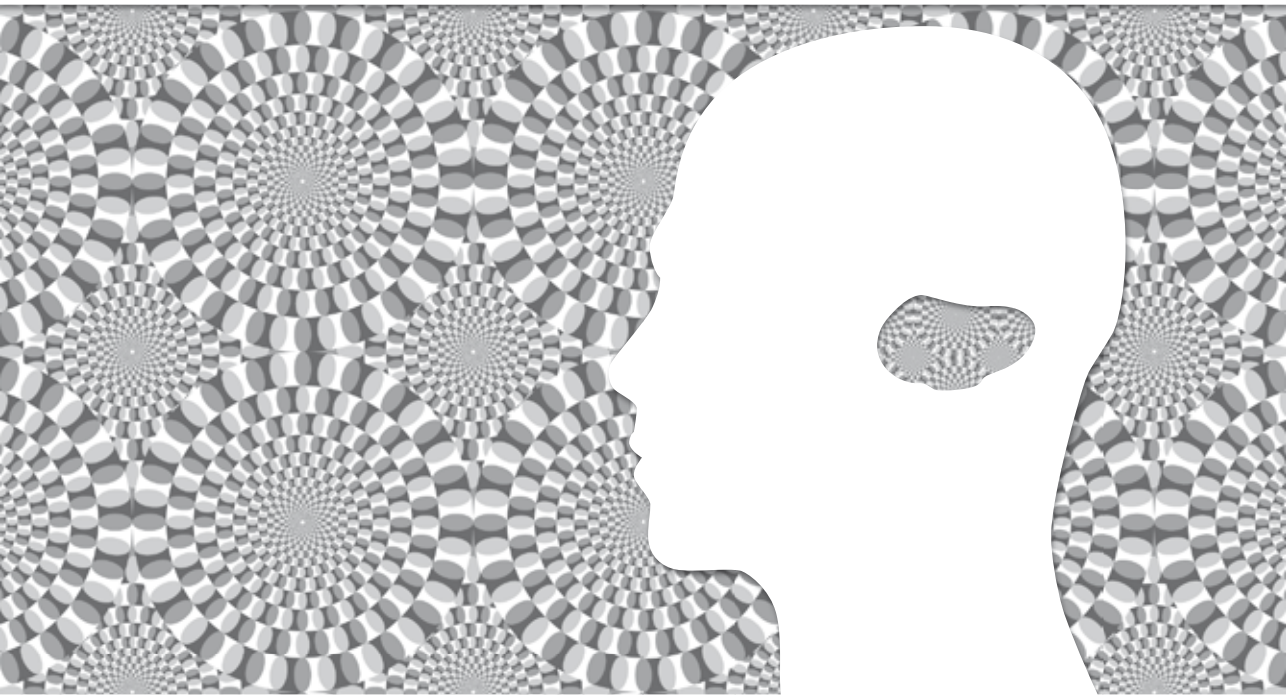

Adult-onset Cerebellar Ataxia

a clinical and genetic Survey

Esther Brusse

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Adult-onset cerebellar Ataxia

a clinical and genetic Survey

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een klinisch en genetisch overzicht

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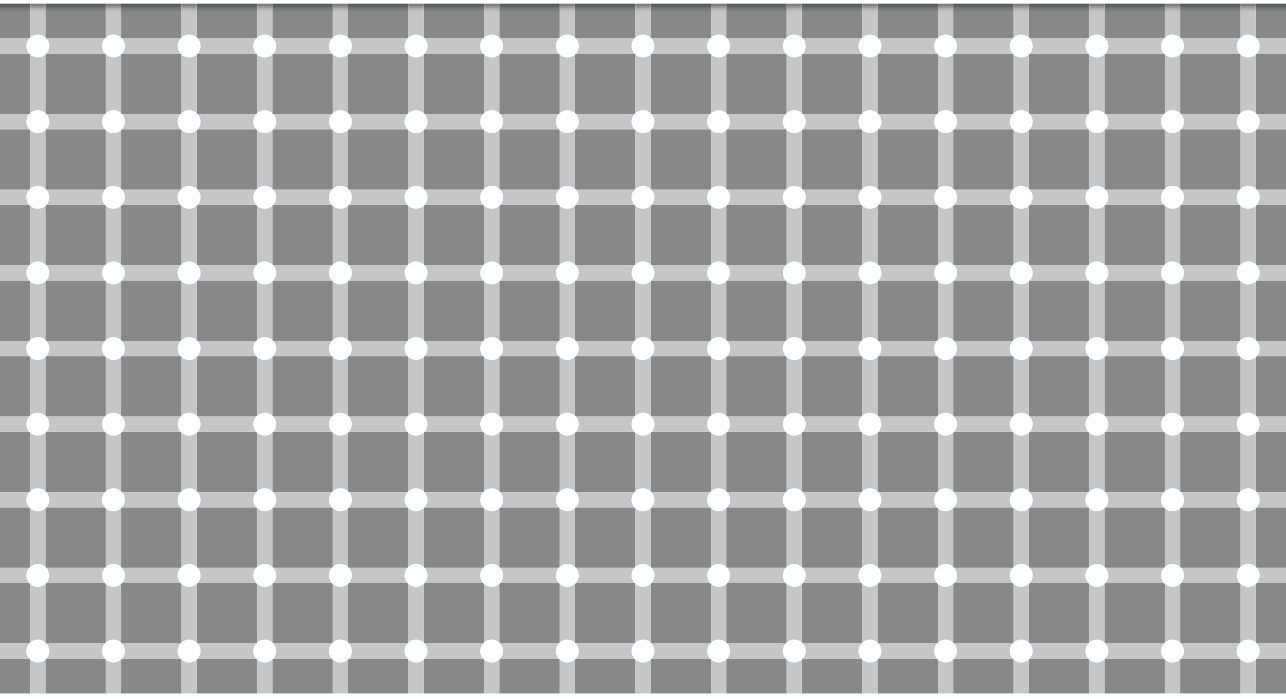
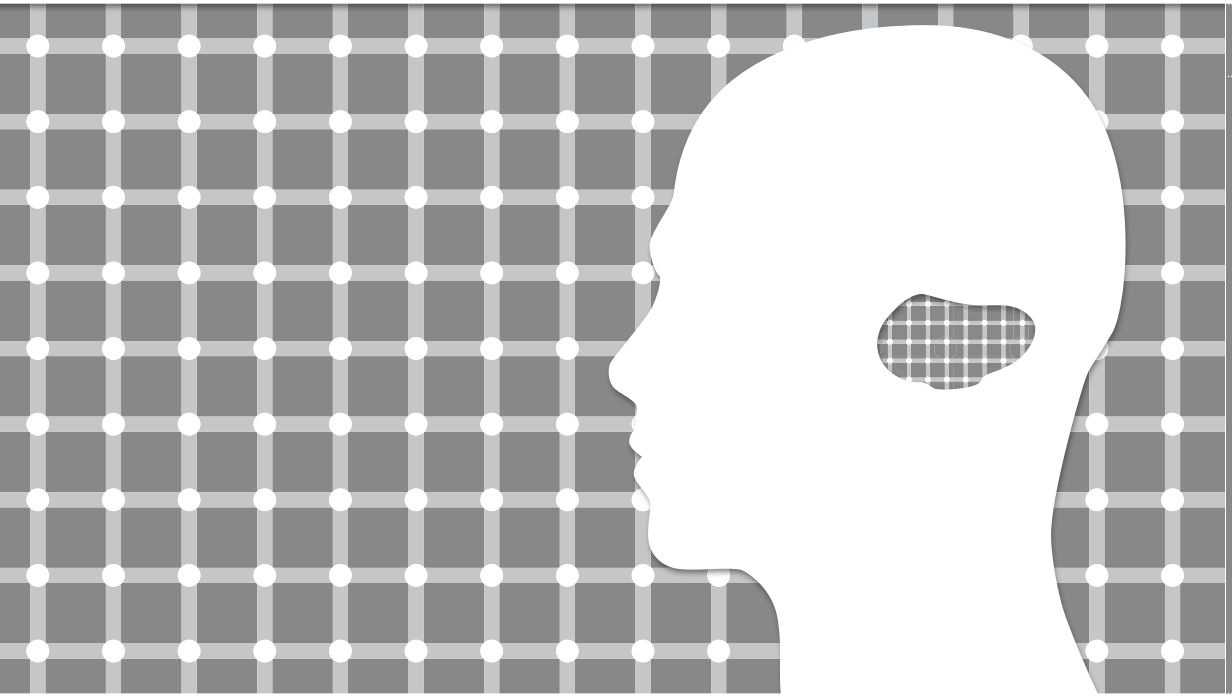


Chapter 1



Introduction and diagnostic classification

1.1 General introduction and scope of the thesis

The cerebellar ataxias (ataxia/ατα ξίς: ”lack of order” in Greek) represent a group of hereditary and sporadic neurodegenerative disorders, with progressive gait- and limb-ataxia or -incoordination as the most prominent clinical sign, although a variety of other neurological or systemic signs may co-exist.

The cerebellum plays a fundamental role in action control, regulating movement rate, smoothness and coordination. Both acute motor timing and long-term adaptation of movements are presumed to require fast signal processing in the cerebellum: the cerebellar cortex is able to transform external sensory information into a motor control signal in the millisecond range. To reach such a velocity, the cerebellum is hypothesized to use neural representations or “internal models,” enabling the motor cortex to use an internal feedback to perform an accurate movement. In this operational model, the cerebellar granular layer is thought to act as a timing control system by processing incoming signals and generating patterns of timed circuits. These patterns can be adapted and fine-tuned through learning: the cerebellar network can learn and make appropriate predictions using error information, for instance about the discrepancies between the actual and predicted sensory effects of limb movements and postural adjustments. Distortion of these internal feedback and predictive feedforward control systems, processed via the corticopontocerebellar tract, inferior olive and thalamus, causes cerebellar dysmetria.[1,2,3]

The hereditary cerebellar ataxias comprise disorders with autosomal dominant, autosomal recessive and X-linked inheritance, as well as disorders related to mitochondrial DNA mutations. Most cases of cerebellar ataxia are sporadic. Sporadic cerebellar degeneration is represented by a wide range of toxic, metabolic, paraneoplastic and multisystem neurodegenerative disorders, like multisystem atrophy (MSA). There may likely be up to 100 different causes of cerebellar ataxia, each with a distinct genetic or molecular etiology and this causative heterogeneity is still expanding: in autosomal dominant cerebellar ataxia (ADCA), for instance, already 28 genetic SCA-loci have been identified until September 2010.[4,5] The precise incidence and prevalence of adult-onset cerebellar ataxias are unknown, however, a population-based study of adult-onset (>18 years) cerebellar ataxia in Wales resulted in an estimated minimum prevalence of 11.3 per 100.000.[6] The lowest estimated prevalence of autosomal dominant cerebellar ataxia (ADCA) in The Netherlands is 2.8 per 100.000.[7] There are founder effects in the genes for SCA2 (40/100 000 cases) in Cuba and SCA3 in Portugal.[8-10] The prevalence of Friedreich ataxia and MSA are estimated 2-4 and 4.4 per 100.000 respectively.[4,11]

Due to the ongoing identification of genes associated with hereditary cerebellar ataxias, the understanding of the pathophysiological mechanisms likely to be involved in cerebellar dysfunction, is considerably expanding. Although this has not resulted in curative therapeutic strategies yet, this expanding knowledge will hopefully facilitate the development of therapy.

