figure 11.1). Is there evidence for an autosomal dominant disorder? In patients with a proven autosomal dominant mode of inheritance, diagnostic tests can be restricted to a small number of molecular genetic tests. If tests for SCA mutations are negative, dentatorubral-pallidoluysin atrophy (DRPLA) or Gerstmann-Straussler-Scheinkler disease (GSS) should be considered. After the exclusion of these two disorders, on the basis of clinical features, or DNA analysis (Table 11.2), available molecular tests will remain negative in up to 30% of ADCA families. If this is the case, the family probably suffers from an as yet unidentified SCA mutation. In these patients further classification will only be possible in a large pedigree that allows linkage studies. In the absence of a family history suggestive of an autosomal dominant disorder, de novo SCA mutations are not excluded. They have been described for various SCAs but they are considered rare. Therefore, in patients above 50, we would not propagate further DNA analysis. In these patients the most likely diagnosis is multiple system atrophy (MSA) or idiopathic late-onset cerebellar atrophy (ILOCA). These two disorders can be differentiated by the absence (ILOCA) or presence (MSA) of autonomic failure. In those patients with a tremor, fragile-X tremor ataxia syndrome (FXTAS) might also be considered. In patients with an age at onset between 35 and 50 we would usually perform DNA analysis to exclude SCA1, 2, 3, 6, 7, 14 and 17. Certainly at this age, a diagnosis of MSA and ILOCA can only be made 'per exclusionem'.

Early-onset cerebellar ataxia

Most cases of early-onset sporadic ataxia are manifestations of an autosomal recessive disorder. Although early-onset cerebellar ataxia may occur as an acquired disease, (Klockgether et al. 1993) or may be due to

Figure 11.1 Tentative algorithm for the clinical approach to patients with late-onset (>30 years), non-focal, slowly progressive, spinocerebellar syndromes.



maternal (mitochondrial disease), X-chromosomal (adrenoleukodystrophy) (Kurihara et al. 1993), or autosomal dominant inheritance, this is considered rare and will usually be evident from the patient's history. As a first step in the clinical approach of these patients, additional studies should be performed, the extent of which is much debated (figure 11.2). As FRDA is the most common early-onset hereditary cerebellar syndrome, even if the phenotype is not classic, we would propagate DNA analysis to exclude a GAA repeat expansion in the *frataxin* gene (Table 11.2).

In our approach nerve conduction studies are recommended as the second step in the work up of these patients. The reason for this is that the presence and type of neuropathy has important differential diagnostic consequences. The differential diagnostic considerations that are brought up by the presence or absence of neuropathy should guide the next set of additional diagnostic tests (third step). For example, in case of a severe demyelinating neuropathy, lysosomale analysis to exclude Krabbe's disease should be performed.

In the absence of a neuropathy the next step is to see whether the neurological and non-neurological features are characteristic of a specific clinical syndrome. Although most of these syndromes are clinically and genetically heterogeneous, the combination of features might provide information about the underlying cause. Examples of some, more generally accepted, clinical syndromes, together with their differential diagnoses, are given in Table 11.1.

Table 11.1 Differential diagnosis of clinical syndromes with early-onset cerebellar ataxia as a prominent feature.

Syndrome	Additional clinical symptom(s)	Differential diagnostic considerations
Behr	Optic atrophy and mental retardation	3-methylglutaconic aciduria (especially type I and type III)
Boucher Neuhauser	Hypogonadotrophic hypogonadism and choroideo-retinal degeneration	Hypocalciuric hypercalcemia. 'Mitochondrial disorders'
Holmes	Hypogonadism	'Mitochondrial disorders', infantile onset spinocerebellar ataxia
Marinesco-Sjogren	Congenital cataract, mental retardation and muscle weakness	CCFDN, 'mitochondrial disorders'
Ramsay Hunt	Myoclonus	Lafora disease, progressive myoclonus epilepsy of Unverricht-Lundborg (EPM1), MERRF, sialidosis type 1, ceroid lipofuscinosis, DRPLA

For abbreviations: see last page of this thesis. For diagnostic tests see table 11.2. For references: see also chapter 9.

this work-up does not provide a classifying diagnosis, again additional test have to be performed to exclude some treatable disorders. Examples of these are vitamin E analysis (reduced in AVED and abetalipoproteinaemia), phytanic acid (Refsum disease), ceruloplasmin (Wilson disease) and anti-gliadin/anti-endomysium antibodies (celiac disease).

Figure 11.2 Tentative algorithm for the clinical approach to patients with an early-onset (< 30 years), non-focal, slowly progressive spinocerebellar syndrome.

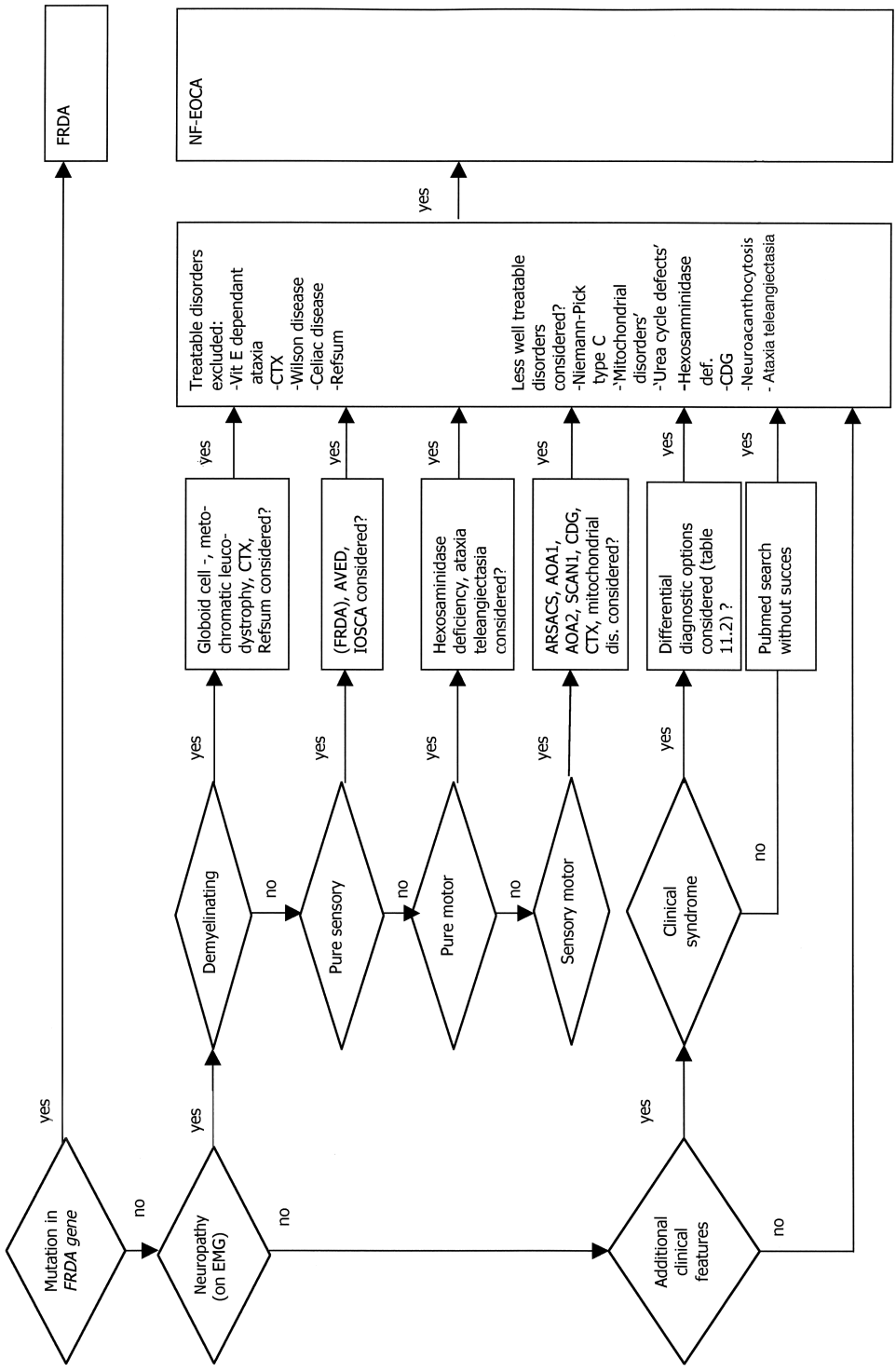


Table 11.2 Differential diagnostic considerations of hereditary cerebellar syndromes.

Syndrome	Additional prominent clinical symptom(s)	Diagnostic tests
Abetalipoproteinemia	Failure to thrive, malabsorbtion syndrome	Acanthocytes in blood smear, low total cholesterol, undetectable triglycerides, absent low-density and very low-density lipoprotein
AOA1	Oculomotor apraxia	DNA analysis
AOA2	Oculomotor apraxia	Alpha fetoprotein in serum (always ↑)
ARSACS	Spasticity, prominent myelinated fibers in the optic fundus	Combination of clinical features, DNA analysis (research)
AT	Oculomotor apraxia	↑ Alpha fetoprotein (90 percent of patients)
AVED	Friedreich-like	↓ Vit E, normal levels of vit A,D,K, triglycerides, cholesterol, DNA analysis
CCFDN	Congenital cataract, mental retardation, muscle weakness	Combination of clinical features and DNA analysis (Varon et al. 2003)
CDG	Extreme variable	Transferin isofocussing
Ceroid lipofuscinose	Psychiatric and extrapyramidal features, myoclonus	Lysosomal analysis, skin or muscle biopsy
Celiac disease	Extreme variable, gastrointestinal disturbances, neuropathy	Anti endomysisium-, anti gliadin antibodies, ↓ Vit E, intestinal biopsy
CTX	Cataract, chronic diarrhea, tendon xanthomas, psychiatric and pyramidal features	↑ cholestanol in urine
DRPLA	Autosomal dominant inheritance, myoclonus	DNA analysis
EA-1	Myokymia	DNA analysis
EA-2	Interictal nystagmus	DNA analysis
EPM1	Myoclonus	Clinical features, regional distribution (Finland), DNA analysis (Lalioti et al. 1997)
FRDA	Sensory neuropathy	DNA analysis
FXTAS	Age>50, intention tremor, cognitive decline (Hagerman et al. 2001a)	DNA analysis
GSS	Autosomal dominant inheritance, dementia	CSF: ↑ Tau, ↑ 14-3-3 protein, DNA analysis
Hexosaminidase deficiency	Motor neuron disease, cognitive impairment, dystonia	Lysosomal analysis

Syndrome	Additional prominent clinical symptom(s)	Diagnostic tests
Hypocalciuric hypercalcaemia	Hypogonadotrophic hypogonadism and choroideo-retinal degeneration	Elevation of serum calcium levels, low urinary Ca excretion, DNA analysis
IOSCA	Sensory neuropathy, hypogonadism	Diagnosis is based on the combination of clinical features and genetic background (only encountered in Finland)
Krabbe (globoid cell leukodystrophy)	Pyramidal features	Lysosomal analysis
Lafora disease	Myoclonus	Skin biopsy (lafora bodies)
3-Methylglutaconic aciduria (especially type I and type III)	Optic atrophy and mental retardation	Elevated urinary levels of 3-methylglutaconic acid, 3-methylglutaric acid and 3-hydroxyisovaleric acid
'Mitochondrial disorders'	Extreme pleiomorphic	↑ lactate, pyruvate and alanin in serum. Increased aminoaciduria, muscle biopsy
MERFF	Myoclonus, hearing loss, muscle weakness	See 'mitochondrial disorders', DNA analysis (serum)
Metachromatic leukodystrophy (adult onset)	Psychiatric features, cognitive impairment, white matter lesions	Lysosomal analysis (arylsulfatase A deficiency)
Neuroacanthocytosis	Chorea and cognitive changes	Acanthocytes in fresh blood smear
NF-EOCA	Slow progression, cerebellar atrophy on MRI	Diagnosis per exclusionem
Niemann Pick type C	Mental impairment, supranuclear vertical gaze paralysis	Lipid storage in macrophages (bone marrow aspiration, liver biopsy)
Refsum disease	Retinitis pigmentosa, anosmia, neuropathy	↑ Phytanic acid
SCAN1	Severe axonal neuropathy	↑ Cholesterol, ↓ albumin. DNA test only available in research setting
Sialidose type 1	Myoclonus, retina: 'cherry-red spot'	Lysosomal analysis
'Urea cycle defects'	Various and often related to ↑ammonium	Hyperammonemia
Vit E dependent ataxia (see also AVED, Abetalipoproteinemia)	Sensory neuropathy	↓ Vit E
Wilson	Extrapyramidal features	↓ Ceruloplasmin in serum, ↑urine copper excretion, increased hepatic enzymes

For abbreviations see last page of this thesis.

Finally, if additional tests remain negative the ultimate step is to realize that a definite diagnosis cannot be made in a considerable number of patients. These patients are usually diagnosed as early-onset cerebellar ataxia (EOCA). To prevent confusion with the original description of EOCA, we prefer to use the term NF-EOCA to describe this heterogeneous group of patients. The clinical syndrome of these patients is characterized by an early onset (first-, second-, or, rarely, third-decade), slowly progressive cerebellar syndrome (over decades), with or without pyramidal features and often, but not always, a relatively severe axonal, neuropathy. (*Chapter 9*) Typically, cerebral MRI in these patients show prominent cerebellar atrophy. Follow up of these patients is recommended as rapid progression excludes a 'diagnosis' of NF-EOCA.

Conclusions and future research

The degenerative ataxias are a clinically and pathophysiologically heterogeneous group of neurological disorders. After decades of classificatory confusion, molecular analysis has identified some of the loci and genes involved, providing the basis for a new classification. This is particularly true for the autosomal dominant spinocerebellar ataxias as about 70 percent of these families can now be classified in terms of the genetic mutation involved (SCA). For the remaining 30 percent of these families, the identification of additional loci and genes, such as the *SCA19* locus, is important in order to improve diagnostic possibilities and to provide adequate genetic counseling. The identification of new genes will undoubtedly also provide further insight into the pathophysiology of the autosomal dominant spinocerebellar ataxias.

Now the basis of the genetic classification for autosomal dominant spinocerebellar ataxias is well established, research has focused on the pathophysiological process that starts with the genetic mutation and which results in the selective degeneration. A major breakthrough came with the discovery of the distinguishing pathological hallmark of these disorders, that is, neuronal intranuclear inclusions (NII). Although an increased propensity to form aggregates is a common feature of nearly all polyglutamine disease proteins, the role of NII is still far from clear. In this thesis, we showed that the presence of NII and enhanced cytokine expression are cellular events that are associated with neurodegeneration in the specific brain regions affected in SCA3. However, neither NII nor enhanced cytokine expression appeared to be necessary or sufficient to cause neuronal cell death. Indeed, although current evidence suggests that specific polyglutamine-dependent, NII-associated, protein-protein interactions contribute to disease pathogenesis, the mechanisms underlying the neurodegeneration seen in the SCAs remain to be identified.

Finally, the ultimate step of clinical research is to look for treatment options, in this case for the degenerative ataxias. Although suggestions for treatment are often greeted with skepticism, the successful treatment of extrapyramidal features in patients with SCA2 and SCA3 (Svetal et al. 2003), the transient improvement seen in patients with SCA2 treated with zolpidem (Clauss et al. 2004), and the responsiveness of patients with SCA 6 to acetazolamine (Jenn 1998) should be considered as a first step in the right direction.

It is anticipated that a similar development will occur for the autosomal recessive cerebellar ataxias, which are suspected to show an even greater genetic heterogeneity than the autosomal dominant phenotype. At the moment, emphasis is still on clinical recognition and the update of clinical classification systems; however, the discovery of the genes for FRDA, ARSACS, AOA1, AOA2, and SCAN1 suggests that a genetic classification system will only be a matter of time. Ultimately, again in line with the ADCAs, such a genetic classification will lead to a better understanding of the underlying pathophysiology and to the development of treatment options.

References

Adamec J, Rusnak F, Owen WG, Naylor S, Benson LM, Gacy AM and Isaya G. Iron-dependent self-assembly of recombinant yeast frataxin: implications for Friedreich ataxia. *Am J Hum Genet* 67: 549-562, 2000.

Albrecht M, Hoffmann D, Evert BO, Schmitt I, Wullner U and Lengauer T. Structural modeling of ataxin-3 reveals distant homology to adaptins. *Proteins* 50: 355-370, 2003.

Allikmets R, Raskind WH, Hutchinson A, Schueck ND, Dean M and Koeller DM. Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A). *Hum Mol Genet* 8: 743-749, 1999.

Babcock M, de Silva D, Oaks R, Davis-Kaplan S, Jiralerspong S, Montermini L, Pandolfo M and Kaplan J. Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science* 276: 1709-1712, 1997.

Banfi S, Servadio A, Chung MY, Kwiatkowski TJ, Jr., McCall AE, Duvick LA, Shen Y, Roth EJ, Orr HT and Zoghbi HY. Identification and characterization of the gene causing type 1 spinocerebellar ataxia. *Nat Genet* 7: 513-520, 1994.

Bang OY, Lee PH, Kim SY, Kim HJ and Huh K. Pontine atrophy precedes cerebellar degeneration in spinocerebellar ataxia 7: MRI-based volumetric analysis. *J Neurol Neurosurg Psychiatry* 75: 1452-1456, 2004.

Barbot C, Coutinho P, Chorao R, Ferreira C, Barros J, Fineza I, Dias K, Monteiro J, Guimaraes A, Mendonca P, do Ceu MM and Sequeiros J. Recessive ataxia with ocular apraxia: review of 22 Portuguese patients. *Arch Neurol* 58: 201-205, 2001.

Bauer I, Gencik M, Laccone F, Peters H, Weber BH, Feder EH, Weirich H, Morris-Rosendahl DJ, Rolfs A, Gencikova A, Bauer P, Wenning GK, Epplen JT, Holmes SE, Margolis RL, Ross CA and Riess O. Trinucleotide repeat expansions in the junctophilin-3 gene are not found in Caucasian patients with a Huntington's disease-like phenotype. *Ann Neurol* 51: 662, 2002.

Beck AT, Ward CH, Mendelson M, Mock J and Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry* 4: 561-571, 1961.

Ben Hamida C, Doerflinger N, Belal S, Linder C, Reutenauer L, Dib C, Gyapay G, Vignal A, Le Paslier D, Cohen D and . Localization of Friedreich ataxia phenotype with selective vitamin E deficiency to chromosome 8q by homozygosity mapping. *Nat Genet* 5: 195-200, 1993.

Boder E and Sdwick RP. Ataxia-telangiectasia; a familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. *Pediatrics* 21: 526-554, 1958.

Bolthauser. Ataxia in rare metabolic disordes. In: Handbook of ataxia disorders, edited by Klockgether. New York: Marcel Dekker, Inc., 2000, p. 271-292.

Bomont P, Watanabe M, Gershoni-Barush R, Shizuka M, Tanaka M, Sugano J, Guiraud-Chaumeil C and Koenig M. Homozygosity mapping of spinocerebellar ataxia with cerebellar atrophy and peripheral neuropathy to 9q33-34, and with hearing impairment and optic atrophy to 6p21-23. *Eur J Hum Genet* 8: 986-990, 2000.

Bouchard JP, Barbeau A, Bouchard R and Bouchard RW. Autosomal recessive spastic ataxia of Charlevoix-Saguenay. *Can J Neurol Sci* 5: 61-69, 1978.

Boutin H, Kimber I, Rothwell NJ and Pinteaux E. The expanding interleukin-1 family and its receptors: do alternative IL-1 receptor/signaling pathways exist in the brain? *Mol Neurobiol* 27: 239-248, 2003.

Brais B, Bouchard JP, Xie YG, Rochefort DL, Chretien N, Tome FM, Lafreniere RG, Rommens JM, Uyama E, Nohira O, Blumen S, Korczyn AD, Heutink P, Mathieu J, Duranceau A, Codere F, Fardeau M, Rouleau GA and Korcyn AD. Short GCG expansions in the PABP2 gene cause oculopharyngeal muscular dystrophy. *Nat Genet* 18: 164-167, 1998.

Brusco A, Gellera C, Cagnoli C, Saluto A, Castucci A, Michielotto C, Fetoni V, Mariotti C, Migone N, Di Donato S and Taroni F. Molecular genetics of hereditary spinocerebellar ataxia: mutation analysis of spinocerebellar ataxia genes and CAG/CTG repeat expansion detection in 225 Italian families. *Arch Neurol* 61: 727-733, 2004.

Burck U, Goebel HH, Kuhlendahl HD, Meier C and Goebel KM. Neuromyopathy and vitamin E deficiency in man. *Neuropediatrics* 12: 267-278, 1981.

Burk K, Abele M, Fetter M, Dichgans J, Skalej M, Laccone F, Didierjean O, Brice A and Klockgether T. Autosomal dominant cerebellar ataxia type I clinical features and MRI in families with SCA1, SCA2 and SCA3. *Brain* 119 (Pt 5): 1497-1505, 1996.

Burk K, Globas C, Bosch S, Graber S, Abele M, Brice A, Dichgans J, Daum I and Klockgether T. Cognitive deficits in spinocerebellar ataxia 2. *Brain* 122 (Pt 4): 769-777, 1999.

Buxton J, Shelbourne P, Davies J, Jones C, Van Tongeren T, Aslanidis C, de Jong P, Jansen G, Anvret M and Riley B. Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. *Nature* 355: 547-548, 1992.

Campuzano V, Montermini L, Lutz Y, Cova L, Hindelang C, Jiralerspong S, Trottier Y, Kish SJ, Faucheux B, Trouillas P, Authier FJ, Durr A, Mandel JL, Vescovi A, Pandolfo M and Koenig M. Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. *Hum Mol Genet* 6: 1771-1780, 1997.

Campuzano V, Montermini L, Molto MD, Pianese L, Cossee M, Cavalcanti F, Monros E, Rodius F, Duclos F, Monticelli A, Zara F, Canizares J, Koutnikova H, Bidichandani SI, Gellera C, Brice A, Trouillas P, De Michele G, Filla A, De Frutos R, Palau F, Patel PI, Di Donato S, Mandel JL, Cocozza S, Koenig M, Pandolfo M. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 271: 1423-1427, 1996.

Cardinaux JR, Allaman I and Magistretti PJ. Pro-inflammatory cytokines induce the transcription factors C/EBPbeta and C/EBPdelta in astrocytes. *Glia* 29: 91-97, 2000.

Chai Y, Berke SS, Cohen RE, Paulson HL. Poly-ubiquitin binding by the polyglutamin diseae protein ataxin-3 links its normal function to protein surveillance pathways. *J Biol Chem* 279: 3605-3611, 2004.

Chamberlain S, Shaw J, Rowland A, Wallis J, South S, Nakamura Y, von Gabain A, Farrall M and Williamson R. Mapping of mutation causing Friedreich's ataxia to human chromosome 9. *Nature* 334: 248-250, 1988.

Chen DH, Brkanac Z, Verlinde CL. Tan XJ, Bylenok L, Nochlin D, Matsushita M, Lipe H, Wolff J, Fernandez M, Cimino PJ, Bird TD, Raskind WH. Missense mutations in the regulatory domain of PKC gamma: a new mechanism for dominant nonepisodic cerebellar ataxia. *Am J Hum Genet* 72:191-199, 2003

Chen HK, Fernandez-Funez P, Acevedo SF, Lam YC, Kaytor MD, Fernandez MH, Aitken A, Skoulakis EM, Orr HT, Botas J and Zoghbi HY. Interaction of Akt-phosphorylated ataxin-1 with 14-3-3 mediates neurodegeneration in spinocerebellar ataxia type 1. *Cell* 113: 457-468, 2003.

Christodoulou K, Deymeer F, Serdaroglu P, Ozdemir C, Poda M, Georgiou DM, Ioannou P, Tsingis M, Zamba E and Middleton LT. Mapping of the second Friedreich's ataxia (FRDA2) locus to chromosome 9p23-p11: evidence for further locus heterogeneity. *Neurogenetics* 3: 127-132, 2001.

Chung MY, Lu YC, Cheng NC and Soong BW. A novel autosomal dominant spinocerebellar ataxia (SCA22) linked to chromosome 1p21-q23. *Brain* 126: 1293-1299, 2003.

Claus R, Sathekge M, Nel W. Transient improvement of spinocerebellar ataxia with Zolpidem. *N Eng J Med* 29:511-512, 2004

Cossee M, Durr A, Schmitt M, Dahl N, Trouillas P, Allinson P, Kostrzewa M, Nivelon-Chevallier A, Gustavson KH, Kohlschutter A, Muller U, Mandel JL, Brice A, Koenig M, Cavalcanti F, Tammaro A, De Michele G, Filla A, Cocozza S, Labuda M, Montermini L, Poirier J and Pandolfo M. Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes. *Ann Neurol* 45: 200-206, 1999.

Cossee M, Schmitt M, Campuzano V, Reutenauer L, Moutou C, Mandel JL and Koenig M. Evolution of the Friedreich's ataxia trinucleotide repeat expansion: founder effect and premutations. *Proc Natl Acad Sci U S A* 94: 7452-7457, 1997.

Criscuolo C, Banfi S, Orio M, Gasparini P, Monticelli A, Scarano V, Santorelli FM, Perretti A, Santoro L, De Michele G and Filla A. A novel mutation in SACs gene in a family from southern Italy. *Neurology* 62: 100-102, 2004.

Cummings CJ, Reinstein E, Sun Y, Antalffy B, Jiang Y, Ciechanover A, Orr HT, Beaudet AL and Zoghbi HY. Mutation of the E6-AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA1 mice. *Neuron* 24: 879-892, 1999.