An accurate diagnosis in cerebellar ataxia is important to provide proper genetic counseling and insight in prognosis. Furthermore, specific diagnoses may have therapeutic implications: sporadic cerebellar ataxias for example, may be associated with treatable disorders, such as vitamin deficiency, intoxications with specific drugs or paraneoplastic syndromes such as anti-Yo and anti-Hu associated paraneoplastic cerebellar degeneration or a cerebellar variant of Lambert-Eaton myasthenic syndrome. Also, in some hereditary cerebellar ataxias early treatment is warranted to stabilize or improve symptoms, like in ataxia with vitamine E deficiency (AVED) and in cerebrotendinous xanthochromatosis (CTX). In general, when therapeutic strategies will become available, an early diagnosis may be important to prevent further cerebellar degeneration.[11]

Scope of the thesis

In 2001, the departments of Neurology and Clinical Genetics of the Erasmus MC University Medical Center collaborated to set up a combined neurogenetic consultancy for patients with hereditary neurological disorders. This combined neurologic and genetic clinical evaluation of patients with cerebellar ataxia served as the basis of this thesis. Our goals were ambiguous: we aimed to improve diagnostic and, when possible, therapeutic strategies for patients with cerebellar ataxia, as well as to identify new genes in families with unknown genetic deficits and subsequently, to perform genotype-phenotype correlations.

In **chapter 1.2**, a diagnostic algorithm for early- and late-onset cerebellar ataxia is presented, based on published expert opinions and diagnostic criteria, that we processed in a consensus meeting, joined by neurologists and geneticists from our clinic. The algorithm aimed to facilitate and standardize the diagnostic process in hereditary and sporadic cerebellar ataxias. This is combined with an overview of the clinical and genetic characteristics of these cerebellar syndromes. In **chapter 2**, we describe the results of a study on fatigue in cerebellar ataxia, since fatigue has frequently been reported as a severe and disabling complaint in our patients. This study analyzed the presence of fatigue and predictive factors for its severity in autosomal dominant cerebellar ataxia, using self-assessment scales.
The second part of this thesis describes genotype-phenotype analyses in several families with cerebellar ataxia of unknown genotype, that we identified from the neurogenetic consultation population. Linkage analysis in a family with autosomal dominant

cerebellar ataxia (ADCA) resulted in the identification of SCA27, with a mutation in the *FGF14* gene, as a novel SCA genotype. This is described in **chapter 3.1**. An extensive clinical description of this family is presented in **chapter 3.2**, including brain imaging, neurophysiological and neuropsychological examination. The SCA27 phenotype in our family displayed a remarkable early onset, predominant tremor and cognitive dysfunction. In **chapter 3.3**, a family with a childhood-onset, slowly progressive autosomal recessive spinocerebellar ataxia is described, in which a new locus was identified, mapping to chromosome 11p15. **Chapter 3.4** encounters a clinical and genetic evaluation of a large family with a cerebellar ataxia related to the mitochondrial G8363A mutation in the tRNA(Lys) gene. We distinguished a cerebellar and a neuromuscular onset phenotype, further delineating the phenotypical variation in this tRNA mutation, and we related the phenotype to the mutational load. Finally, the implications of the studies presented in this thesis for clinical practice, and indications for further research are delineated in **chapter 4**.

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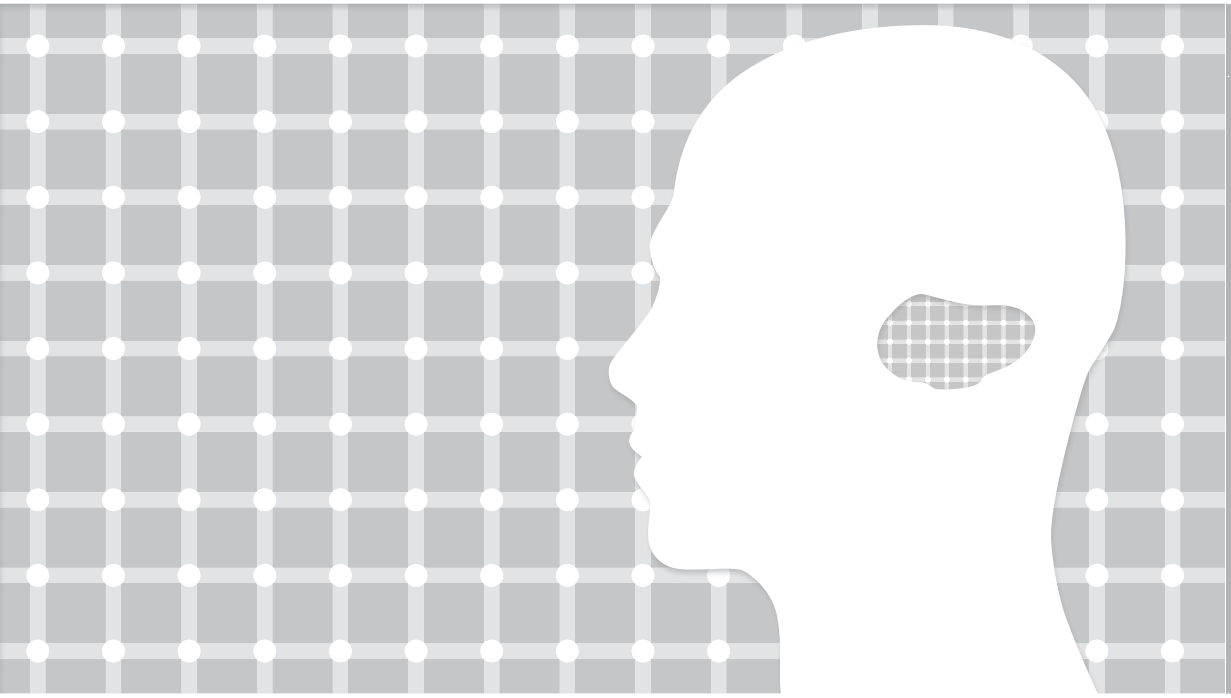


Diagnosis and management of early- and late-onset cerebellar ataxia

E. Brusse, J.A. Maat-Kievit, J.C. van Swieten

Clin Genet. 2007 Jan;71(1):12-24

Abstract

Cerebellar ataxias represent a heterogeneous group of neurodegenerative disorders. Two main categories are distinguished: hereditary and sporadic ataxias. Sporadic ataxias may be symptomatic or idiopathic. The clinical classification of hereditary ataxias is nowadays being replaced by an expanding genotype-based classification. A large spectrum of degenerative and metabolic disorders may also present with ataxia early or late in the course of disease. We present a diagnostic algorithm for the adult patient presenting with subacute cerebellar ataxia, based on family history and straightforward clinical characteristics of the patient. Along with the algorithm, an overview of the autosomal dominant, autosomal recessive, X-linked, mitochondrial, symptomatic and idiopathic subtypes of cerebellar ataxia is presented. An appropriate diagnosis is of utmost importance to such considerations as prognosis, genetic counseling and possible therapeutic implications.

1.2 Diagnosis and management of early- and late-onset cerebellar ataxia

Cerebellar ataxias are a group of neurodegenerative disorders characterized by progressive degeneration of the cerebellum and often accompanied by a variety of neurological and other systemic symptoms.
Originally, nosology of ataxia was based on neuropathologic criteria, distinguishing spinocerebellar degeneration, and olivopontocerebellar atrophy.[1] More recently, Harding introduced a clinical classification of the hereditary ataxias. She also introduced the term idiopathic late onset cerebellar ataxia (IDCA, also referred to as ILOCA) for sporadic adult-onset ataxias.[2–4] Nowadays, this clinical classification is being replaced by an expanding genotype-based classification. For example, 26 different genetic subtypes of spinocerebellar ataxia (SCA) can now be distinguished in autosomal dominant cerebellar ataxias (ADCA).

In the diagnostic work-up of a patient presenting with subacute cerebellar ataxia, a large spectrum of neurodegenerative and metabolic disorders may also be considered. First of all, we distinguish two major categories: hereditary and sporadic ataxias. Hereditary ataxias can have an autosomal dominant, autosomal recessive, X-linked or mitochondrial mode of inheritance. Sporadic ataxias can be symptomatic or idiopathic *(Table 1)*. We present a diagnostic algorithm that can provide assistance in the challenging diagnostic process of subacute cerebellar ataxias with onset in (early) adulthood. This algorithm is based on straightforward clinical characteristics of the patient and family history *(Fig. 1)*. A selection of clinical and genetic characteristics of the hereditary and sporadic ataxias, as listed in *Table 1*, will be included in the discussion of the algorithm.
This review, including the algorithm, focuses on ataxias presenting in (early) adulthood. Although there is an overlap with childhood ataxias, the latter are more often of congenital, metabolic or syndrome-associated origin. This requires other diagnostic considerations, which are beyond the scope of this review.