Cummings CJ and Zoghbi HY. Fourteen and counting: unraveling trinucleotide repeat diseases. *Hum Mol Genet* 9: 909-916, 2000.

David G, Abbas N, Stevanin G, Durr A, Yvert G, Cancel G, Weber C, Imbert G, Saudou F, Antoniou E, Drabkin H, Gemmill R, Giunti P, Benomar A, Wood N, Ruberg M, Agid Y, Mandel JL and Brice A. Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. *Nat Genet* 17: 65-70, 1997.

David G, Giunti P, Abbas N, Coullin P, Stevanin G, Horta W, Gemmill R, Weissenbach J, Wood N, Cunha S, Drabkin H, Harding AE, Agid Y and Brice A. The gene for autosomal dominant cerebellar ataxia type II is located in a 5-cM region in 3p12-p13: genetic and physical mapping of the SCA7 locus. *Am J Hum Genet* 59: 1328-1336, 1996.

Davies SW, Beardsall K, Turmaine M, DiFiglia M, Aronin N and Bates GP. Are neuronal intranuclear inclusions the common neuropathology of triplet-repeat disorders with polyglutamine-repeat expansions? *Lancet* 351: 131-133, 1998.

De Angelis MV, Gatta V, Stuppia L, Passamonti L, Gambi D and Uncini A. Autosomal dominant distal spinal muscular atrophy: an Italian family not linked to 12q24 and 7p14. *Neuromuscul Disord* 12: 26-30, 2002.

Deelman BG, Koning-Haanstra M, Liebrand WBG, and Van den Burg W. SAN test een afasie test voor auditief en mondeling taalgebruik (in Dutch). Lisse, Swets and Zeitlinger. 2004.

Dejerine-Thomas. L'atrophie olivo-ponto-cerebelleuse. Nouv Iconogr Salpet 13, 330-370. 1900.

Denier C, Ducros A, Vahedi K, Joutel A, Thierry P, Ritz A, Castelnovo G, Deonna T, Gerard P, Devoize JL, Gayou A, Perrouty B, Soisson T, Autret A, Warter JM, Vighetto A, Van Bogaert P, Alamowitch S, Roullet E and Tournier-Lasserve E. High prevalence of CACNA1A truncations and broader clinical spectrum in episodic ataxia type 2. *Neurology* 52: 1816-1821, 1999.

Deuschl G, Bain P and Brin M. Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. *Mov Disord* 13 Suppl 3: 2-23, 1998.

Dijkstra U, Gabreels F, Joosten E, Wevers R, Lamers K, Doesburg W and Renier W. Friedreich's ataxia: intravenous pyruvate load to demonstrate a defect in pyruvate metabolism. *Neurology* 34: 1493-1497, 1984.

Ducros A, Denier C, Joutel A, Vahedi K, Michel A, Darcel F, Madigand M, Guerouaou D, Tison F, Julien J, Hirsch E, Chedru F, Bisgard C, Lucotte G, Despres P, Billard C, Barthez MA, Ponsot G, Bousser MG and Tournier-Lasserve E. Recurrence of the T666M calcium channel CACNA1A gene mutation in familial hemiplegic migraine with progressive cerebellar ataxia. *Am J Hum Genet* 64: 89-98, 1999.

Ducros A, Joutel A, Vahedi K, Cecillon M, Ferreira A, Bernard E, Verier A, Echenne B, Lopez dM, Bousser MG and Tournier-Lasserve E. Mapping of a second locus for familial hemiplegic migraine to 1q21-q23 and evidence of further heterogeneity. *Ann Neurol* 42: 885-890, 1997.

Dumitru D, Amato AA and Zwarts MJ. *Electrodiagnostic medicine*. Philadelphia: Hanley and Belfus, 2002.

Durr A. Friedreich's ataxia: treatment within reach. *Lancet Neurol* 1: 370-374, 2002.

Durr A, Cossee M, Agid Y, Campuzano V, Mignard C, Penet C, Mandel JL, Brice A and Koenig M. Clinical and genetic abnormalities in patients with Friedreich's ataxia. *N Engl J Med* 335: 1169-1175, 1996.

Durr A, Stevanin G, Cancel G, Duyckaerts C, Abbas N, Didierjean O, Chneiweiss H, Benomar A, Lyon-Caen O, Julien J, Serdaru M, Penet C, Agid Y and Brice A. Spinocerebellar ataxia 3 and Machado-Joseph disease: clinical, molecular, and neuropathological features. *Ann Neurol* 39: 490-499, 1996.

Dutka DP, Donnelly JE, Nihoyannopoulos P, Oakley CM and Nunez DJ. Marked variation in the cardiomyopathy associated with Friedreich's ataxia. *Heart* 81: 141-147, 1999.

Engert JC, Berube P, Mercier J, Dore C, Lepage P, Ge B, Bouchard JP, Mathieu J, Melancon SB, Schalling M, Lander ES, Morgan K, Hudson TJ and Richter A. ARSACS, a spastic ataxia common in northeastern Quebec, is caused by mutations in a new gene encoding an 11.5-kb ORF. *Nat Genet* 24: 120-125, 2000.

Epplen C, Epplen JT, Frank G, Miterski B, Santos EJ and Schols L. Differential stability of the (GAA)n tract in the Friedreich ataxia (STM7) gene. *Hum Genet* 99: 834-836, 1997.

Escayg A, De Waard M, Lee DD, Bichet D, Wolf P, Mayer T, Johnston J, Baloh R, Sander T and Meisler MH. Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. *Am J Hum Genet* 66: 1531-1539, 2000.

Evert BO, Vogt IR, Kindermann C, Ozimek L, de Vos RA, Brunt ER, Schmitt I, Klockgether T and Wullner U. Inflammatory genes are upregulated in expanded ataxin-3-expressing cell lines and spinocerebellar ataxia type 3 brains. *J Neurosci* 21: 5389-5396, 2001.

Evert BO, Vogt IR, Vieira-Saecker AM, Ozimek L, de Vos RA, Brunt ER, Klockgether T and Wullner U. Gene expression profiling in ataxin-3 expressing cell lines reveals distinct effects of normal and mutant ataxin-3. *J Neuropathol Exp Neurol* 62: 1006-1018, 2003.

Filla A, De Michele G, Cavalcanti F, Perretti A, Santoro L, Barbieri F, D'Arienzo G and Campanella G. Clinical and genetic heterogeneity in early onset cerebellar ataxia with retained tendon reflexes. *J Neurol Neurosurg Psychiatry* 53: 667-670, 1990.

Flanigan K, Gardner K, Alderson K, Galster B, Otterud B, Leppert MF, Kaplan C and Ptacek LJ. Autosomal dominant spinocerebellar ataxia with sensory axonal neuropathy (SCA4): clinical description and genetic localization to chromosome 16q22.1. *Am J Hum Genet* 59: 392-399, 1996.

Friedreich N. Uber ataxie mit besoderer Berucksichtigung der heriditairen Formen. Virchovs Arach Pathol Anat Physiolol 70, 140-152. 1877.

Friedreich N. Uber ataxie mit besoderer Berucksichtigung der hereditaren Formen. Virchovs Arch Pathol Anat Physiol. 68, 145-245. 1876.

Friedreich N. Uber degenerative atrophie der spinalen hinterstrange. Virchovs Arach Pathol Anat Physiolol 22, 1-26. 1863.

Friedreich N. Uber degenerative atrophie der spinalen hinterstrange. Virchovs Arach Pathol Anat Physiolol 26, 391-419. 1863.

Friedreich N. Uber degenerative atrophie der spinalen hinterstrange. Virchovs Arach Pathol Anat Physiolol 26, 433-459. 1863.

Fujigasaki H, Martin JJ, De Deyn PP, Camuzat A, Deffond D, Stevanin G, Dermaut B, Van Broeckhoven C, Durr A and Brice A. CAG repeat expansion in the TATA box-binding protein gene causes autosomal dominant cerebellar ataxia. *Brain* 124: 1939-1947, 2001a.

Fujigasaki H, Verma IC, Camuzat A, Margolis RL, Zander C, Lebre AS, Jamot L, Saxena R, Anand I, Holmes SE, Ross CA, Durr A and Brice A. SCA12 is a rare locus for autosomal dominant cerebellar ataxia: a study of an Indian family. *Ann Neurol* 49: 117-121, 2001b.

Fujigasaki H, Uchihara T, Koyano S, Iwabuchi K, Yagishita S, Makifuchi T, Nakamura A, Ishida K, Toru S, Hirai S, Ishikawa K, Tanabe T and Mizusawa H. Ataxin-3 is translocated into the nucleus for the formation of intranuclear inclusions in normal and Machado-Joseph disease brains. *Exp Neurol* 165: 248-256, 2000.

Gabreëls-Festen AA, Gabreels FJ, Jennekens FG, Joosten EM and Janssen-van Kempen TW. Autosomal recessive form of hereditary motor and sensory neuropathy type I. *Neurology* 42: 1755-1761, 1992.

Gabsi S, Gouider-Khouja N, Belal S, Fki M, Kefi M, Turki I, Ben Hamida M, Kayden H, Mebazaa R and Hentati F. Effect of vitamin E supplementation in patients with ataxia with vitamin E deficiency. *Eur J Neurol* 8: 477-481, 2001.

Gambardella A, Annesi G, Bono F, Spadafora P, Valentino P, Pasqua AA, Mazzei R, Montesanti R, Conforti FL, Oliveri RL, Zappia M, Aguglia U and Quattrone A. CAG repeat length and clinical features in three Italian families with spinocerebellar ataxia type 2 (SCA2): early impairment of Wisconsin Card Sorting Test and saccade velocity. *J Neurol* 245: 647-652, 1998.

Geoffroy G, Barbeau A, Breton G, Lemieux B, Aube M, Leger C and Bouchard JP. Clinical description and roentgenologic evaluation of patients with Friedreich's ataxia. *Can J Neurol Sci* 3: 279-286, 1976a.

Gilman S, Low PA, Quinn N, Albanese A, Ben Shlomo Y, Fowler CJ, Kaufmann H, Klockgether T, Lang AE, Lantos PL, Litvan I, Mathias CJ, Oliver E, Robertson D, Schatz I and Wenning GK. Consensus statement on the diagnosis of multiple system atrophy. *J Neurol Sci* 163: 94-98, 1999.

Gispert S, Twells R, Orozco G, Brice A, Weber J, Heredero L, Scheufler K, Riley B, Allotey R, Nothers C and. Chromosomal assignment of the second locus for autosomal dominant cerebellar ataxia (SCA2) to chromosome 12q23-24.1. *Nat Genet* 4: 295-299, 1993.

Gomez CM. ARSACS goes global. *Neurology* 62: 10-11, 2004.

Grant DA and Berg EA. A behavioral analysis of degree of reinforcement and ease of shifting to new responses in a Weigl-type card sorting problem. *J Clin Exp Neuropsychol* 34, 404-411. 1948.

Greenfield JG. The spino-cerebellar degenerations. Oxford, Blackwell. 1954.

Grieco GS, Malandrini A, Comanducci G, Leuzzi V, Valoppi M, Tessa A, Palmeri S, Benedetti L, Pierallini A, Gambelli S, Federico A, Pierelli F, Bertini E, Casali C and Santorelli FM. Novel SACs mutations in autosomal recessive spastic ataxia of Charlevoix-Saguenay type. *Neurology* 62: 103-106, 2004.

Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM and Li XJ. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *J Neurosci* 19: 2522-2534, 1999.

Hagerman PJ and Hagerman RJ. The fragile-X premutation: a maturing perspective. *Am J Hum Genet* 74: 805-816, 2004.

Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B and Hagerman PJ. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology* 57: 127-130, 2001.

Hanna MG, Davis MB, Sweeney MG, Noursadeghi M, Ellis CJ, Elliot P, Wood NW and Marsden CD. Generalized chorea in two patients harboring the Friedreich's ataxia gene trinucleotide repeat expansion. *Mov Disord* 13: 339-340, 1998.

Harding AE. Clinical features and classification of inherited ataxias. *Adv Neurol* 61: 1-14, 1993.

Harding AE. Ataxic disorders associated with defective DNA repair. In: Hereditary ataxias and related disorders, edited by Harding AE. Edinburgh: Livingstone, 1984a, p. 52-56.

Harding AE. *The hereditary ataxias and related disorders*. Edinburgh: Churchill Livingstone, 1984b.

Harding AE. Classification of the hereditary ataxias and paraplegias. *Lancet* 1: 1151-1155, 1983.

Harding AE. The clinical features and classification of the late onset autosomal dominant cerebellar ataxias. A study of 11 families, including descendants of the 'the Drew family of Walworth'. *Brain* 105: 1-28, 1982a.

Harding AE, Muller DP, Thomas PK and Willison HJ. Spinocerebellar degeneration secondary to chronic intestinal malabsorption: a vitamin E deficiency syndrome. *Ann Neurol* 12: 419-424, 1982b.

Harding AE. Early onset cerebellar ataxia with retained tendon reflexes: a clinical and genetic study of a disorder distinct from Friedreich's ataxia. *J Neurol Neurosurg Psychiatry* 44: 503-508, 1981a.

Harding AE. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain* 104: 589-620, 1981b.

Hardy J and Gwinn-Hardy K. Genetic classification of primary neurodegenerative disease. *Science* 282: 1075-1079, 1998b.

Hardy J and Gwinn-Hardy K. Genetic classification of primary neurodegenerative disease. *Science* 282: 1075-1079, 1998a.

Harley HG, Brook JD, Rundle SA, Crow S, Reardon W, Buckler AJ, Harper PS, Housman DE and Shaw DJ. Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. *Nature* 355: 545-546, 1992.

Hayashi M, Kobayashi K and Furuta H. Immunohistochemical study of neuronal intranuclear and cytoplasmic inclusions in Machado-Joseph disease. *Psychiatry Clin Neurosci* 57: 205-213, 2003.

Helmlinger D, Hardy S, Sasorith S, Klein F, Robert F, Weber C, Miguet L, Potier N, Van Dorsselaer A, Wurtz JM, Mandel JL, Tora L and Devys D. Ataxin-7 is a subunit of GCN5 histone acetyltransferase-containing complexes. *Hum Mol Genet* 13: 1257-1265, 2004.

Hentati A, Deng HX, Hung WY, Nayer M, Ahmed MS, He X, Tim R, Stumpf DA, Siddique T and Ahmed. Human alpha-tocopherol transfer protein: gene structure and mutations in familial vitamin E deficiency. *Ann Neurol* 39: 295-300, 1996.

Herman-Bert A, Stevanin G, Netter JC, Rascol O, Brassat D, Calvas P, Camuzat A, Yuan Q, Schalling M, Durr A and Brice A. Mapping of spinocerebellar ataxia 13 to chromosome 19q13.3-q13.4 in a family with autosomal dominant cerebellar ataxia and mental retardation. *Am J Hum Genet* 67: 229-235, 2000.

Heslinga HW, Van den Burg W, and Saan RJ. De nieuwe 15-woorden test (the 15-Words-test) in een gezonde populatie (in Dutch). Internal report no 8324. Groningen, Department of Medical Psychology, State University Groningen , The Netherlands. 1983.

Hirayama K and Tokumaru Y. Cervical dural sac and spinal cord in juvenile muscular atrophy of distal upper extremity. *Neurology* 54: 1922-1926, 2000.

Hirayama K, TSubaki T, Toyokura Y and Okinakas S. Juvenile muscular atrophy of unilateral upper extremity. *Neurology* 13: 373-380, 1963.

Holmes G. An attempt to classify cerebellar disease, with a notion to Marie's hereditary cerebellar ataxia. *Brain* 30, 545-567. 1907.

Holmes SE, O'Hearn EE, McInnis MG, Gorelick-Feldman DA, Kleiderlein JJ, Callahan C, Kwak NG, Ingersoll-Ashworth RG, Sherr M, Sumner AJ, Sharp AH, Ananth U, Seltzer WK, Boss MA, Vieria-Saecker AM, Epplen JT, Riess O, Ross CA and Margolis RL. Expansion of a novel CAG trinucleotide repeat in the 5' region of PPP2R2B is associated with SCA12. *Nat Genet* 23: 391-392, 1999.

Hull M, Fiebich BL, Lieb K, Strauss S, Berger SS, Volk B and Bauer J. Interleukin-6-associated inflammatory processes in Alzheimer's disease: new therapeutic options. *Neurobiol Aging* 17: 795-800, 1996.

Huynh DP, Del Bigio MR, Ho DH and Pulst SM. Expression of ataxin-2 in brains from normal individuals and patients with Alzheimer's disease and spinocerebellar ataxia 2. *Ann Neurol* 45: 232-241, 1999.

Isnard R, Kalotka H, Durr A, Cossee M, Schmitt M, Pousset F, Thomas D, Brice A, Koenig M and Komajda M. Correlation between left ventricular hypertrophy and GAA trinucleotide repeat length in Friedreich's ataxia. *Circulation* 95: 2247-2249, 1997.

Jen J, Wan J, Graves M, Yu H, Mock AF, Coulin CJ, Kim G, Yue Q, Papazian DM and Baloh RW. Loss-of-function EA2 mutations are associated with impaired neuromuscular transmission. *Neurology* 57: 1843-1848, 2001.

Jen JC, Yue Q, Karrim J, Nelson SF, Baloh RW. Spinocerebellar ataxia type 6 with positional vertigo and acetazolamide responsive episodic ataxia. *J Neurol Neurosurg Psychiatry* 65: 565-568, 1998.

Jobsis GJ, Weber JW, Barth PG, Keizers H, Baas F, van Schooneveld MJ, van Hilten JJ, Troost D, Geesink HH and Bolhuis PA. Autosomal dominant cerebellar ataxia with retinal degeneration (ADCA II): clinical and neuropathological findings in two pedigrees and genetic linkage to 3p12-p21.1. *J Neurol Neurosurg Psychiatry* 62: 367-371, 1997.

Jodice C, Mantuano E, Veneziano L, Trettel F, Sabbadini G, Calandriello L, Francia A, Spadaro M, Pierelli F, Salvi F, Ophoff RA, Frants RR and Frontali M. Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNA1A gene on chromosome 19p. *Hum Mol Genet* 6: 1973-1978, 1997.

Karthikeyan G, Lewis LK and Resnick MA. The mitochondrial protein frataxin prevents nuclear damage. *Hum Mol Genet* 11: 1351-1362, 2002.

Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, Nakamura S, Nishimura M, Akiguchi I and . CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat Genet* 8: 221-228, 1994.

Kinoshita A, Hayashi M, Oda M and Tanabe H. Clinicopathological study of the peripheral nervous system in Machado-Joseph disease. *J Neurol Sci* 130: 48-58, 1995.

Kish SJ, el Awar M, Stuss D, Nobrega J, Currier R, Aita JF, Schut L, Zoghbi HY and Freedman M. Neuropsychological test performance in patients with dominantly inherited spinocerebellar ataxia: relationship to ataxia severity. *Neurology* 44: 1738-1746, 1994.

Klockgether T, Doller G, Wullner U, Petersen D and Dichgans J. Cerebellar encephalitis in adults. *J Neurol* 240: 17-20, 1993.

Klockgether T, Ludtke R, Kramer B, Abele M, Burk K, Schols L, Riess O, Laccone F, Boesch S, Lopes-Cendes I, Brice A, Inzelberg R, Zilber N and Dichgans J. The natural history of degenerative ataxia: a retrospective study in 466 patients. *Brain* 121 (Pt 4): 589-600, 1998.

Klockgether T, Petersen D, Grodd W and Dichgans J. Early onset cerebellar ataxia with retained tendon reflexes. Clinical, electrophysiological and MRI observations in comparison with Friedreich's ataxia. *Brain* 114 (Pt 4): 1559-1573, 1991.

Kobayashi T and Kakizuka A. Molecular analyses of Machado-Joseph disease. *Cytogenet Genome Res* 100: 261-275, 2003.

Koide R, Kobayashi S, Shimohata T, Ikeuchi T, Maruyama M, Saito M, Yamada M, Takahashi H and Tsuji S. A neurological disease caused by an expanded CAG trinucleotide repeat in the TATA-binding protein gene: a new polyglutamine disease? *Hum Mol Genet* 8: 2047-2053, 1999.

Koningsmark BW and Weiner LP. The olivopontocerebellar atrophies: a review. *Medicine (Baltimore)* 49: 227-241, 1970.

Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW and Ranum LP. An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). *Nat Genet* 21: 379-384, 1999.

Koskinen T, Santavuori P, Sainio K, Lappi M, Kallio AK and Pihko H. Infantile onset spinocerebellar ataxia with sensory neuropathy: a new inherited disease. *J Neurol Sci* 121: 50-56, 1994.

Koutnikova H, Campuzano V, Foury F, Dolle P, Cazzalini O and Koenig M. Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. *Nat Genet* 16: 345-351, 1997.

Koyano S, Iwabuchi K, Yagishita S, Kuroiwa Y and Uchihara T. Paradoxical absence of nuclear inclusion in cerebellar Purkinje cells of hereditary ataxias linked to CAG expansion. *J Neurol Neurosurg Psychiatry* 73: 450-452, 2002.

Kruglyak L, Daly MJ, Reeve-Daly MP and Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58: 1347-1363, 1996.

Kubis N, Durr A, Gugenheim M, Chneiweiss H, Mazzetti P, Brice A and Bouche P. Polyneuropathy in autosomal dominant cerebellar ataxias: phenotype-genotype correlation. *Muscle Nerve* 22: 712-717, 1999.

Kurihara M, Kumagai K, Yagishita S, Imai M, Watanabe M, Suzuki Y and Orii T. Adrenoleukomyeloneuropathy presenting as cerebellar ataxia in a young child: a probable variant of adrenoleukodystrophy. *Brain Dev* 15: 377-380, 1993.

La Spada AR, Roling DB, Harding AE, Warner CL, Spiegel R, Hausmanowa-Petrusewicz I, Yee WC and Fischbeck KH. Meiotic stability and genotype-phenotype correlation of the trinucleotide repeat in X-linked spinal and bulbar muscular atrophy. *Nat Genet* 2: 301-304, 1992.

Labuda M, Labuda D, Miranda C, Poirier J, Soong BW, Barucha NE and Pandolfo M. Unique origin and specific ethnic distribution of the Friedreich ataxia GAA expansion. *Neurology* 54: 2322-2324, 2000.

Lalioti MD, Scott HS, Buresi C, Rossier C, Bottani A, Morris MA, Malafosse A and Antonarakis SE. Dodecamer repeat expansion in cystatin B gene in progressive myoclonus epilepsy. *Nature* 386: 847-851, 1997.

Lamarche JB, Lemieux B and Lieu HB. The neuropathology of "typical" Friedreich's ataxia in Quebec. *Can J Neurol Sci* 11: 592-600, 1984.

Larnaout A, Belal S, Ben Hamida C, Ben Hamida M and Hentati F. Atypical ataxia telangiectasia with early childhood lower motor neuron degeneration: a clinicopathological observation in three siblings. *J Neurol* 245: 231-235, 1998.

Lathrop GM and Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 36: 460-465, 1984.

Le Ber I, Bouslam N, Rivaud-Pechoux S, Guimaraes J, Benomar A, Chamayou C, Goizet C, Moreira MC, Klur S, Yahyaoui M, Agid Y, Koenig M, Stevanin G, Brice A and Durr A. Frequency and phenotypic spectrum of ataxia with oculomotor apraxia 2: a clinical and genetic study in 18 patients. *Brain* 127: 759-767, 2004.

Le Ber I,Moreira MC, Riveaud-Pechoux S, Chamayou C, Ochsner F, Kuntzer T, Tardieu M, Said G, Habert MO, Demarquay G, Tannier C, Beis JM, Brice A, Koenig M and Durr A. erebellar ataxia with oculomotor zpraxia type 1: clinical and genetic studies. *Brain* 126:2761-2772, 2003.

Leone M, Brignolio F, Rosso MG, Curtoni ES, Moroni A, Tribolo A and Schiffer D. Friedreich's ataxia: a descriptive epidemiological study in an Italian population. *Clin Genet* 38: 161-169, 1990.

Li F, Macfarlan T, Pittman RN and Chakravarti D. Ataxin-3 is a histone-binding protein with two independent transcriptional corepressor activities. *J Biol Chem* 277: 45004-45012, 2002.

Lopes-Cendes I, Silveira I, Maciel P, Gaspar C, Radvany J, Chitayat D, Babul R, Stewart J, Dolliver M, Robitaille Y, Rouleau GA and Sequeiros J. Limits of clinical assessment in the accurate diagnosis of Machado-Joseph disease. *Arch Neurol* 53: 1168-1174, 1996.

Louis-Bar D. Sur un syndrome progressif comprenant des telangiectasies capilaires cutanées et conjuntivales symmetriques, à dispostion naevoide et des troubles cérébelleux. *Con Neurol* 4: 32-34, 1941.

Luteyn F. De Groninger Intelligentie Test (The Groninger Intelligence Test). Van der Ploeg FAE. 1983. Lisse, Swets and Zeitlinger.

Luthi-Carter R, Strand A, Peters NL, Solano SM, Hollingsworth ZR, Menon AS, Frey AS, Spektor BS, Penney EB, Schilling G, Ross CA, Borchelt DR, Tapscott SJ, Young AB, Cha JH and Olson JM. Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. *Hum Mol Genet* 9: 1259-1271, 2000.

Lynch DR, Farmer JM, Balcer LJ and Wilson RB. Friedreich ataxia: effects of genetic understanding on clinical evaluation and therapy. *Arch Neurol* 59: 743-747, 2002.

Marie P. Sur l'heredoataxie cerebelleuse. Sem in Med (Paris) 13, 444-447. 1893.

Marquardt T and Denecke J. Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies. *Eur J Pediatr* 162: 359-379, 2003.