Table 1 Classification of cerebellar ataxias

I. Hereditary cerebellar ataxias

a. Autosomal Dominant Cerebellar Ataxias (ADCA)

1. Episodic Ataxias (types 1 - 6)
2. Spinocerebellar Ataxia (SCA) subtypes 1-28
3. Dentatorubral-pallidoluysian atrophy (DRPLA)

b. Autosomal Recessive cerebellar ataxias

1. With identified gene-defect
2. With identified gene-locus
3. As part of metabolic disorder, extended disease
4. Other metabolic and degenerative disease with congenital or childhood onset

c. X-linked cerebellar ataxia

1. Adrenoleukodystrophy
2. Fragile-X tremor ataxia syndrome (FXTAS)
3. Hereditary sideroblastic anaemia and ataxia
4. Other X-linked congenital and childhood ataxias

d. Mitochondrial cerebellar Ataxia

II. Sporadic cerebellar ataxia

a. Symptomatic cerebellar ataxia

1. Structural lesions, malformations
2. Toxic
   - Alcohol
   - Drugs
     a. Anti-epileptic drugs
     b. Benzodiazepines
     c. Lithium
     d. Antineoplastics
     e. Others (see text)
   - Others
     a. Heavy metals (mercury, lead)
     b. Chemicals (solvents, pesticides)
3. Endocrine
   - Hypothyroidism
4. Malabsorption
   - Celiac disease (gluten ataxia)
   - Vitamin deficiency
5. Miscellaneous
   - Paraneoplastic syndromes
   - Demyelinating disorders
6. Inflammatory
   - Whipple disease
   - Postviral/ immune-mediated ataxia

b. Idiopathic

1. Multiple system atrophy (MSA)
2. Idiopathic late-onset cerebellar ataxia (ILOCA)

The diagnostic algorithm

Magnetic resonance imaging of the brain

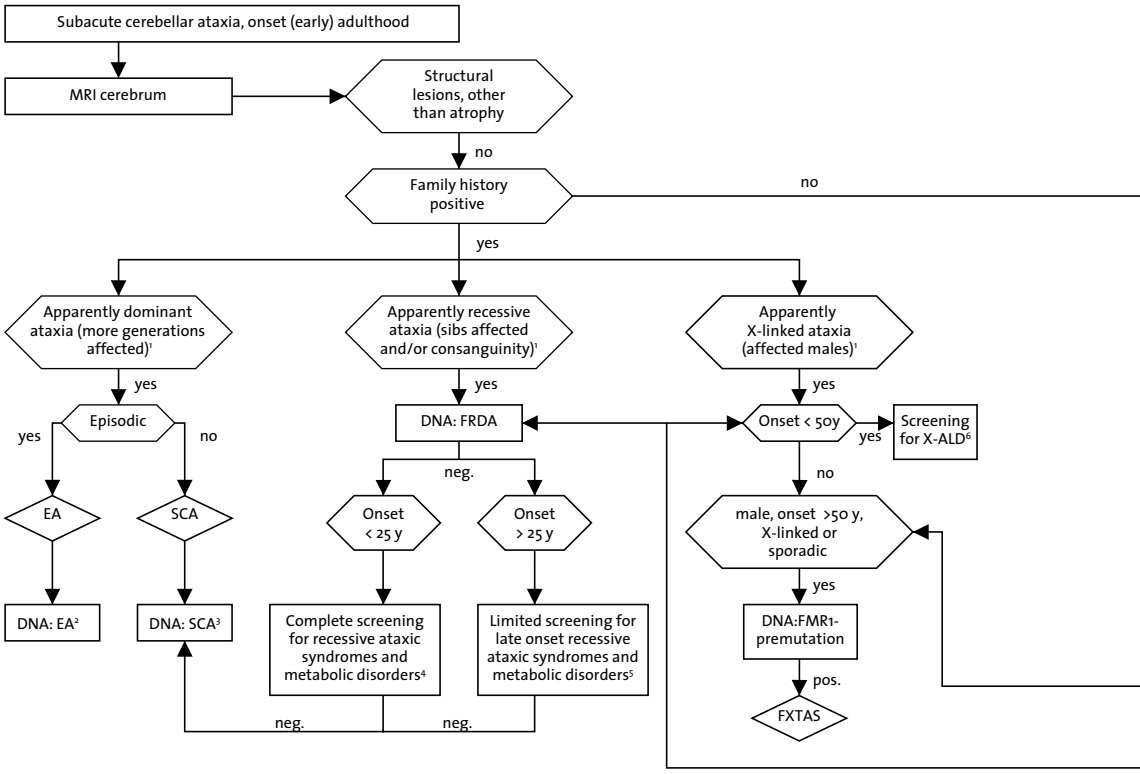
The diagnostic process starts with magnetic resonance imaging (MRI) of the brain. This may identify structural lesions that may be the cause of symptomatic ataxias. Cerebellar atrophy is a nonspecific feature. Other features may be more specific, such as white matter lesions in the cerebellar pedunculi, seen in fragile-X-associated tremor ataxia syndrome (FXTAS).

Family history

In patients with a positive family history, a detailed genealogy will often reveal an apparent mode of inheritance. The presence of ataxia in consecutive generations or the presence of male-to-male inheritance is suggestive of an autosomal dominant inheritance. The presence of multiple affected sibs in a single generation, or consanguinity in the parents, supports the idea of an autosomal recessive mode of inheritance. In a disorder affecting only males in one or more generations in the maternal line, X-linked inheritance can be considered. It is important to realize that an ADCA may present as an apparently sporadic or recessive ataxia: relatives who carry the mutation may be clinically unaffected due to anticipation with later onset and milder phenotype in elderly generations, or due to reduced penetrance. Furthermore, a negative family history cannot rule out a hereditary ataxia, for example in relation with a de novo mutation. A genetic defect may be identified in 2–19% of the sporadic ataxias, most frequently representing Friedreich ataxia (FA) or SCA6.[5–7]

Mitochondrial diseases may represent different modes of inheritance because mutations may originate in nuclear or mitochondrial DNA (mtDNA). Furthermore, mtDNA mutations may mimic autosomal dominant, recessive or X-linked inheritance due to phenomena such as heteroplasmy and threshold effects for biochemical mutation expression. Moreover, deletions and insertions in mtDNA are often sporadic.[8] Consequently, the algorithm does not provide a diagnostic tool for mitochondrial disease based on family history. Specific phenotypic features of the patient (and family) may suggest mitochondrial ataxia, often associated with typical mitochondriopathies, as described later.

Figure 1 **Diagnostic algorithm for patients with (early) adult onset subacute cerebellar ataxia.**



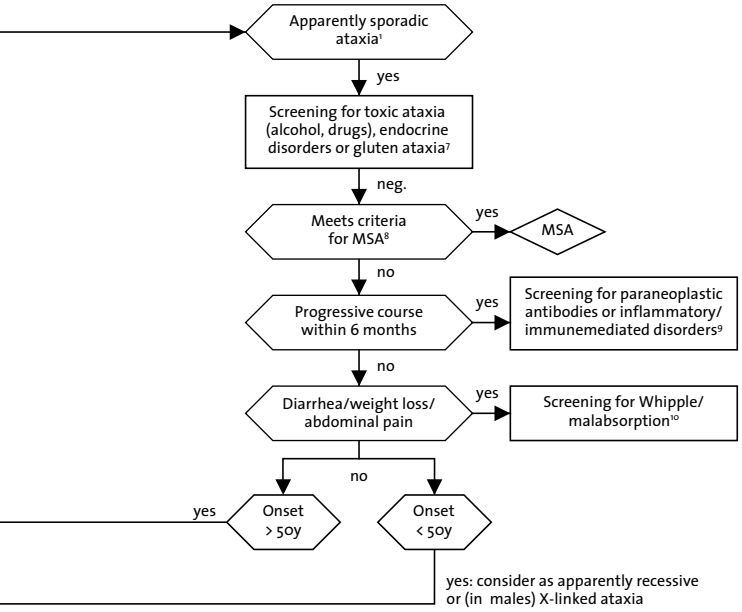
Notes (Supplementary information for Figure 1)

1. To obtain further phenotypic clues, consider for example electromyography, ophthalmologic or cardiologic screening or consultation of clinical geneticist.

2. EA1 versus EA2

EA1	EA2
KCNA1-gene	CACNA1A-gene
Kinesiogenic EA	Non-kinesiogenic EA
Brief attacks (< 15 minutes)	Variable attacks: minutes-hours (days)
Interictal myokemia	Eventually interictal cerebellar signs (nystagmus, ataxia)
Acetazolamide may be effective	Acetazolamide very effective

3. SCA with retinopathy: start with SCA7; otherwise start with SCA 1,2,3,6, and 7; second line testing: DRPLA, SCA(8),12,14, 17, 27 (adjust for local situation, depending on regional prevalences of SCA-subtypes and availability of gene-analysis).



4. Laboratory studies for recessive ataxia, onset < 25 years

Study	Indicative for
Vitamin E, acanthocytes	AVED, abetalipoproteinaemia, chorea-acanthocytosis
Cholesterol, triglycerides, LDL, VDL	abetalipoproteinaemia
Lactate, pyruvate	Mitochondrial disease
Protein, albumin, immunoglobulins IgA, IgG, IgE, α-fetoprotein	Ataxia teleangiectasia, AOAI/II
24 hours urine sample: metabolic screening and bile alcohols	General screening and cerebrotendinous xanthomatosis
Plasma: phytanic acid; copper, ceruloplasmin	Refsum; Wilson, aceruloplasminaemia.
Heparin: beta-hexosaminidase; beta-galactosidase; arylsulfatase-A; beta-galactocerebrosidase; neuraminidase	GM2 gangliosidosis (hexosaminidase deficiency); GM1 gangliosidosis; MLD (metachromatic leucodystrophy); Krabbe; sialidosis
Skin biopsy	Niemann-Pick type C

When metabolic screening is indicative, check the availability of DNA-screening for this disorder with a clinical geneticist or on the internet.