Maruff P, Tyler P, Burt T, Currie B, Burns C and Currie J. Cognitive deficits in Machado-Joseph disease. *Ann Neurol* 40: 421-427, 1996.

Matsuura T, Ranum LP, Volpini V, Pandolfo M, Sasaki H, Tashiro K, Watase K, Zoghbi HY and Ashizawa T. Spinocerebellar ataxia type 10 is rare in populations other than Mexicans. *Neurology* 58: 983-984, 2002.

Matsuura T, Yamagata T, Burgess DL, Rasmussen A, Grewal RP, Watase K, Khajavi M, McCall AE, Davis CF, Zu L, Achari M, Pulst SM, Alonso E, Noebels JL, Nelson DL, Zoghbi HY and Ashizawa T. Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. *Nat Genet* 26: 191-194, 2000.

Matsuura T, Achari M, Khajavi M, Bachinski LL, Zoghbi HY and Ashizawa T. Mapping of the gene for a novel spinocerebellar ataxia with pure cerebellar signs and epilepsy. *Ann Neurol* 45: 407-411, 1999.

Miyoshi Y, Yamada T, Tanimura M, Taniwaki T, Arakawa K, Ohyagi Y, Furuya H, Yamamoto K, Sakai K, Sasazuki T and Kira J. A novel autosomal dominant spinocerebellar ataxia (SCA16) linked to chromosome 8q22.1-24.1. *Neurology* 57: 96-100, 2001.

Moreira MC, Barbot C, Tachi N, Kozuka N, Mendonca P, Barros J, Coutinho P, Sequeiros J and Koenig M. Homozygosity mapping of Portuguese and Japanese forms of ataxia-oculomotor apraxia to 9p13, and evidence for genetic heterogeneity. *Am J Hum Genet* 68: 501-508, 2001a.

Moreira MC, Barbot C, Tachi N, Kozuka N, Uchida E, Gibson T, Mendonca P, Costa M, Barros J, Yanagisawa T, Watanabe M, Ikeda Y, Aoki M, Nagata T, Coutinho P, Sequeiros J and Koenig M. The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. *Nat Genet* 29: 189-193, 2001b.

Moreira MC, Klur S, Watanabe M, Nemeth AH, Le B, I, Moniz JC, Tranchant C, Aubourg P, Tazir M, Schols L, Pandolfo M, Schulz JB, Pouget J, Calvas P, Shizuka-Ikeda M, Shoji M, Tanaka M, Izatt L, Shaw CE, M'Zahem A, Dunne E, Bomont P, Benhassine T, Bouslam N, Stevanin G, Brice A, Guimaraes J, Mendonca P, Barbot C, Coutinho P, Sequeiros J, Durr A, Warter JM and Koenig M. Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. *Nat Genet* 36: 225-227, 2004.

Mrissa N, Belal S, Hamida CB, Amouri R, Turki I, Mrissa R, Hamida MB and Hentati F. Linkage to chromosome 13q11-12 of an autosomal recessive cerebellar ataxia in a Tunisian family. *Neurology* 54: 1408-1414, 2000.

Muhlenhoff U, Richhardt N, Ristow M, Kispal G and Lill R. The yeast frataxin homolog Yfh1p plays a specific role in the maturation of cellular Fe/S proteins. *Hum Mol Genet* 11: 2025-2036, 2002.

Murata Y, Yamaguchi S, Kawakami H, Imon Y, Maruyama H, Sakai T, Kazuta T, Ohtake T, Nishimura M, Saida T, Chiba S, Oh-i T and Nakamura S. Characteristic magnetic resonance imaging findings in Machado-Joseph disease. *Arch Neurol* 55: 33-37, 1998.

Nagafuchi S, Yanagisawa H, Sato K, Shirayama T, Ohsaki E, Bundo M, Takeda T, Tadokoro K, Kondo I and Murayama N. Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. *Nat Genet* 6: 14-18, 1994.

Nagahama Y, Fukuyama H, Yamauchi H, Matsuzaki S, Konishi J, Shibasaki H and Kimura J. Cerebral activation during performance of a card sorting test. *Brain* 119 (Pt 5): 1667-1675, 1996.

Nakamura K, Jeong SY, Uchihara T, Anno M, Nagashima K, Nagashima T, Ikeda S, Tsuji S and Kanazawa I. SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. *Hum Mol Genet* 10: 1441-1448, 2001.

Navon R, Khosravi R, Melki J, Drucker L, Fontaine B, Turpin JC, N'Guyen B, Fardeau M, Rondot P and Baumann N. Juvenile-onset spinal muscular atrophy caused by compound heterozygosity for mutations in the HEXA gene. *Ann Neurol* 41: 631-638, 1997.

Nemeth AH, Bochukova E, Dunne E, Huson SM, Elston J, Hannan MA, Jackson M, Chapman CJ and Taylor AM. Autosomal recessive cerebellar ataxia with oculomotor apraxia (ataxia-telangiectasia-like syndrome) is linked to chromosome 9q34. *Am J Hum Genet* 67: 1320-1326, 2000.

Nikali K, Suomalainen A, Terwilliger J, Koskinen T, Weissenbach J and Peltonen L. Random search for shared chromosomal regions in four affected individuals: the assignment of a new hereditary ataxia locus. *Am J Hum Genet* 56: 1088-1095, 1995.

Ogawa T, Takiyama Y, Sakoe K, Mori K, Namekawa M, Shimazaki H, Nakano I and Nishizawa M. Identification of a SACs gene missense mutation in ARSACS. *Neurology* 62: 107-109, 2004.

Ophoff RA, van Eijk R, Sandkuijl LA, Terwindt GM, Grubben CP, Haan J, Lindhout D, Ferrari MD and Frants RR. Genetic heterogeneity of familial hemiplegic migraine. *Genomics* 22: 21-26, 1994.

Orr HT, Chung MY, Banfi S, Kwiatkowski TJ, Jr., Servadio A, Beaudet AL, McCall AE, Duvick LA, Ranum LP and Zoghbi HY. Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nat Genet* 4: 221-226, 1993.

Ouahchi K, Arita M, Kayden H, Hentati F, Ben Hamida M, Sokol R, Arai H, Inoue K, Mandel JL and Koenig M. Ataxia with isolated vitamin E deficiency is caused by mutations in the alpha-tocopherol transfer protein. *Nat Genet* 9: 141-145, 1995.

Pandolfo M, Van de Warrenburg B.P.C. Spinocerebellar ataxia type 14. Opening a new door in the dominant ataxia research? *Neurology* 64: 1113-1114, 2005

Pareyson D, Gellera C, Castellotti B, Antonelli A, Riggio MC, Mazzucchelli F, Girotti F, Pietrini V, Mariotti C and Di Donato S. Clinical and molecular studies of 73 Italian families with autosomal dominant cerebellar ataxia type I: SCA1 and SCA2 are the most common genotypes. *J Neurol* 246: 389-393, 1999.

Paulson HL, Das SS, Crino PB, Perez MK, Patel SC, Gotsdiner D, Fischbeck KH and Pittman RN. Machado-Joseph disease gene product is a cytoplasmic protein widely expressed in brain. *Ann Neurol* 41: 453-462, 1997a.

Paulson HL, Perez MK, Trottier Y, Trojanowski JQ, Subramony SH, Das SS, Vig P, Mandel JL, Fischbeck KH and Pittman RN. Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron* 19: 333-344, 1997b.

Pearson CE, Eichler EE, Lorenzetti D, Kramer SF, Zoghbi HY, Nelson DL and Sinden RR. Interruptions in the triplet repeats of SCA1 and FRAXA reduce the propensity and complexity of slipped strand DNA (S-DNA) formation. *Biochemistry* 37: 2701-2708, 1998.

Perez MK, Paulson HL and Pittman RN. Ataxin-3 with an altered conformation that exposes the polyglutamine domain is associated with the nuclear matrix. *Hum Mol Genet* 8: 2377-2385, 1999.

Peyronnaud JM, charron L, and Barbeau A. The neuropathy of Charlevoix-Saguenay ataxia: an electrophysiological and pathological study. *Can J Neurol Sci* 6, 199-203. 1979.

Piedras-Renteria ES, Watase K, Harata N, Zhuchenko O, Zoghbi HY, Lee CC and Tsien RW. Increased expression of alpha 1A Ca2+ channel currents arising from expanded trinucleotide repeats in spinocerebellar ataxia type 6. *J Neurosci* 21: 9185-9193, 2001.

Pulst SM, Nechiporuk A, Nechiporuk T, Gispert S, Chen XN, Lopes-Cendes I, Pearlman S, Starkman S, Orozco-Diaz G, Lunkes A, DeJong P, Rouleau GA, Auburger G, Korenberg JR, Figueroa C and Sahba S. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. *Nat Genet* 14: 269-276, 1996.

Pulst SM, Perlman S. Hereditary ataxias. In: *Neurogenetics (CNS Contemporary Neurology series)*. Oxford University Press, New York, 2000.

Ramesar RS, Bardien S, Beighton P and Bryer A. Expanded CAG repeats in spinocerebellar ataxia (SCA1) segregate with distinct haplotypes in South african families. *Hum Genet* 100: 131-137, 1997.

Ranum LP and Day JW. Pathogenic RNA repeats: an expanding role in genetic disease. *Trends Genet* 20: 506-512, 2004.

Ranum LP, Schut LJ, Lundgren JK, Orr HT and Livingston DM. Spinocerebellar ataxia type 5 in a family descended from the grandparents of President Lincoln maps to chromosome 11. *Nat Genet* 8: 280-284, 1994.

Raven JC. Guide to the standard progressive matrices. London, Lewis. 1960.

Read AP. Huntington's disease: testing the test. Nat Genet 4: 329-330, 1993.

Restituito S, Thompson RM, Eliet J, Raike RS, Riedl M, Charnet P and Gomez CM. The polyglutamine expansion in spinocerebellar ataxia type 6 causes a beta subunit-specific enhanced activation of P/Q-type calcium channels in Xenopus oocytes. *J Neurosci* 20: 6394-6403, 2000.

Richards RI. Dynamic mutations: a decade of unstable expanded repeats in human genetic disease. *Hum Mol Genet* 10: 2187-2194, 2001.

Richter A, Rioux JD, Bouchard JP, Mercier J, Mathieu J, Ge B, Poirier J, Julien D, Gyapay G, Weissenbach J, Hudson TJ, Melancon SB and Morgan K. Location score and haplotype analyses of the locus for autosomal recessive spastic ataxia of Charlevoix-Saguenay, in chromosome region 13q11. *Am J Hum Genet* 64: 768-775, 1999.

Rolfs A, Koeppen AH, Bauer I, Bauer P, Buhlmann S, Topka H, Schols L and Riess O. Clinical features and neuropathology of autosomal dominant spinocerebellar ataxia (SCA17). *Ann Neurol* 54: 367-375, 2003.

Ross CA and Poirier MA. Protein aggregation and neurodegenerative disease. *Nat Med* 10 Suppl: S10-S17, 2004.

Rothwell N. Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential. *Brain Behav Immun* 17: 152-157, 2003.

Rüb U, de Vos RA, Brunt ER, Schultz C, Paulson H, Del Tredici K and Braak H. Degeneration of the external cuneate nucleus in spinocerebellar ataxia type 3 (Machado-Joseph disease). *Brain Res* 953: 126-134, 2002.

Rubinsztein DC, Leggo J, Coles R, Almqvist E, Biancalana V, Cassiman JJ, Chotai K, Connarty M, Crauford D, Curtis A, Curtis D, Davidson MJ, Differ AM, Dode C, Dodge A, Frontali M, Ranen NG, Stine OC, Sherr M, Abbott MH, Franz ML, Graham CA, Harper PS, Hedreen JC and Hayden MR. Phenotypic characterization of individuals with 30-40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36-39 repeats. *Am J Hum Genet* 59: 16-22, 1996.

Sano Y, Date H, Igarashi S, Onodera O, Oyake M, Takahashi T, Hayashi S, Morimatsu M, Takahashi H, Makifuchi T, Fukuhara N and Tsuji S. Aprataxin, the causative protein for AOA1 is a nuclear protein with a potential role as a DNA repair protein. *Ann Neurol* 55: 241-249, 2004.

Sanpei K, Takano H, Igarashi S, Sato T, Oyake M, Sasaki H, Wakisaka A, Tashiro K, Ishida Y, Ikeuchi T, Koide R, Saito M, Sato A, Tanaka T, Hanyu S, Takiyama Y, Nishizawa M, Shimizu N, Nomura Y, Segawa M, Iwabuchi K, Eguchi I, Tanaka H, Takahashi H and Tsuji S. Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nat Genet* 14: 277-284, 1996.

Santoro L, De Michele G, Perretti A, Crisci C, Cocozza S, Cavalcanti F, Ragno M, Monticelli A, Filla A and Caruso G. Relation between trinucleotide GAA repeat length and sensory neuropathy in Friedreich's ataxia. *J Neurol Neurosurg Psychiatry* 66: 93-96, 1999.