Notes (Supplementary information for Figure 1) *continued*

5. Limited screening for recessive ataxia, onset > 25 years

Study	Indicative for
Vitamin E, acanthocytes	AVED, chorea-acanthocytosis
Lactate, pyruvate	Mitochondrial disease
Protein, albumin, immunoglobulins IgA, IgG, IgE, α-fetoprotein	Ataxia teleangiectasia,
Plasma: phytanic acid; copper, ceruloplasmin	Refsum; Wilson, aceruloplasminaemia.
Heparin: beta-hexosaminidase; arylsulfatase-A; beta-galactocerebrosidase.	GM2 gangliosidosis (hexosaminidase deficiency); GM1 gangliosidosis MLD, Krabbe
Skin biopsy	Niemann-Pick type C

6. Plasma: VLCFA (very long chain fatty acids) (screening leukodystrofies only when white matter lesions on brain-MRI).

7. Screening for toxic ataxia, endocrine disorders or gluten ataxia

Study	Indicative for
Hb, Ht, electrolytes, liver tests (blood levels of anti-epileptic drugs)	Intoxications (alcohol, drugs)
Glucose, TSH	Endocrine disorders
Antigliadin antibodies, anti-endomysial antibodies	Celiac disease, gluten ataxia

8. Criteria for MSA *(see ref. 58)*

I. Possible MSA	One criterion plus two features from separate other domains
II. Probable MSA	Criterion for autonomic failure or urinary dysfunction plus poorly levodopa-responsive parkinsonism or cerebellar dysfunction
III Definite MSA	Pathologically confirmed

Clinical domains, features and criteria, used in the diagnosis of MSA *(see ref 58)*

Domain	Features	Criteria
I. Autonomic and urinary dysfunction	1. orthostatic hypotension 2. urinary incontinence or incomplete bladder emptying	orthostatic fall in bloodpressure (by 30 mm Hg systolic or 15 mm Hg diastolic) or urinary incontinence (persistent, involuntary partial or total bladder emptying, accompanied by erectile dysfunction in men) or both.
II. Parkinsonism	1. bradykinesia 2. rigidity 3. postural instability (not caused by visual, vestibular cerebellar or proprioceptive dysfunction) 4. tremor (postural, resting or both)	bradykineasia plus at least one of features 2-4
III. Cerebellar dysfunction	1. gait ataxia 2. ataxic dysarthria 3. limb ataxia 4. sustained gaze-evoked nystagmus	gait ataxia plus at least one of features 2-4
IV. Corticospinal tract dysfunction	1. extensor plantar responses with hyperreflexia	no corticospinal tract features are used in defining the diagnosis of MSA

Exclusion criteria for MSA

I. History	Onset < 30 years, positive family history, other identifiable (systemic) causes, hallucinations unrelated to medication.
II. Physical examination	DSM IV criteria for dementia, prominent slowing of vertical saccades or supranuclear gaze palsy, evidence of focal cortical dysfunction such as aphasia, alien limb syndrome
III Laboratory investigation	Metabolic, molecular genetic and imaging evidence of an alternative cause or features

Notes (Supplementary information for Figure 1) *continued*

9. Paraneoplastic antibodies

antibody	Co-existing symptoms (apart from ataxia)	Associated cancers
Anti-Yo	-	Gynaecological, breast
Anti-Tr	-	Hodgkin Lymphoma
Anti-mGluR1-α	-	Hodgkin Lymphoma
Anti-Zic4	-	Small cell lung carcinoma (SCLC)
Anti-Hu	Encephalomyelitis, limbic encephalitis, sensory neuronopathy, autonomic dysfunction	SCLC, neuroblastoma, sarcoma, other
Anti-Ri	Opsoclonus-myoclonus, brainstem encephalitis	Breast, gynaecological, SCLC
Anti-Ma	Limbic and brainstem-encephalitis, Opsoclonus-myoclonus	Breast
Anti-PCA2	Encephalomyelitis	SCLC
Anti-CRMP	Encephalomyelitis, chorea, polyneuropathy	SCLC, thymoma, germ cell tumours of testis
Anti-VGCC	Autonomic dysfunction, Lambert Eaton myastenic syndrome	SCLC

Lumbar puncture in screening for inflammatory/ immune-mediated disorders

Study	Indicative for
Mononuclear and polynuclear lymphocytes, glucose	Infections, immune-mediated disorders
Protein, IgG-index (immune-electrophoresis)	Inflammation, immune-mediated disorders, demyelinisation
Consider: lactate, pyruvate	Mitochondrial disorders
Consider: viral/infectious screening	Borrelia, Cryptococcus, toxoplasmosis, leptospirosis, tuberculosis, herpes, EBV, Coxsackie, Echo, HIV, HTLV1

10. Laboratory studies for Whipple and malabsorption

Study	Indicative for
Vitamin E, B1, B6, B12, cholesterol triglycerides, LDL, VDL	Malabsorption, vitamin deficiency
PCR tropheryma whippelii	Whipple disease

Apparently dominant ataxias

Autosomal dominant cerebellar ataxias

SCA constitutes the main group in the genetic classification of ADCA, with the current identification of 26 subtypes. Furthermore, a clinically complex form (dentatorubral-pallidoluysian atrophy, DRPLA) and six episodic ataxias (EA) are distinguished *(Tables 1 and 2)*.[9–11] Other rare autosomal dominant disorders, like hereditary spastic ataxias, sensory motor neuropathy with ataxia and adult-onset leukodystrophy, may also present with ataxia, but these heterogeneous, rare disorders will not be considered in this overview.[11,12] Although most patients with EA2 develop interictal cerebellar signs, the episodic occurrence of symptoms differentiates EA from SCA.[13]

Episodic ataxias.

EA1, EA2 and EA5 are channelopathies associated with mutations in genes encoding voltage-dependent potassium (EA1) and calcium (EA2 and 5) channel subunits. In EA6, mutations in *SLC1A3* have been found, a sodium-dependent transporter molecule regulating neurotransmitter concentrations.[14] The genetic locus in EA3 has been found, whereas the genetic defect in EA4 still awaits identification *(Table 2)*.

EA1 and EA2 can be distinguished on clinical grounds, EA5 has an EA2 phenotype and EA6 is associated with seizures.[13, 14] Movement-induced or the so-called kinesigenic attacks of ataxia, being provoked by exercise, startle and change of position, are characteristic for EA1, whereas caffeine, alcohol and phenytoin often trigger attacks in the nonkinesigenic EA2. In both subtypes, stress may also induce attacks. Typically, onset of EA occurs in childhood or early adulthood, although the onset of EA2 may be up to the fifth decade. Attacks in EA2 can last hours to days, whereas the attacks in EA1 are shorter (<15 min) but more frequent (up to 15 times a day). Tremor, muscle cramps and stiffening of extremities often accompany ataxia in EA1. In EA2, ataxia is often associated with nausea, migrainous headache and (hemi)paresis and sometimes also with dystonia, diplopia and tinnitus. Interictal myokymia of the face, arms and legs is seen in EA1, whereas permanent interictal cerebellar signs (nystagmus, limb ataxia) develop during the course of EA2.

Acetazolamide is an effective therapy in most patients with EA2 and in half of the patients with EA1; phenytoin is an alternative therapy in EA1*(Fig. 1, note 2)*.[13,15]

Apart from EA2, mutations in the *CACNA1A* gene are also associated with familial hemiplegic migraine (FHM) and SCA6. Evident clinical overlap exists between EA2, FHM and SCA6, even within families.[13,15]

Spinocerebellar ataxias and dentatorubral-pallidoluysian atrophy.

The relative prevalence of various SCA subtypes varies considerably between different countries due to founder effects. However, worldwide SCA1, 2, 3, 6 and 7 explain

Table 2 The autosomal dominant cerebellar ataxias

name	Gene/gene-product	locus	Mean age at onset (range)	Clinical testing available
SCA1	*ATXN1*/ataxin-1	6p23	37 (<10 to >70)	+
SCA2	*ATXN2*/ataxin-2	12q24	32 (<10 to >60)	+
SCA3	*ATXN3*/ataxin-3; Machado Joseph disease (MJD) protein 1	14q24.3-q31	36 (<10 to >60)	+
SCA4	*Q9H7K4*/puratrophin-1	16q22.1	? (19-72)	+
SCA5	*SPTBN2*/spectrin beta chain, brain 2	11q13	30 (10-68)	+
SCA6	*CACNA1A*/voltage-dependent P/Q-type calcium channel alpha-1A subunit	19p13	52 (20 to >70)	+
SCA 7	*ATXN7*/ataxin-7	3p21.1-p12	35 (<1 to >60)	+
SCA 8	*KLHL1AS*	13q21	40 (1 to >60)	+
SCA 9	reserved			
SCA 10	*ATXN10*/ataxin-10/E46L	22q13	36 (26-45)	+
SCA 11	-	15q14-q21.3	25 (15 to > 60)	
SCA 12	*PPP2R2B*/ brain specific regulatory subunit of protein phosphatase 2A (serine threonine phosphatase)	5q31-q33	35 (8-55)	+
SCA 13		19q13.3-q13.4	Childhood (< 1 to < 45)	
SCA 14	*PRKCG*/protein kinase C, gamma subtype	19q13.4	27 (12-42)	+
SCA 15		3p26.1-p25.3	26 (10-50)	
SCA 16		8q22.1-q24.1	40 (20-66)	
SCA 17	*TBP*/TATA-box binding-protein	6q27	33 (6 to > 40)	+
SCA 18		7q22-q32	15 (12-25)	
SCA 19		1p21-q21	34 (11-45)	
SCA 20		11p13-q11	46 (19-64)	
SCA 21		7p21.3-p15.1	18 (7-30)	
SCA 22		1p21-q21	? (10-46)	
SCA 23		20p13-12.3	? (40-60)	

name	Gene/gene-product	locus	Mean age at onset (range)	Clinical testing available
SCA 24	reserved			
SCA 25		2p21-p13	? (1-39)	
SCA 26		19p13.3		
SCA 27	*FGF14*/fibroblast growth factor 14	13q34	11 (7-20)	+
SCA 28		18p11.22-q11.2		
DRPLA	*DRPLA*/atrophin-1 related protein	12p13.31	30 (0 to >60)	+
EA 1	*KCNA1*/potassium voltage-gated channel component	12p13	? (2-15)	+
EA 2	*CACNA1A*/voltage-dependent P/Q-type calcium channel alpha-1A subunit	19p13	? (3 to > 50)	+
EA 3	-	1q42	variable	
EA 4	-	-	30-60	
EA 5	*CACNB4*/ voltage-dependend L-type calcium channel beta-4 subunit	2q22-q23	?	
EA6	*SCL1A3*/sodium dependent gluatamate transporter (EAAT1)	5p13	?	

50–65% of all cases of autosomal dominant ataxia, with SCA3 as the most common subtype. Other known SCA genotypes are rare (<1%), whereas in the remaining 35–50% of SCA families, the genotype is unknown.[9–11,16]

Cerebellar dysfunction in SCA is most often associated with a variety of other clinical features. Involvement of the long spinal tracts is common, with signs of diminished vibratory sense of the legs and hyperreflexia. Extrapyramidal features, spasticity, cognitive impairment, polyneuropathy, ophthalmoplegia and epilepsy are also seen. Although there is a considerable overlap in clinical features between different SCA genotypes, some distinctive clinical features may be useful in the genetic screening of a specific patient. The presence of pigmentary retinopathy is almost always associated with SCA7. A pure cerebellar, late-onset subtype is most often described in SCA6, 10, 11 and 12 *(Fig. 1, note 3)*.[9–12]

The mean age of onset in SCA is in the third or fourth decade, but the onset varies considerably between the SCA genotypes and also between patients of the same SCA subtype or same family *(Table 2)*. This is partly due to the phenomenon of anticipation, described below. A childhood onset with severe phenotype due to extreme anticipation is indicative of SCA2, SCA7 and SCA17,[9,11] while a childhood onset with tremor is characteristic of SCA27.[17] In all SCA subtypes, MRI of the brain shows progressive cerebellar atrophy in the course of the disease, sometimes combined with atrophy of the brain stem or spinal cord.[9] The underlying gene defect is known in 13 of the 26 SCA genotypes and DRPLA *(Table 2)*.

Most of the known SCA mutations give rise to CAG trinucleotide repeat expansions. There is evident correlation between the size of this expansion and age at onset and severity of the disease. This explains the phenomenon of anticipation, where instability of larger repeats gives rise to further expansions in subsequent generations, resulting in earlier onset and a more severe phenotype. Repeat instability is more obvious in paternal transmission.[9,11] SCA1,2, 3, 6, 7, 17 and DRPLA are caused by CAG repeat expansions in coding regions, resulting in polyglutamine tracts. SCA12 gives rise to CAG repeat expansion in a promotor region of the *PP2R2B* gene, not encoding a polyglutamine tract.[9,11] In SCA10, an ATTCT pentanucleotide repeat expansion has been identified in intron 9 of the *ATXN10* gene. The functional effect of this expansion is still under investigation.[18] In SCA8, it is still questioned whether the CTG trinucleotide expansion in the noncoding 3' untranslated region of the *KLHL1AS* gene is causative of or only associative to the disorder because there is no relationship between repeat size and age at onset or disease severity.[9, 10] Four other SCA subtypes are associated with point mutations or deletions *(Table 2)*.[17,19–21]

The unraveling of the complex genetic background of SCA has not yet resulted in finding a final common pathogenic mechanism. The CAG trinucleotide repeat variants give rise to polyglutamine tracts, aggregating in cells. Many other proteins co-localize with intranuclear inclusions or interact with the mutant polyglutamine stretches. These proteins may be pathogenic but may also block the toxic properties of polyglutamine. The ubiquitin–proteasome pathway is presumed to play a protective role against the toxicity of mutant polyglutamine proteins by sequestering them into inclusions.[9,22] Furthermore, an RNA-mediated pathophysiological mechanism, like the toxic gain of function described in myotonic dystrophia and FXTAS, has been suggested.[23] In SCA subtypes associated with point mutations, even less is known about pathogenesis.

Apparently recessive cerebellar ataxias

The autosomal recessive ataxias represent a heterogeneous group of rare neurodegenerative disorders. Harding's original clinical classification is gradually replaced by a genetic classification *(Table 3)*.[2] Congenital and childhood ataxias are reviewed in several recent publications and will not be discussed in this review.[24,25]
FA and ataxia telangiectasia (AT) are the most frequent subtypes of recessive ataxia, with an estimated prevalence of 1 in 50,000 and 1 in 100,000, respectively. Some subtypes of recessive ataxia show a regional distribution: for example, ataxia oculomotor apraxia type I (AOA1) is most frequent in Portugal and Japan.[26,27] In the algorithm for recessive ataxia, FA, being the most frequent cause, is excluded first. The age at onset determines the next step in the screening process. The onset age of 25 years is an arbitrary but reasonable cutoff point because most recessive ataxias and metabolic neurodegenerative disorders have a childhood onset. A minority of recessive ataxias and metabolic disorders can have an adult onset, often associated with milder phenotypes due to residual enzyme activity, and these disorders should be excluded patients older than 25 years *(Fig. 1, note 5)*.

When screening indicates a recessive ataxia or metabolic disorder, it is advisable to check the local availability of DNA screening for this disorder.[9,11,12] Ultimately, screening for SCA mutations is advised in all patients with apparently recessive ataxia and a negative screening for recessive disorders because an autosomal dominant inheritance cannot be ruled out.[5–7]

Friedreich ataxia
Ataxia, dysarthria, absent deep tendon reflexes, pyramidal signs and an early-onset (<25 years) are the classical clinical features of FA. Cardiomyopathy is often present, and scoliosis, distal muscle atrophy, sensorineural deafness, optic atrophy and diabetes are common features.[26] Since the introduction of genetic testing, the phenotype has appeared to be more variable, and up to 25% does not meet the original diagnostic criteria for FA.[28] A late-onset variant of FA, presenting at the age of 25–48 years, with a milder, slowly progressive phenotype, often without cardiomyopathy, has been described and also a phenotype with spastic paraplegia but without ataxia or neuropathy.[26,28,29] This variation in phenotype is related to the size of the GAA trinucleotide repeat expansion in the *FRDA1* gene, varying from 90 to 1300 repeats (normal range 6–33 repeats). The expansion size is inversely related to age at onset and impairment of mobility and directly related to the incidence of cardiomyopathy.[26,28] The *FRDA1* gene encodes frataxin, a mitochondrial protein, and FA is believed to be a disease of mitochondrial dysfunction.[26]

Table 3 The autosomal recessive cerebellar ataxiasa

Name	Gene/locus	Protein	Age at onset
1. With identified gene-defect			
Friedreich ataxia (FA)	*FRDA1*/9q13-q21	Frataxin	2-48
Ataxia Teleangiectasia (AT)	*ATM*/11q22-q23	Phosphoinositol-3-kinase type enzyme	2-22
Ataxia with oculomotor apraxia type 1 (AOA1)	*APTX*/9p13	Aprataxin	2-16
Ataxia with oculomotor apraxia type 2 (AOA2)	*SETX*/9q34	Senataxin	10-22
Spinocerebellar ataxia with axonal neuropathy (SCAN)	*TDP1*/14q31	Tyrosyl-DNA-phosphodiesterase 1	teenage
Ataxia with vitamin E deficiency (AVED)	α-*TTP*/8q13	α-tocopherol transfer protein	2-52
Abetalipoproteinemia (ABL)	*MPT*/4q22-24	Microsomal triglyceride transfer protein MPT	2nd decade
Spastic ataxia Charlevoix-Saugenay (ARSACS)	*SACS*/13q12	Sacsin	1st decade
Infantile onset spinocerebellar ataxia (IOSCA)	*C10orf2*/10q24	Mitochondrial proteins Twinkle and Twinky	9-18 months
Autosomal recessive mitochondrial ataxic syndrome (MIRAS)	*POLG* (DNA Polymerase γ)		juvenile or adult
Marinesco-Sjögren syndrome (MSS)	*SIL1*/5q31		infancy
Cayman ataxia	*ATCAY*/19p13.3	Caytaxin	childhood
Nonprogresive cerebellar ataxia, mental retardation and cerebral gyral simplification	*VLDLR* (Very low densitity lipoprotein receptor) gene		

Name	Gene/locus	Protein	Age at onset
2. With identified gene-locus			
Childhood onset slow progressive ataxia	11p15		childhood
Early onset ataxia with hearing impairment and optic atrophy	6p21-23		
Early onset ataxia with developmental delay and failure to thrive	22q11		infancy
Congenital ataxia with mental retardation, optic atrophy and skin abnormalities (CAMOS)	15q24-q26		congenital
Non-progressive infantile ataxia	20q11-q13		infancy
3. As part of metabolic disorder, extended disease			
Metachromatic leucodystrophy (MLD)	*ARSA*/22q13	Arylsulfatase A	infancy to adulthood
Krabbe	*GALC*/14q31	Galactocylceramidase	infancy to 5th decade
Cerebrotendinous xantomatosis (CTX)	*CYP27A1*/2q33-ter	Sterol 27- hydroxylase	infancy to juvenile
Niemann-Pick-C (sphingomyelin storage disorder)	*NPC1*/18q11-121	NPC1 protein	childhood to adulthood
GM1 gangliosidosis	*GLB1*/3p21.33	Beta-galactosidase	childhood
Tay-Sachs disease (GM2-gangliosidosis, hexosaminidase-deficiency	*HEXA*/15q23-24	Hexosaminidase A	childhood to adulthood
Wilson's disease	*ATP7B*/13q14-21	ATP-ase Cu transporting β-polypeptide	3-50
Aceruloplasminemia	*CP*	Ceruloplasmin	25-60
Refsum's disease (RD)	*PHYH,PEX7*/10p11-pter, 6q22-24	Phytanoyl-CoA-hydroxylase, peroxin 7	infancy to 28(>50)

Table 3 The autosomal recessive cerebellar ataxiasa *continued*

Name	Gene/locus	Protein	Age at onset
Sialidosis	*Neu1*/6p21.3	Neuraminidase	childhood
Chorea-acanthocytosis	*CHAC*/9q21	chorein	23-59
Gamma-glutamyl cysteine synthetase	*GCLC*/6p21	Gamma-glutamyl cysteine synthetase	
Leucoencephalopathy with vanishing white matter	*EIF2B1, B2, B3, B4, B5*/12, 14q24, 1, 2p23, 3q27	Translocation initiation factor EIF2B 5 subunits	variable

4. Other metabolic and degenerative disease of childhood onset and congenital ataxias are given in references 11, 24 and 25

Other recessive ataxias

The algorithm indicates an age-dependent screening for recessive ataxic syndromes and metabolic diseases. Obviously, specific clinical findings may help differentiate the recessive cerebellar ataxias as classified in *Table 3* and may indicate a selective use of diagnostic tests.

Therefore, the following part presents a brief overview of most recessive ataxias, based on prominent clinical characteristics apart from ataxia, to determine first-line testing. In the absence of such characteristics, a complete screening is advised *(Fig. 1, notes 4 and 5)*.

Oculomotor apraxia.

Oculomotor apraxia is a common sign in AT and AOA1 and 2. AT is further characterized by ataxia and oculocutaneous telangiectases and often choreoathetosis and dystonia are present. Immunodeficiency, hypersensitivity to ionizing radiation (IR) and predisposition to malignancy are also specific for AT. In AOA1 and 2, polyneuropathy is also seen. In AOA1, mild mental retardation may be present. Onset of AT is usually in early childhood, patients are wheelchair bound from their early teenage years and die between 20 and 30 years. Onset of AOA is mostly in childhood or early teens. An elevated α-fetoprotein level is found in AT and AOA2.[26, 30, 31]

AT is associated with mutations in the *AT mutated (ATM)* gene, which encodes the phosphatidylinositol 3-kinase protein, which is related to processes of DNA repair. The clinical variation in AT is partly related to the relative preservation of ATM protein expression in specific *ATM* mutations, leading to milder phenotypes with later onset, slower progression and no sensitivity to IR.[32,33] AOA1 is associated with the recently identified *aprataxin* gene *(APTX)*; the *senataxin (SETX)* gene is involved in AOA2. Both

genes are believed to play a role in the DNA repair pathway.[31,32,34] The frequency of AOA2 in non-Friedreich autosomal recessive cerebellar ataxia (ARCA) is estimated at 8%, suggesting AOA2 to be a frequent cause of ARCA.[31]

Polyneuropathy

Areflexia and loss of vibration sense of the lower limbs, often combined with pyramidal signs, are common in FA and also in ataxia with vitamin E deficiency (AVED). However, in AVED, cardiomyopathy and diabetes are uncommon. The onset is usually before the age of 20 years, but this may range from 2 to 52 years.[26] The defect in the gene coding for α-tocopherol transfer protein, associated with AVED, causes impairment of incorporation of dietary vitamin E (α-tocopherol) into very low density lipoproteins, leading to a reduction of plasma vitamin E.[24,26]

Peripheral neuropathy with sensory loss, distal muscle atrophy and areflexia is also seen in abetalipoproteinemia. This disorder starts in childhood or early teens with intestinal celiac-like symptoms, followed by ataxia. Retinopathy is common, probably caused by vitamin A deficiency. On fresh blood smears, acanthocytes are often present.[24,26,35] The disorder is caused by mutations in the *microsomal triglyceride transfer protein (MTP)* gene, ultimately leading to fat malabsorption with vitamin A, E and K deficiency.