Saudou F, Finkbeiner S, Devys D and Greenberg ME. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* 95: 55-66, 1998.

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S and . A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 268: 1749-1753, 1995.

Scheel H, Tomiuk S and Hofmann K. Elucidation of ataxin-3 and ataxin-7 function by integrative bioinformatics. *Hum Mol Genet* 12: 2845-2852, 2003.

Schelhaas HJ, Ippel PF, Hageman G, Sinke RJ, van der Laan EN and Beemer FA. Clinical and genetic analysis of a four-generation family with a distinct autosomal dominant cerebellar ataxia. *J Neurol* 248: 113-120, 2001.

Schelhaas HJ, Verbeek DS, van de Warrenburg BP and Sinke RJ. SCA19 and SCA22: evidence for one locus with a worldwide distribution. *Brain* 127: E6, 2004.

Schelhaas HJ, Van de Warrenburg BP. Clinical, psychological, and genetic characteristics of spinocerebellar ataxia type 19 (SCA19). Cerebellum (in press)

Schelhaas HJ, van de Warrenburg BP, Hageman G, Ippel EE, van Hout M, Kremer B. Cognitive impairment in SCA-19. *Acta Neurol Belg* 103: 199-205, 2003.

Schelhaas HJ, Van de Warrenburg BP, Kremer HP, Zwarts MJ. Neuromuscular transmission in SCA6. *Ann Neurol* 55: 451-452, 2004.

Schelhaas HJ, van de Warrenburg BP, Kremer HP, Zwarts MJ. The "split hand" phenomenon: evidence of a spinal origin. *Neurology* 61: 1619-1620, 2004.

Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, Bates GP, Davies SW, Lehrach H and Wanker EE. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. *Cell* 90: 549-558, 1997.

Schmidt T, Landwehrmeyer GB, Schmitt I, Trottier Y, Auburger G, Laccone F, Klockgether T, Volpel M, Epplen JT, Schols L and Riess O. An isoform of ataxin-3 accumulates in the nucleus of neuronal cells in affected brain regions of SCA3 patients. *Brain Pathol* 8: 669-679, 1998.

Schols L, Bauer P, Schmidt T, Schulte T and Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. *Lancet Neurol* 3: 291-304, 2004.

Schols L, Bauer I, Zuhlke C, Schulte T, Kolmel C, Burk K, Topka H, Bauer P, Przuntek H and Riess O. Do CTG expansions at the SCA8 locus cause ataxia? *Ann Neurol* 54: 110-115, 2003.

Schols L, Gispert S, Vorgerd M, Menezes Vieira-Saecker AM, Blanke P, Auburger G, Amoiridis G, Meves S, Epplen JT, Przuntek H, Pulst SM and Riess O. Spinocerebellar ataxia type 2. Genotype and phenotype in German kindreds. *Arch Neurol* 54: 1073-1080, 1997a.

Schols L, Amoiridis G, Buttner T, Przuntek H, Epplen JT and Riess O. Autosomal dominant cerebellar ataxia: phenotypic differences in genetically defined subtypes? *Ann Neurol* 42: 924-932, 1997b.

Schroder R, Keller E, Flacke S, Schmidt S, Pohl C, Klockgether T and Schlegel U. MRI findings in Hirayama's disease: flexion-induced cervical myelopathy or intrinsic motor neuron disease? *J Neurol* 246: 1069-1074, 1999.

Servadio A, Koshy B, Armstrong D, Antalffy B, Orr HT and Zoghbi HY. Expression analysis of the ataxin-1 protein in tissues from normal and spinocerebellar ataxia type 1 individuals. *Nat Genet* 10: 94-98, 1995.

Shiloh Y. ATM: sounding the double-strand break alarm. *Cold Spring Harb Symp Quant Biol* 65: 527-533, 2000.

Shimazaki H, Takiyama Y, Sakoe K, Ikeguchi K, Niijima K, Kaneko J, Namekawa M, Ogawa T, Date H, Tsuji S, Nakano I and Nishizawa M. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia: the aprataxin gene mutations. *Neurology* 59: 590-595, 2002.

Sinke RJ, Ippel EF, Diepstraten CM, Beemer FA, Wokke JH, van Hilten BJ, Knoers NV, van Amstel HK and Kremer HP. Clinical and molecular correlations in spinocerebellar ataxia type 6: a study of 24 Dutch families. *Arch Neurol* 58: 1839-1844, 2001.

Sitrin MD and Bengoa JM. Intestinal absorption of cholecalciferol and 25-hydroxycholecalciferol in chronic cholestatic liver disease. *Am J Clin Nutr* 46: 1011-1015, 1987.

Svetel M, Djarmati A, Dragasevic N, Savic D, Culjkovic B, Romac S, Kostic VS. SCA2 and SCA3 mutations in young-onset dopa-responsive parkinsonism. *Eur J Neurol* 10: 597, 2003

Sobrido MJ, Cholfin JA, Perlman S, Pulst SM and Geschwind DH. SCA8 repeat expansions in ataxia: a controversial association. *Neurology* 57: 1310-1312, 2001.

Stevanin G, Herman A, Durr A, Jodice C, Frontali M, Agid Y and Brice A. Are (CTG)n expansions at the SCA8 locus rare polymorphisms? *Nat Genet* 24: 213, 2000.

Storey E, Forrest SM, Shaw JH, Mitchell P and Gardner RJ. Spinocerebellar ataxia type 2: clinical features of a pedigree displaying prominent frontal-executive dysfunction. *Arch Neurol* 56: 43-50, 1999.

Swartz BE, Burmeister M, Somers JT, Rottach KG, Bespalova IN and Leigh RJ. A form of inherited cerebellar ataxia with saccadic intrusions, increased saccadic speed, sensory neuropathy, and myoclonus. *Ann N Y Acad Sci* 956: 441-444, 2002.

Swift M, Morrell D, Cromartie E, Chamberlin AR, Skolnick MH and Bishop DT. The incidence and gene frequency of ataxia-telangiectasia in the United States. *Am J Hum Genet* 39: 573-583, 1986a.

Syllaba L, Henner K. Contribution a l' indépendence de l' athétose double idiopatique et congénitale. *Rev Neurol* 4 : 541-562, 1926.

Taga T and Kishimoto T. Gp130 and the interleukin-6 family of cytokines. *Annu Rev Immunol* 15: 797-819, 1997.

Tait D, Riccio M, Sittler A, Scherzinger E, Santi S, Ognibene A, Maraldi NM, Lehrach H and Wanker EE. Ataxin-3 is transported into the nucleus and associates with the nuclear matrix. *Hum Mol Genet* 7: 991-997, 1998.

Takashima H, Boerkoel CF, John J, Saifi GM, Salih MA, Armstrong D, Mao Y, Quiocho FA, Roa BB, Nakagawa M, Stockton DW and Lupski JR. Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. *Nat Genet* 32: 267-272, 2002.

Taniguchi T, Ogasawara K, Takaoka A and Tanaka N. IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol* 19: 623-655, 2001.

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* 72: 971-983, 1993.

Trottier Y, Cancel G, An-Gourfinkel I, Lutz Y, Weber C, Brice A, Hirsch E and Mandel JL. Heterogeneous intracellular localization and expression of ataxin-3. *Neurobiol Dis* 5: 335-347, 1998.

Trouillas P, Takayanagi T, Hallett M, Currier RD, Subramony SH, Wessel K, Bryer A, Diener HC, Massaquoi S, Gomez CM, Coutinho P, Ben Hamida M, Campanella G, Filla A, Schut L, Timann D, Honnorat J, Nighoghossian N and Manyam B. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. The Ataxia Neuropharmacology Committee of the World Federation of Neurology. *J Neurol Sci* 145: 205-211, 1997.

Uchitel OD, Protti DA, Sanchez V, Cherksey BD, Sugimori M and Llinas R. P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. *Proc Natl Acad Sci U S A* 89: 3330-3333, 1992.

Uversky VN. Protein folding revisited. A polypeptide chain at the folding-misfolding-nonfolding cross-roads: which way to go? *Cell Mol Life Sci* 60: 1852-1871, 2003.

Vales LD and Friedl EM. Binding of C/EBP and RBP (CBF1) to overlapping sites regulates interleukin-6 gene expression. *J Biol Chem* 277: 42438-42446, 2002.

Van de Warrenburg BP, Notermans NC, Schelhaas HJ, van Alfen N, Sinke RJ, Knoers NV, Zwarts MJ and Kremer BP. Peripheral nerve involvement in spinocerebellar ataxias. *Arch Neurol* 61: 257-261, 2004a.

Van de Warrenburg BP, Sinke RJ, Verschuuren-Bemelmans CC, Scheffer H, Brunt ER, Ippel PF, Maat-Kievit JA, Dooijes D, Notermans NC, Lindhout D, Knoers NV and Kremer HP. Spinocerebellar ataxias in the Netherlands: prevalence and age at onset variance analysis. *Neurology* 58: 702-708, 2002.

Van de Warrenburg BP, Verbeek DS, Piersma SJ, Hennekam FA, Pearson PL, Knoers NV, Kremer HP and Sinke RJ. Identification of a novel SCA14 mutation in a Dutch autosomal dominant cerebellar ataxia family. *Neurology* 61: 1760-1765, 2003.

Varilo T, Nikali K, Suomalainen A, Lonnqvist T and Peltonen L. Tracing an ancestral mutation: genealogical and haplotype analysis of the infantile onset spinocerebellar ataxia locus. *Genome Res* 6: 870-875, 1996.

Varon R, Gooding R, Steglich C, Marns L, Tang H, Angelicheva D, Yong KK, Ambrugger P, Reinhold A, Morar B, Baas F, Kwa M, Tournev I, Guerguelcheva V, Kremensky I, Lochmuller H, Mullner-Eidenbock A, Merlini L, Neumann L, Burger J, Walter M, Swoboda K, Thomas PK, von Moers A, Risch N and Kalaydjieva L. Partial deficiency of the C-terminal-domain phosphatase of RNA polymerase II is associated with congenital cataracts facial dysmorphism neuropathy syndrome. *Nat Genet* 35: 185-189, 2003.

Verbeek DS, Schelhaas JH, Ippel EF, Beemer FA, Pearson PL and Sinke RJ. Identification of a novel SCA locus (SCA19) in a Dutch autosomal dominant cerebellar ataxia family on chromosome region 1p21-q21. *Hum Genet* 111: 388-393, 2002.

Verbeek DS, van de Warrenburg BP, Wesseling P, Pearson PL, Kremer HP and Sinke RJ. Mapping of the SCA23 locus involved in autosomal dominant cerebellar ataxia to chromosome region 20p13-12.3. *Brain* 127: 2551-2557, 2004.

Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF and Zhang FP. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65: 905-914, 1991.

Wadia N, Pang J, Desai J, Mankodi A, Desai M and Chamberlain S. A clinicogenetic analysis of six Indian spinocere-bellar ataxia (SCA2) pedigrees. The significance of slow saccades in diagnosis. Brain 121 (Pt 12): 2341-2355, 1998.

Warrick JM, Chan HY, Gray-Board, Chai Y, Paulson HL and Bonini NM. Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. *Nat Genet* 23: 425-428, 1999.

Warrington EK. Recognition memory test. NFER-Nelson. Windsor. 1984.

Watanabe H, Tanaka F, Matsumoto M, Doyu M, Ando T, Mitsuma T and Sobue G. Frequency analysis of autosomal dominant cerebellar ataxias in Japanese patients and clinical characterization of spinocerebellar ataxia type 6. *Clin Genet* 53: 13-19, 1998a.

Watanabe M, Sugai Y, Concannon P, Koenig M, Schmitt M, Sato M, Shizuka M, Mizushima K, Ikeda Y, Tomidokoro Y, Okamoto K and Shoji M. Familial spinocerebellar ataxia with cerebellar atrophy, peripheral neuropathy, and elevated level of serum creatine kinase, gamma-globulin, and alpha-fetoprotein. *Ann Neurol* 44: 265-269, 1998b.

Weber M, Eisen A, Stewart H and Hirota N. The split hand in ALS has a cortical basis. *J Neurol Sci* 180: 66-70, 2000.

Wechsler D. WAIS-R manual. New York, Psychological Corporation. 1974.

Wenning GK, Ben Shlomo Y, Magalhaes M, Daniel SE and Quinn NP. Clinical features and natural history of multiple system atrophy. An analysis of 100 cases. *Brain* 117 (Pt 4): 835-845, 1994.

Wichman A, Buchthal F, Pezeshkpour GH and Gregg RE. Peripheral neuropathy in abetalipoproteinemia. *Neurology* 35: 1279-1289, 1985.

Wilbourn AJ. Dissociated wasting of medial and lateral hand muscles with motor neuron disease. *Can J Neurol Sci* 21 (suppl 2):S9: 1994.

Wilbourn AJ. The "split hand syndrome". *Muscle Nerve* 23: 138, 2000.