SCA with axonal neuropathy (SCAN1), caused by a mutation in the DNA repair protein tyrosyl DNA phosphodiesterase 1, is a rare variant, overlapping the AT phenotype, with onset in teenage years but without oculomotor apraxia.[32] Late-onset hexosaminidase A deficiency or GM2 gangliosidosis may present as a FA-like phenotype. Motor neuron disease with muscular atrophy often occurs and dementia or psychiatric features may develop later.[35,36]

Ophthalmologic signs

A Kaiser-Fleischer ring of the cornea is characteristic of Wilson disease, a disorder of copper metabolism, which may also show ataxia, chorea, dystonia and hepatic and psychiatric symptoms.[37] Retinal degeneration represents one of the characteristic signs of aceruloplasminemia, another disorder of copper metabolism, in a triad with diabetes mellitus and neurological symptoms, especially ataxia and dementia. Heterozygotes of ceruloplasmin gene mutations may be symptomatic, showing hypoceruloplasminaemia and mild ataxia.[38]

Retinitis pigmentosa, combined with anosmia, polyneuropathy, cerebellar ataxia and often also deafness and ichthyosis, is characteristic for Refsum's disease. Retinitis pigmentosa always develops before the age of 28 years, whereas other symptoms develop gradually over decades up to the fifth decade. Deficiency of phytanoyl-CoA hydroxylase causes accumulation of phytanic acid.[26]

Juvenile cataracts are a clinical hallmark in cerebrotendinous xanthomatosis (CTX). CTX is one of the leukodystrophies, also characterized by white matter lesions on MRI *(see below)*.[39]

Cerebral white matter lesions on MRI

Apart from (recessive) mitochondrial disorders, white matter lesions on MRI of the brain or myelum are also seen in all the leukodystrophies.[36] Subtypes with adult onset are well described in metachromatic leukodystrophy (MLD), X-linked adrenoleukodystrophy (X-ALD) and also in Krabbe disease, which may occasionally present just in the fifth decade, with symptoms of weakness, paresthesias of the extremities and ataxia. Mental deterioration is often seen in late-onset forms of MLD and Krabbe disease and also in CTX. Clinical hallmarks of CTX are juvenile cataract, tendon xanthomas, chronic diarrhoea and progressive neurological symptoms of ataxia, pyramidal signs, dementia, epilepsia and axonal polyneuropathy. CTX is caused by a deficiency of the enzyme sterol 27-hydroxylase, MLD is associated with defectsin the *arylsulfatase* gene and Krabbe disease is caused by galactocerebrosidase deficiency.[36,39]

Other indicative neurological characteristics

Manifestations of mitochondrial disorders, with a combination of sensory axonal polyneuropathy, progressive external ophthalmoplegia (PEO), optic atrophy and seizures, are seen in infantile-onset spinocerebellar ataxia (IOSCA), a rare disease, described in Finnish patients.[40] Ataxia with a variable combination of migraine, epilepsy, myoclonus, neuropathy, late-onset PEO and cognitive decline is seen in autosomal recessive mitochondrial ataxic syndrome (MIRAS). Both MIRAS and IOSCA are associated with nuclear gene mutations leading to mitochondrial dysfunction *(Table 3)*.[41–43]

Dementia or psychiatric features are often associated with adult-onset subtypes of Niemann-Pick C, a lipid storage disorder, which may present with ataxia.[44] Spasticity, combined with early-onset cerebellar ataxia and peripheral neuropathy, is characteristic of autosomal recessive spastic ataxia of Charlevoix-Saguenay, associated with the sacsin (SACS) gene. Recently, a phenotype without spasticity was described in Japan, associated with a novel SACS mutation.[26,45] Dystonia is the most prominent feature in the adult-onset variant of GM1 gangliosidosis, which may present with abnormal gait and dysarthria.[46]

Apparently X-linked cerebellar ataxia

X-linked ataxia, onset <50 years

X-linked congenital and childhood ataxic syndromes as X-linked cerebellar ataxia and sideroblastic anemia, usually nonprogressive disorders, are beyond the scope of the algorithm and will not be discussed.[25,47]

Adrenoleukodystrophy

Cerebellar ataxia is present in 5–10% of adult and childhood patients with X-ALD and may even be the presenting symptom. Impaired adrenocortical function and sometimes cognitive decline are present. White matter lesions, especially in the parieto-occipital region, are well observed on MRI of the brain. An increased level of very long chain fatty acids in plasma is diagnostic. An adrenomyeloneuropathy subtype can be present in adult males, characterized by a progressive spastic paraparesis with sphincter and sexual dysfunction. X-ALD is associated with over 400 different mutations in the *ATP-binding cassette, subfamily D (ABCD)1* gene.[48]

X-linked ataxia, onset >50 years

Fragile-X-associated tremor ataxia syndrome

Progressive intention tremor, gait ataxia, parkinsonism and autonomic dysfunction are characteristic features in FXTAS. Polyneuropathy and dementia may be associated features. It has been described in elderly male carriers of premutation alleles in the *fragile-X mental retardation 1(FMR1)* gene, resulting in a CGG repeat expansion of 55–200 repeats. A full mutation in this *FMR1* gene (over 200 repeats) causes fragile-X syndrome, a relatively frequent cause of mental retardation in boys.[49] Diagnostic criteria for FXTAS are based on clinical signs (ataxia, tremor, parkinsonism), radiological findings (symmetric hyperintense lesions in the middle cerebellar peduncles visible on T2-weighted MRI) and pathological findings (specific neuronal and astrocytic intranuclear inclusions).[49]

FXTAS is estimated to form a considerable contribution to the causes of sporadic ataxia in older adult males: it is seen in 30% of male FMR1 premutation carriers over 50 years and even in 75% of male carriers over 80 years. Moreover, the premutation carrier frequency is relatively high (1:259 females and 1:810 males).[49] Therefore, the algorithm suggests the screening of all males with onset of ataxia above 50 years for FXTAS. In several studies in older male populations with SCA, the prevalence of FMR1 premutation was reported to be around 5%, although lower numbers were also found.[49,50] A few female premutation carriers with FXTAS have been described, but screening of females for FXTAS is not considered necessary.[49]

Increased FMR1 messenger RNA (mRNA) levels are found in individuals with the *FMR1* gene premutation. A toxic gain of function of these mRNA levels is suspected in the pathogenesis of FXTAS, discrepant with the loss of function of the *FMR1* gene in the full-mutation fragile-X syndrome.[51]

Mitochondrial cerebellar ataxia

Cerebellar ataxia may also be associated with maternally transmitted mutations in the mtDNA. On clinical grounds, different subtypes of mitochondriopathies are distinguished, often presenting as multisystem disorders, with involvement of the peripheral nervous system, endocrinium, heart, eyes, ears, guts, kidney and bone marrow. Ataxia is associated with Kearns Sayre syndrome (chronic PEO or CPEO, pigmentary retinopathy, cardiac conduction defects, ataxia), MELAS (myopathy, encephalopathy, lactic acidosis and stroke-like episodes), MERFF (myoclonic epilepsy and ragged red fibers, with symptoms of myopathy, ataxia, dementia, CPEO, deafness, epilepsy), Leigh syndrome (developmental delay, seizures, optic atrophy, retinitis pigmentosa, CPEO, lactic acidosis, hypotonia), NARP (neuropathy, ataxia, retinitis pigmentosa) and May-White syndrome (myoclonus, ataxia, deafness).[8] Although rare, cerebellar ataxia has been described as the presenting symptom in these mitochondriopathies, for example in MELAS.[52]

Apparently sporadic ataxia

According to the algorithm, after exclusion of symptomatic cerebellar ataxia, a hereditary ataxia should be excluded in patients younger than 50 years because a negative family history cannot rule out a hereditary cause of ataxia. First, it is advised to screen for recessive ataxias, in male patients combined with screening for X-linked ataxia. Then, screening for autosomal dominant SCA mutations is also advised. Although the yield of this screening may be relatively low (2–19%, see above)[5–7] the finding of a gene mutation is important for genetic counseling of patients and their relatives. Furthermore, this may indicate preventive screening, as for instance with cardiomyopathy and diabetes in patients with FA. When all diagnostic tests are negative, the descriptive acronym ILOCA can be used.

Symptomatic cerebellar ataxia

Ataxia may be caused by toxic agents including alcohol, mercury, lead, solvents and pesticides. Specific drugs may induce cerebellar symptoms as side effects: antiepileptic drugs like carbamazepine, phenytoin, valproic acid; chemotherapeutic agents; sedative

drugs; lithium; antidepressants, amiodarone, cyclosporine, isoniazid, metronidazole, nitrofurantoin and procainamide.[53] Endocrine disorders such as hypothyroidism and diabetes are treatable causes of ataxia. Vitamin deficiencies, resulting from malabsorption syndromes may cause neurotoxic effects, as was described before in hereditary vitamin E deficiencies and abetalipoproteinemia. Combined spinal cord degeneration may follow vitamin B12 avitaminosis. Careful history and simple laboratory tests will exclude the most common symptomatic ataxias *(Fig. 1, note 7)*. Sometimes it is helpful to discontinue specific medication and evaluate the effect on ataxia.

Gluten ataxia may manifest in patients with gluten sensitivity or celiac disease and is believed to display an organ-specific manifestation of gluten sensitivity, rather than a secondary symptom due to malabsorption.[54] Patients with gluten ataxia often do not have gastrointestinal symptoms. Antigliadin antibodies (a diagnostic test for celiac disease) are found in 30–40% of patients with sporadic idiopathic cerebellar ataxia, compared with 12% in the normal population.[54] However, the concept of gluten ataxia is still under discussion. Other studies could not confirm the higher prevalence of antigliadin antibodies in idiopathic cerebellar ataxia. Also, a high prevalence of antigliadin antibodies was found in hereditary ataxia (SCA) and Huntington disease. Gluten sensitivity may therefore be an epiphenomenon in neurodegenerative disorders rather than a pathogenic factor.[55]

Cerebellar ataxia is a well-known paraneoplastic phenomenon, and it should be considered in patients developing a progressive ataxic syndrome in less than 6 months *(Fig. 1, note 9)*.[56] Demyelinating diseases often display a relapsing remitting course, and MRI of the brain can support the diagnosis. Cerebellar ataxia may occur as a manifestation of whipple disease, caused by the bacillus Tropheryma whippelii. Systemic manifestations include diarrhoea, weight loss and abdominal pain. Central nervous system involvement further includes supranuclear ophthalmoplegia, movement disorders, memory loss and sleep disturbances.[57] Post-viral ataxia can present as a rather acute ataxia in children, with a benign course. Ataxia can also occur in Bickerstaff's brain stem encephalitis, a post-viral inflammatory disease with a clinical spectrum overlapping Miller-Fisher syndrome and Guillain Barre syndrome.

Multiple system atrophy

Multiple system atrophy (MSA) is the most common cause (30%) of isolated late-onset cerebellar ataxia.[58] The prevalence of MSA has been estimated as 1.9–4.9 cases per 100,000 people. Clinical hallmarks are autonomic and urinary dysfunction, parkinsonism, cerebellar dysfunction and corticospinal tract dysfunction. The diagnosis of possible, probable or definite MSA is established on consensus diagnostic criteria regarding clinical symptoms and signs. The diagnostic criteria also provide exclusion criteria,

based on history, physical examination and laboratory investigation *(Fig. 1, note 8)*.[58] MSA usually starts in the sixth decade with rapid progression and a mean survival of 6–9 years. Patients who initially do not meet this criteria may still develop MSA in a later phase of the disease. Two major subtypes can be distinguished: MSA-P, with predominating parkinsonian features, and MSA-C, in which cerebellar ataxia is the main motor feature. MRI of the brain may display aspecific olivopontocerebellar and putaminal atrophy, but also signal hyperintensities of the pons and middle cerebellar peduncles on T2-weighted images. Increased putaminal hypointensities on T2-weighted gradient echo imaging are more common in MSA than in Parkinson disease, and combination with a hyperintense slit-like band lateral to the putamen may be specific for MSA. Pathologically, MSA is an α-synucleinopathy, with glial cytoplasmic inclusions.[58]

Therapeutic strategies

Treatment of the underlying disorder is the first step in symptomatic ataxia. Intoxications, vitamin deficiencies and endocrine disorders, for example, are treatable causes of ataxia. Treatment of underlying malignancy may improve or cure paraneoplastic syndromes. Although there is an increasing insight into genetic and pathophysiological mechanisms underlying hereditary ataxias, therapeutic options modifying neurodegeneration are still very limited.[59] This does not, however, diminish the importance of an adequate diagnosis for the identification of potentially treatable disorders. In autosomal dominant EA, acetazolamide is very effective, especially in EA2. Acetazolamide and also gabapentin have shown some improvement in cerebellar signs in SCA in open trials, but these effects have to be confirmed in further studies.[59] In the autosomal recessive ataxia subtypes, daily supplementation of vitamin E in AVED prevents further neurodegeneration.[26] Dietary treatment in Refsum's disease, the restriction of intake of phytanic acid, may prevent onset of symptoms.[26] Treatment of CTX with chenodeoxycholic acid stabilizes or lessens the symptoms.[39]

In all patients with cerebellar ataxia, symptomatic therapy may relieve symptoms. Spasmolytic drugs such as baclofen may be used to treat spasticity, and in selected patients botulinum toxin may be considered, which is also effective in treatment of dystonia. Dystonia, tremor or bradykinesia may be an indication for dopaminergic and anticholinergic therapy. Anticholinergic drugs are also successful in treatment of hypersalivation due to swallowing difficulties. Intention tremor has been treated with betablockers, benzodiazepines or even thalamic stimulation.[59] Muscle cramps can be relieved with for instance benzodiazepines (clonazepam).

Conclusive remarks

This review illustrates the broad clinical spectrum of disorders associated with cerebellar ataxia. For the individual patients and their family, it is very important to come to an appropriate diagnosis. This provides insights into prognosis, enables adequate genetic counselling in hereditary ataxias and may have implications for therapy or preventive screening, as described above. We developed a diagnostic algorithm to facilitate the diagnostic process. We do not suggest this algorithm to be absolute or applicable for every patient. Obviously, specific diagnostic clues should lead to the specific use of diagnostic tests. The cutoff point for ages at onset in the algorithm needs flexible interpretation. For example, some SCA subtypes, especially SCA6, may have an onset at age above 50 years. Of course, genetic testing is dependent on local availability. Furthermore, frequent updating is essential with the ongoing identification of genes associated with ataxia. In this context, it should be mentioned that identifying families with undiagnosed hereditary ataxia may be valuable for linkage studies.

It is reasonable to expect that the increasing insight into the genetic background of ataxias will eventually lead to considerable therapeutic options in the future. Currently, optimal treatment of patients with ataxia requires the multidisciplinary expertise of neurologist, professionals in the field of revalidation and clinical geneticist. In addition, foundations of patients with (hereditary) ataxias may be very useful as a source of information and support for patients and their families.

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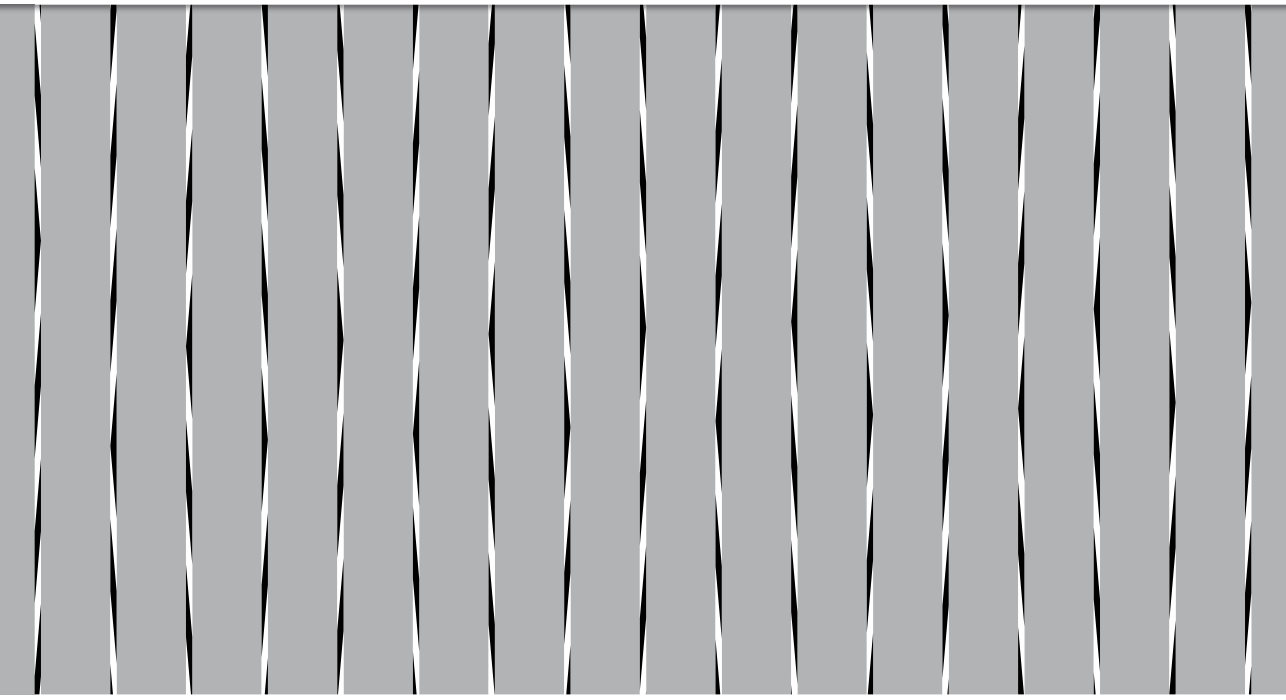
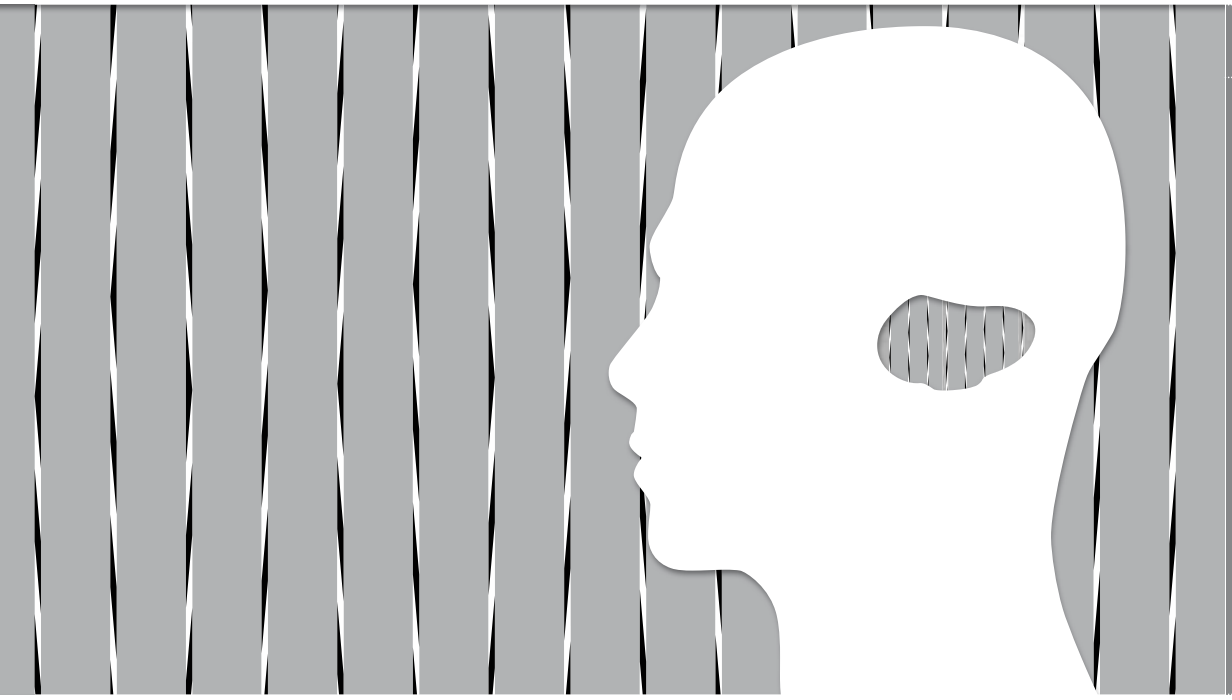