Wilson RB and Roof DM. Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. *Nat Genet* 16: 352-357, 1997.

Woods CG and Taylor AM. Ataxia telangiectasia in the British Isles: the clinical and laboratory features of 70 affected individuals. *Q J Med* 82: 169-179, 1992.

Worth PF, Giunti P, Gardner-Thorpe C, Dixon PH, Davis MB and Wood NW. Autosomal dominant cerebellar ataxia type III: linkage in a large British family to a 7.6-cM region on chromosome 15q14-21.3. *Am J Hum Genet* 65: 420-426, 1999.

Worth PF, Houlden H, Giunti P, Davis MB and Wood NW. Large, expanded repeats in SCA8 are not confined to patients with cerebellar ataxia. *Nat Genet* 24: 214-215, 2000.

Yamada M, Sato T, Shimohata T, Hayashi S, Igarashi S, Tsuji S and Takahashi H. Interaction between neuronal intranuclear inclusions and promyelocytic leukemia protein nuclear and coiled bodies in CAG repeat diseases. *Am J Pathol* 159: 1785-1795, 2001.

Yamashita I, Sasaki H, Yabe I, Fukazawa T, Nogoshi S, Komeichi K, Takada A, Shiraishi K, Takiyama Y, Nishizawa M, Kaneko J, Tanaka H, Tsuji S and Tashiro K. A novel locus for dominant cerebellar ataxia (SCA14) maps to a 10.2-cM interval flanked by D19S206 and D19S605 on chromosome 19q13.4-qter. *Ann Neurol* 48: 156-163, 2000.

Yue Q, Jen JC, Nelson SF and Baloh RW. Progressive ataxia due to a missense mutation in a calcium-channel gene. *Am J Hum Genet* 61: 1078-1087, 1997.

Yue S, Serra HG, Zoghbi HY and Orr HT. The spinocerebellar ataxia type 1 protein, ataxin-1, has RNA-binding activity that is inversely affected by the length of its polyglutamine tract. *Hum Mol Genet* 10: 25-30, 2001.

Zeman A, Stone J, Porteous M, Burns E, Barron L and Warner J. Spinocerebellar ataxia type 8 in Scotland: genetic and clinical features in seven unrelated cases and a review of published reports. *J Neurol Neurosurg Psychiatry* 75: 459-465, 2004.

Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY and Lee CC. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat Genet* 15: 62-69, 1997.

Zoghbi HY. CAG repeats in SCA6. Anticipating new clues. *Neurology* 49: 1196-1199, 1997.

Zoghbi HY, Sandkuyl LA, Ott J, Daiger SP, Pollack M, O'Brien WE and Beaudet AL. Assignment of autosomal dominant spinocerebellar ataxia (SCA1) centromeric to the HLA region on the short arm of chromosome 6, using multilocus linkage analysis. *Am J Hum Genet* 44: 255-263, 1989.

Zouari M, Feki M, Ben Hamida C, Larnaout A, Turki I, Belal S, Mebazaa A, Ben Hamida M and Hentati F. Electrophysiology and nerve biopsy: comparative study in Friedreich's ataxia and Friedreich's ataxia phenotype with vitamin E deficiency. *Neuromuscul Disord* 8: 416-425, 1998.

Zu L, Figueroa KP, Grewal R and Pulst SM. Mapping of a new autosomal dominant spinocerebellar ataxia to chromosome 22. *Am J Hum Genet* 64: 594-599, 1999.

Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, Cataudella T, Leavitt BR, Hayden MR, Timmusk T, Rigamonti D and Cattaneo E. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat Genet* 35: 76-83, 2003.

Zung WW. A Self-rating depression scale. *Arch Gen Psychiatry* 12: 63-70, 1965.

Samenvatting

De patiëntengroep die lijdt aan een erfelijke aandoening van de kleine hersenen (cerebellum) is zeer heterogeen. De overeenkomst in deze groep is het verschijnsel ataxie (taxis: orde): het ontbreken van geordende spraak, schrift (slordig en groot), oogbewegingen en bewegingen van romp en extremiteitspieren ("lopen als een dronkeman"). De verschillen binnen deze groep van aandoeningen bestaan onder andere uit een verschil in de beginleeftijd waarop de klachten zich presenteren (vroege beginleeftijd, versus late begin leeftijd), de manier van overerving (meestal autosomaal recessief bij een vroeg debuut en autosomaal dominant bij een laat debuut) en bijkomende verschijnselen (bijvoorbeeld spasticiteit).

Om kennis over deze groep van aandoeningen te vermeerderen zonder de rode draad te verliezen start het proefschrift in **sectie I** met een literatuuroverzicht van de autosomaal dominante (*hoofdstuk 2*) en autosomaal recessieve (*hoofdstuk 3*) cerebellaire aandoeningen. Daarna worden verschillende klinische, neurofysiologische, genetische en pathologische aspecten van deze groep van aandoeningen behandeld in drie secties.

In **sectie II** wordt ingegaan op het feit dat in ongeveer 30 procent van de autosomaal dominante cerebellaire ataxie (ADCA) families geen mutatie kan worden aangetoond. Deze families kunnen niet worden geclassificeerd volgens de zogenaamde spinocerebellaire ataxie (SCA) classificatie. Door het verrichten van klinisch en genetisch onderzoek in een grote Nederlandse ADCA familie waarin de bekende SCA mutaties waren uitgesloten probeerden wij een nieuw gen te vinden dat wellicht een deel van deze 30 procent zou kunnen verklaren.
Hiertoe beschrijven wij in *hoofdstuk 4* de klinische, neuropsychologische, neurofysiologische en MRI gegevens van deze familie. De klinische verschijnselen worden gedomineerd door frontaal cognitieve disfunctie, een relatief mild cerebellair syndroom en myoclonus.
In *hoofdstuk 5* beschrijven we het genetisch onderzoek in de bovengenoemde Nederlandse familie. Zowel bekende mutaties met een verlengde (geëxpandeerde) trinucleotide herhaalsequentie ('repeat'), als overige SCA loci waarbij het gen nog niet geïdentificeerd is, konden worden uitgesloten. Bij verder koppelingsonderzoek werden er harde aanwijzigen gevonden voor een nieuw locus op chromosoom 1 (1p21-1q21). Het nieuwe locus werd aangemeld bij de 'HUGO Genome Nomenclature Committee' en toegekend als SCA19. Inmiddels werd dit locus ook gevonden in een Chinese SCA familie, hetgeen suggereert dat de aandoening wellicht wereldwijd zou kunnen voorkomen.
In **sectie III** wordt de aandacht verlegd van genetische mutatie naar het fenotype en de pathofysiologie van de twee meest voorkomende SCAs in Nederland; SCA3 en SCA6.

Met betrekking tot het fenotype van SCA3 ontdekten wij dat veel patiënten met SCA3 het verschijnsel vertoonden van wat in de Angelsaksische literatuur 'the split hand' wordt genoemd (*hoofdstuk 6*).
De term 'split hand' verwijst naar een patroon van handspieratrofie waarbij het laterale deel van de hand meer is aangedaan dan het mediale deel. Aanvankelijk werd dit fenomeen in de literatuur beschouwd als zijnde specifiek voor een aandoening waarbij de motorische voorhoorn cel betrokken is. Later werd, op theoretische basis en ondersteund door onderzoek, gesuggereerd dat de 'split hand' een corticale origine zou hebben. Bij overleden patiënten met SCA3 blijkt nagenoeg altijd dat de hersenschors gespaard blijft terwijl het ruggenmerg, en dan met name de motorische voorhoorn cel, degenereert. De aanwezigheid van een 'split hand' bij patiënten met SCA3 suggereert daarom veeleer een spinale (motorische voorhoorn), dan corticale origine van dit klinisch fenomeen. Deze suggestie werd later bevestigd bij tien patiënten met een andere aandoening maar met een vergelijkbaar patroon van neurodegeneratie (cortex niet aangedaan, motorische voorhoorn wel aangedaan).

In *hoofdstuk 7* gaan we in op een pathofysiologisch aspect van de SCAs. Net als in veel andere SCAs wordt ook SCA3 veroorzaakt door een CAG trinucleotide repeatverlenging in het coderende deel van het gen. De

translatie van de CAG repeat in een verlengde polyglutamine expansie leidt tot structuurveranderingen van het eiwit (ataxine-3). Deze structuurverandering leidt uiteindelijk tot de formatie van bepaalde neuronale intranucleaire insluitlichaampjes (NII) en celdood. Deze NII's worden dan ook niet alleen aangetroffen in het brein en ruggenmerg van overleden SCA3 patiënten maar ook bij patiënten met SCA1, 2, 7 en 17. Aanvankelijk werd de aanwezigheid van NII beschouwd als een voorwaarde voor celdood in deze groep van aandoeningen. Recente ontwikkelingen suggereren echter een meer complex beeld. Zo lijken additionele factoren, zoals transcriptie disregulatie, eveneens bij te dragen aan de pathogenese. Om nu de relatie tussen transcriptie disregulatie, NII en celdood te verhelderen onderzochten we zowel brein als ruggenmerg van vijf overleden SCA3 patiënten. NII werden nagenoeg alleen gevonden in de hersenstam (pons) en de nucleus dentatus van de kleine hersenen. Disregulatie van genen betrokken bij ontstekingsprocessen bleek niet beperkt tot neuronen met NII en evenmin was er een verschil tussen degenererende neuronen met en zonder NII. Deze resultaten suggereren dat noch de aanwezigheid van NII, noch de expressie van ontstekingsmediatoren, voldoende zijn om celdood te verklaren.

In *hoofdstuk 8* gaat we in op pathofysiologische aspecten van SCA6. SCA6 wordt veroorzaakt door een relatief korte repeatverlenging van CAG in een gen dat codeert voor de α_{1A}-subunit van het P/Q-spanningsafhankelijke calcium kanaal ('voltage-gated calcium channel (P/Q-VGCC)'. P/Q-VGCC komen niet alleen in het centrale zenuwstelsel tot expressie, maar ook ter plaatse van de neuromusculaire overgang. De aanwezigheid van P/Q VGCC ter plaatse van de neuromusculaire overgang suggereert dat neuromusculaire transmissie gecompromitteerd zou kunnen zijn in SCA6. Om deze hypothese te testen onderzochten we de functie van de neuromusculaire overgang met behulp van single fiber EMG bij tien patiënten met SCA6. Afwijkingen in de neuromusculaire overgang werden niet gevonden. Hiermee toont onze studie aan dat SCA6 veroorzaakt wordt door ziekteprocessen die specifiek optreden in het centrale zenuwstelsel.

In **sectie IV** concentreerden wij ons op het fenotype van de autosomaal recessieve cerebellaire syndromen. De ontdekking van het frataxine gen in 1996 en de ontdekking dat andere relatief zeldzame ataxieën wereldwijd voorkomen suggereren dat een genetische classificatie voor de recessieve ataxieën slechts een kwestie van tijd is. Vooralsnog moet de clinicus het echter doen met een vaak lastige klinische work-up en een verouderd klinisch classificatiesysteem. Het ontwikkelen van een acceptabel klinisch classificatiesysteem en een realistisch 'work-up' schema zijn daarom essentieel voor een goede patiëntenzorg.

De aanwezigheid van neuropathie en het type neuropathie hebben differentieel diagnostische consequenties en kunnen daarom een gids zijn voor het al dan niet aanvragen van aanvullend diagnostisch onderzoek. In *hoofdstuk 9* worden hiertoe de resultaten beschreven van klinisch onderzoek en zenuwgeleidingsonderzoek bij 15 patiënten met een 'early onset cerebellar ataxia' (EOCA). Drie groepen konden worden onderscheiden: groep I met behouden reflexen en normale geleidingssnelheden, groep II met een beginleeftijd in het tweede decade, afwezigheid van voetafwijkingen en een milde motore axonale neuropathie en groep III met piramidale en atactische verschijnselen en een ernstige sensorische en motorische axonale neuropathie. In hoeverre dit onderscheid fundamentele fenotypische verschillen reflecteert en dus gebruikt kan worden voor aanvullende DNA research moet verder worden onderzocht.

In *hoofdstuk 10* evalueerden we de klinische verschijnselen, progressie en neurofysiologische bevindingen van EOCA patiënten die onze polikliniek bezochten over een periode van ongeveer 10 jaar.
Patiënten met een ongecompliceerd cerebellair syndroom bleken een heel langzame progressie te vertonen en een relatief goede prognose te hebben. De meeste patiënten met een cerebellaire ataxie en een neuropathie waren rolstoelgebonden; nog slechts een paar waren ambulant. Geconcludeerd werd dat bij patiënten met een autosomaal recessieve ataxie, waarbij middels uitgebreid aanvullende diagnostiek bekende metabole en genetische aandoeningen zijn uitgesloten, een klinische classificatie van belang is voor counseling met betrekking tot de prognose.

Dankwoord

Mijn dank gaat uit naar eenieder die heeft bijgedragen aan de totstandkoming van dit proefschrift. Een aantal personen heeft dat gedaan op een indirecte manier. Zij kennen mijn passie voor onderzoek en gunnen mij het plezier. Zij zijn er voor de lol, de liefde en de vriendschap; de essentie:

Carina: vrolijkheid
Meike en Wouter: verwondering
Klaas: vriendschap
Ouders en schoonouders: onvoorwaardelijk

Anderen waren direct betrokken. Een aantal van hen zou ik hier willen noemen:

Gerard Hageman, voor je humor, bescheidenheid, jarenlange steun en vertrouwen en voor je aanstekelijk enthousiasme bij de patiëntenzorg, het onderwijs en het onderzoek.

Berry Kremer, voor je liefde voor het vak, je buitengewone collegialiteit en je kritische commentaar en morele steun bij de totstandkoming van dit proefschrift.

Machiel Zwarts, voor je vertrouwen, je manier van werken en het feit dat de deur van je werkkamer altijd wagenwijd openstaat.

Baziel van Engelen, voor het evenwicht, de interactie en het feit dat je me naar Nijmegen hebt gehaald.

Martha Huvenaars, voor je kennis en betrokkenheid bij patiënten op de neuromusculaire en neurodegeneratieve polikliniek.

Raymond Menkenhorst en Arnoud Kappelle, de paranimfen. Voor cafébezoek, culinaire kennis en organisatietalent.

Bas Bloem voor borrelhap, Gewürztraminer en tomeloos enthousiasme.

Rianne Esselink, voor je betrokkenheid en collegiale houding: eerst als arts-assistent toen ik co-assistent was, later als 'oudste assistent' toen ik pas begon en nu als collega alhier.

Anneke Ramdhani-Joosten en de andere arts-assistenten van het Medisch Spectrum Twente, Enschede voor solidariteit en tranentrekkende humor.

De afdeling KNF, mijn huiskamer in dit ziekenhuis. May Eikholt en Henny Janssen in het bijzonder, maar ook Netty, Mirjam, Maarten, José, Yvonne en de anderen.
Leo en Norbert, daar waar alles vastliep.

Dorien Langenkamp en Marie-Louise Vennemans-Kropmans voor correctie van de Nederlandse samenvatting van dit proefschrift.
Secretaresses van de vijfde, Emilie Langstraat in het bijzonder, voor hard werken en vrolijk gelach.

De assistenten Neurologie: 'for those who keep the show on the road' met kamer 5135 en 5141 als vraagbaak en IT-ondersteuning.

En tot slot de afdeling Neurologie Nijmegen met als hoofd George Padberg voor het creëren van een uitermate inspirerende, motiverende en vriendelijke werkomgeving.

Curriculum vitae

Helenius Jurgen Schelhaas was born on 15 October 1967 in Hoogeveen, the Netherlands. In 1985 he completed secondary school at the Menso Altinck College in Hoogeveen and started his medical training at the State University of Groningen in 1988. He obtained his medical degree in 1994. In 1995 he moved to England and later to Northern Ireland, where he worked for seven months as house officer internal medicine and for six months as house officer psychiatry, respectively. During this year he not only enjoyed his avocation walking and history but also wrote his first scientific manuscript. On his return to the Netherlands in 1996, he became a resident at the Department of Neurology at the Medical Spectrum Twente, Enschede (Dr. J.A.G. Geelen). While working here, he developed an interest in autosomal dominant and autosomal recessive cerebellar disorders under the encouragement of Dr. G. Hageman. The last year of his residency was spent at Radboud University Nijmegen Medical Center, where he learned more about neuromuscular disorders and neurophysiological techniques, in particular nerve conduction studies and EMG (Prof. G.W.A.M. Padberg and Prof. M.J. Zwarts) On completion of his residency in February 2002 he was appointed member of staff at the Department of Neurology, Radboud University Nijmegen Medical Center, but continued to work one day a week at the Neurophysiology Laboratory. He is an active member of the Dutch Neuromuscular Research Support Center Website Committee since 2003 and was part of the editorial staff of the 'amyotrophic lateral sclerosis manual for patients and relatives' in 2004.

List of publications

Schelhaas HJ, Van de Warrenburg BP. Clinical, psychological, and genetic characteristics of spinocerebellar ataxia type 19 (SCA19). Cerebellum (in press).

Voermans NC, Vaneker M, Hengstman, GJD, ter Laak H, Zimmerman C, Schelhaas HJ, Zwarts MJ. Primary respiratory failure in inclusion body myositis. *Neurology* 63:2191-2192, 2004.

Hengstman GJ, Schelhaas HJ, Zwarts MJ. Auditory dysfunction in chronic inflammatory demyelinating polyradiculoneuropathy. *Neurology* 62: 1446-1448, 2004.

Schelhaas HJ, Van de Warrenburg BP, Kremer HP, Zwarts MJ. Neuromuscular transmission in SCA6. *Ann Neurol* 55: 451-452, 2004.

Van de Warrenburg BP, Notermans NC, Schelhaas HJ, van Alfen N, Sinke RJ, Knoers NV et al. Peripheral nerve involvement in spinocerebellar ataxias. *Arch Neurol* 61: 257-261, 2004.

Van de Warrenburg BP, Schelhaas HJ. Zwarts MJ, Kremer HPH. Spinocerebellar Ataxia and Peripheral Neuropathy. *Author reply Arch Neurol* 61: 1626, 2004.

Schelhaas HJ, Verbeek DS, van de Warrenburg BP, Sinke RJ. SCA19 and SCA22: evidence for one locus with a worldwide distribution. *Brain (electronic version)* 127(Pt 1): E6, 2004.

Kappelle AC, Schelhaas HJ, Pasman JW, Bloem BR. Rotatie van het hoofd: een ongebruikelijke compensatie voor een abductie beperking van een oog. *Nederlands Tijdschrift voor Geneeskunde* 148: 2084-2085, 2004.

Schelhaas HJ, van de Warrenburg BP, Kremer HP, Zwarts MJ. The "split hand" phenomenon: evidence of a spinal origin. Neurology 61: 1619-1620, 2004.

Schelhaas HJ, van de Warrenburg BP, Hageman G, Ippel EE, van Hout M, Kremer B. Cognitive impairment in SCA-19. *Acta Neurol Belg* 103: 199-205, 2003.

Schelhaas HJ, Van Engelen BG, Gabreels-Festen AA, Hageman G, Vliegen JH, Van der Knaap MS, Zwarts MJ. Transient cerebral white matter lesions in a patient with connexin 32 missense mutation. *Neurology* 59: 2007-2008, 2004.

Verbeek DS, Schelhaas JH, Ippel EF, Beemer FA, Pearson PL, Sinke RJ. Identification of a novel SCA locus (SCA19) in a Dutch autosomal dominant cerebellar ataxia family on chromosome region 1p21-q21. *Hum Genet* 111: 388-393, 2002.

Schelhaas HJ, Ippel PF, Hageman G, Sinke RJ, van der Laan EN, Beemer FA. Clinical and genetic analysis of a four-generation family with a distinct autosomal dominant cerebellar ataxia. *Journal of Neurology* 248:113-120, 2001.

Schelhaas HJ, Ippel PF, Beemer FA, Hageman G. Similarities and differences in the phenotype, genotype and pathogenesis of different spinocerebellar ataxias. *European Journal of Neurology* 7: 309-314, 2000.

Schelhaas HJ, Hulst MV, Ippel PF, Prevo RL, Hageman G. Early onset cerebellar ataxia with retained tendon reflexes: foot deformity in a first degree family member. *Clinical J Neurol Neurosurg* 101: 253-255, 1999.

Schelhaas HJ, Brouwers PJ, van der Aa HE, Prevo RL. MRI locates a posterior fossa aneurysm in the fourth ventricle. *Clinical J Neurol Neurosurg* 100: 216-218, 1998.

Vloedbeld MG, Schelhaas HJ, Hageman G, Vreuls RC, Rozeboom AR. Het syndroom van Joubert: kliniek en differentiaaldiagnostische overwegingen. *Nederlands tijdschrift voor kindergeneeskunde* 66: 202-205, 1998.

Schelhaas HJ, Hageman G, Post JG. Cerebellar ataxia, dementia, pyramidal signs, cortical cataract of the posterior pole and a raised IgG index in a patient with a sporadic form of olivopontocerebellar ataxia. *Clinical J Neurol Neurosurg* 99: 99-101, 1997.

Abbreviations

ABC7	*ATP-binding cassette transporter gene* (gene involved in X-linked sideroblastic anemia with ataxia)
ADCA	Autosomal dominant cerebellar ataxia
ADM	Abductor digiti minimi muscle
Alpha-TTP	*Alpha-tocopherol transfer protein gene*
AOA1	Ataxia with oculomotor apraxia type 1
AOA2	Ataxia with oculomotor apraxia type 2
APTX	*Aprataxin* (gene involved in AOA1).
ARSACS	Autosomal recessive spastic ataxia of Charlevoix-Saguenay
AT	Ataxia teleangiectasia
ATLD	Aaxia teleangiectasia like disorder
ATM	The gene responsible for AT (AT mutated)
AVED	Ataxia with isolated vitamin E deficiency
BDI	Beck Depression Inventory
CACNA1A	P/Q- voltage-dependent calcium channel (α_{1A}-subunit)
CCFDN	Congenital cataract, facial dysmorphism and peripheral neuropathy
CDG	Congenital disorders of glycosylation
C/EBP	CCAAT enhancer-binding protein
CMCT	Central motor conduction time
CNS	Central nervous system
CSF	Cerebro spinal fluid
DRPLA	Dentatorubropallidoluysian atrophy
DSMA	Dominant distal spinal muscular atrophy
EA-1	Episodic ataxia type 1
EA-2	Episodic ataxia type 2
EEG	Electroencephalography
EMG	Electromyography
EOCA	Early onset cerebellar ataxia
EPM1	Progressive myoclonus epilepsy of Unverricht-Lundborg
FGF14	Fibroblast growth factor 14 gene
FHM	Familial hemiplegic migraine
FMR-1	*Fragile X mental retardation gene type 1*
FRAXA	Fragile X syndrome (caused by FMR-1 mutation)
FRAXE	Fragile X syndrome (caused by FMR-2 mutation)
FRDA	Friedreich's ataxia
FXTAS	Fragile-X tremor ataxia syndrome (caused by a FMR-1 premutation)
GSS	Gerstmann Strausler Schlenker disease (prion disorder)
HD	Huntington disease
HDL2	Huntington disease like-phenotype-2
HIT	Histidine triad superfamily (involved in AOA1)
ICARS	International ataxia co-operative rating scale
ILOCA	Idiopathic late onset cerebellar ataxia
IL-1ra	Interleukin 1 receptor antagonist
IOSCA	Infantile onset spinocerebellar ataxia
IRF-1	Interferon-regulatory factor-1
JPH3	Junctophilin-3 gene (mutated in Huntington disease like-phenotype-2)
JMA	Juvenile muscular atrophy

LOD	Logaritm of the odds
MCD	Mean consecutive difference
MCV	Motor nerve conduction velocities
MEP	Motor evoked potentials
MERRF	Myoclonus epilepsy with ragged red fibers (mitochondrial disorder)
MJD	Machado Joseph disease (SCA3)
MMSE	Mini Mental State Examination
MRC	Medical Research Counsel examination
MRI	Magnetic resonance imaging
MRE11	Gene involved in Ataxia teleangiectasia like disorder (Meiotic Recombination)
MSA-C	Multiple system atrophy cerebellar type
NF-EOCA	Non-Friedreich early onset cerebellar ataxia
NII	Neuronal intranuclear inclusions
NMJ	Neuromuscular junction
OPCA	Olivopontocerebellar atrophy
OPMD	Oculopharyngeal muscle dystrophy
PI3K	Phosphatidyl-inositol 3-kinase
PIKKs	Phosphatidyl-inositol 3-kinase-like protein kinases
P/Q-VGCC	P/Q voltage gated calcium channel
Raven CPM	Raven colored progressive matrices
RMT-F	Recognition Memory Test for Faces
Raven SPM	Raven standard progressive matrices
SACS	Gene involved in ARSACS
SAN	'Stichting afasie Nederland' (cortical language test)
SBMA	Spinal and bulbar muscular atrophy
SCA	Spinocerebellar ataxia
SCAN1	Spinocerebellar ataxia with axonal neuropathy type 1
SETX	Gene involved in ataxia with oculomotor apraxia type 2 (Senataxin)
SFEMG	Single fiber electromyography
SNAP	Sensory nerve action potential
TBP	TATA binding protein
TFTC	TATA-binding protein-free TAF-containing complex
TDP1	*Tyrosyl-DNA phosphodiesterase 1 gene*
TTP	Tocopherol transfer protein (involved in AVED)
5' UTR	5' untranslated region (that part of DNA that is located before the start codon)
VLDL	Very low-density lipoprotein
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WCST	Wisconsin Card Sorting Test
WMS	Wechsler Memory Scale
XRCC1	X-ray repair cross-complementing group 1
YFH	Yeast frataxin homologue