Chapter 2



Fatigue in spinocerebellar ataxia

Patient self-assessment of an early and disabling symptom

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Abstract

Objective: **To identify the prevalence and severity of fatigue and predicting factors for severe fatigue in autosomal dominant spinocerebellar ataxia (SCA).**

Methods: **We studied a cross-section of 123 SCA patients. Six functional scales were used in a self-assessment: the Fatigue Severity Scale (FSS); the Beck Depression Inventory (BDI); the Rotterdam Handicap Scale (RHS); the short form SF-36 health survey, distinguishing a norm-based physical and mental component score (Nb-PCS and Nb-MCS); the Pittsburgh Sleep Quality Index (PSQI) and the Epworth Sleepiness Scale (ESS). A subset of 58 patients was clinically evaluated, measuring severity of ataxia with the Scale for the Assessment and Rating of Ataxia (SARA) and cognitive functioning with the Mini Mental State Examination (MMSE)**

Results: **Severe fatigue (FSS value ≥ 5) was present in 69% of patients and FSS value correlated with the scores on RHS, Nb-PCS, Nb-MCS, BDI, PSQI and ESS. There was no relation with disease duration, gender or medication use. Multivariate analysis revealed that Nb-PCS and BDI were the best independent predictors for severe fatigue. Interestingly, the presence of visual symptoms was related to FSS value in the clinically evaluated subgroup.**

Conclusion: **Fatigue is a severe and disabling symptom in adult patients with SCA, even early in the course of disease. Physical functioning and depression are the strongest predictors of fatigue. In treatment strategies, all treatable factors for fatigue should be addressed, especially depression, visual symptoms and sleeping disorders.**

2 Fatigue in spinocerebellar ataxia

Patient self-assessment of an early and disabling symptom

Cerebellar ataxias represent a heterogeneous group of neurodegenerative disorders, distinguishing hereditary and sporadic ataxias. The majority of adult onset hereditary ataxias are constituted by autosomal dominant cerebellar ataxias (ADCAs), also referred to as spinocerebellar ataxia's (SCAs), with SCA1, SCA2, SCA3 and SCA6 as the most common genotypes.[1-3] In 30% of Dutch ADCA patients, no SCA genotype can be identified.[4]

Cerebellar ataxia is the predominant symptom in all SCA patients.[5, 6] In our experience, adult cerebellar ataxia patients often complain of fatigue, limiting quality of life apart from physical impairment. To our knowledge, fatigue has been studied in two small series of Friedreich ataxia and SCA3 patients,[7, 8] but without data about predictors of fatigue. Severity of ataxia and noncerebellar symptoms and depression have shown to predict the health status in patients with SCA 1,2,3, and 6 in an European multicenter study, but fatigue was not investigated.[9]

In neurological disorders like multiple sclerosis (MS), Parkinson's disease (PD), head trauma, stroke or neuromuscular disorders, fatigue is increasingly recognized as an important cause of disability.[10-16] Predicting factors of fatigue are depression (MS, PD, stroke), physical impairment (MS, neuromuscular disorders) but not disease duration.[11, 14, 15, 17, 18]

We aimed to investigate the prevalence of fatigue and its predicting factors in Dutch SCA patients. Severity of fatigue was quantified with the Fatigue Severity Scale (FSS). We collected data on disease-specific factors (disease duration, severity of ataxia, physical impairment, SCA subtype) and factors known to be related to fatigue (medication use, sleep disturbances, depression, quality of life).

Methods

Patient assessments

Patients, aged 18 years and older, were recruited between March and October 2009 from our outpatient clinic (n=16) and from the Dutch ADCA patient foundation, by a written invitation. We included ADCA patients displaying one of the known SCA mutations and patients with positive family history but without identifiable SCA genotype,

since these "SCA-negative" ADCA patients represent a significant proportion of SCAs. The clinical diagnosis was established by one of the neurologists (EB, JvS) or it was verified by reviewing medical records. Patients were asked to complete a questionnaire including demographic data, diagnosis, onset age, co-morbidity and use of medication, the Dutch version of the Fatigue Severity Scale (FSS), the Rotterdam Handicap Scale (RHS), the medical outcomes study 36-item short form health survey (SF-36), the Beck Depression Inventory (BDI), the Pittsburgh Sleep Quality Index (PSQI) and the Epworth Sleepiness Scale (ESS).

We were especially interested in medication use and co morbidity known to be related to fatigue: drugs like β-adrenergic blockers, antidepressants or sedatives and disorders like thyroid disease, anemia, cardiac failure, and malignancies. We did not perform new blood tests on thyroid status, hemoglobin level or other metabolic disturbances.

The FSS is a nine-item questionnaire with a score ranging from 1 ("strongly disagree") to 7 ("strongly agree") for each item. The mean FSS value of the nine inquiries ranges from 1 ("no signs of fatigue") to 7 ("most disabling fatigue"). A mean FSS value of 5 or higher indicates severe fatigue, the FSS value of 5 being the 95th percentile in healthy controls.[14, 19, 20] The RHS comprises 9 items, resulting in a total score between 9 ("unable to fulfill any applicable task or activity") to 36 ("able to fulfill all applicable tasks or activities").[21, 22] Both FSS and RHS have been validated in patients with peripheral and central nervous disorders, and have shown good internal consistency, test-retest reliability and discriminative validity.[14, 19, 20]

The SF-36 is a well evaluated, widely used health status survey, comprising eight domains: physical functioning (PF), role limitations due to physical health problems (RP), bodily pain (BP), general health perceptions (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE) and general mental health (MH). We used the standard version. All domain scores were transformed to norm-based scores, related to the eight domain means and standard deviations derived from a Dutch community,[23] summation resulted in a norm-based Physical Component Summary (Nb-PCS) measure (domain 1-4) and a norm-based Mental Component Summary (Nb-MCS) measure (domain 5-8). Higher summary scores indicate higher levels of functioning or well-being.[24]

We included the long form of the BDI with a total score ranging from 0 to 63, defining scores from 0-9 as normal, 10-15 as minimal depression, 16-19 as mild-moderate depression, 20-29 as moderate to severe depression and 30-63 as severe depression.[25, 26] In Parkinson's disease, BDI has proven to be a reliable measure of depression despite the inclusion of somatic items, not just reflecting disease severity.[25, 26] To study fatigue

in relation to the presence of sleep disturbances, we included the PSQI and the ESS. The PSQI assesses sleep quality and disturbances over a 1-month time interval, using seven components, resulting in a global score from 0-21. A global score > 5 indicates poor sleep quality.[27] The ESS measures sleep propensity in eight different daily situations, resulting in a total score ranging from 0 ("no excessive daytime sleepiness") to 24 ("severe excessive daytime sleepiness").[28]

All patients were requested in the informed consent letter to participate in a consecutive clinical evaluation at our clinic. Sixty-two patients were willing to participate and were examined by the same neurologist (EB). History in these patients specifically addressed factors causing or relieving fatigue, presence of gait- and limb ataxia, visual complaints, dysarthria, dysphagia and level of impairment. The severity of ataxia was assessed with the eight-item Scale for the Assessment and Rating of Ataxia (SARA), with a sum score ranging from 0 ("absence of ataxia") to 40 ("most severe ataxia").[6] Global cognitive functioning was expressed in the Mini Mental State Examination (MMSE), with a maximum score of 30.[29]

Standard Protocol Approvals, Registrations, and Patient Consents
The study was approved by the ethics committee of the Erasmus MC University Medical Center and informed consent was obtained from all participants.

Statistical analysis
We estimated the need of including 97 patients to reach a power of 0.80 in analyzing whether duration of the disease, depression and disease severity have the potentials to predict the level of fatigue by multiple regression analysis, assuming an α-level of 0.05 (two-tailed) and further assuming that the explained variance of these three predictive values with respect to fatigue is expected to be 10% above and beyond the estimated 10% of the gender and age as confounding factors.
Outcome for specific ataxia diagnosis was recoded in 4 categories ("ADCA", "SCA3", "SCA6," and "other SCA mutation") reflecting the most frequent SCA subtypes in our population. "ADCA" addresses the category of SCA patients without a mutation in the known SCA genes. "Other SCA mutation" refers to patients with alternative SCA-genotypes apart from SCA3 or SCA6 . Associations between variables and FSS values were tested using Spearman's correlation test for continuous variables and the Mann-Whitney U test or Kruskal-Wallis test, as appropriate, for categorical variables.

Since fatigue had a skewed distribution which could not be transformed, we dichotomized fatigue for the predictive model, with FSS < 5 reflecting mild to moderate fatigue and FSS ≥ 5 reflecting severe fatigue. To identify variables from the self-assessment that were associated with FSS, Chi-square tests or Fisher exact tests for categori-

cal variables and Mann-Whitney U tests for continuous variables were performed. Variables with a significance of $p \leq 0.15$ were considered as candidate variables for multivariate logistic regression analysis and were all entered, except for Nb-MCS. The reason to leave out this variable lies in the fact that the items of the vitality domain (VT) of the Nb-MCS are similar to the FSS items, which will obviously lead to a prediction of FSS score. Subsequently, variables with the highest p-value were eliminated step by step, until the fit of the model decreased significantly (based on the likelihood-ratio test). Evaluation was performed with SPSS 15.0 for Windows (SPSS Inc., 2007).

Results

Clinical characteristics

We distributed 365 self-assessment questionnaires, to which 157 patients responded (response rate 41%). We had to exclude 34 individuals (21% of responders): 28 patients had an alternative or uncertain diagnosis, in 4 individuals data were incomplete, one patient was deceased and one was unable to fill in the assessment. Eventually, we

Table 1 baseline characteristics of the total study population, compared to the subgroup that underwent adjacent clinical evaluation

	self-assessment group (n=123)	clinical evaluation subgroup (n=58)
Gender, number (%)		
-Male	50 (40.7)	23 (39.7)
-Female	73 (59.3)	35 (60.3)
Mean age at examination (SD)	57.9 (12.7)	56.5 (12.9)
Median disease duration (IQR)	13 (7-19)	12.5 (6-19)
Diagnosis, number (%)		
-SCA3	44 (35.8)	21 (36.2)
-ADCA	31 (25.2)	18 (31)
-SCA6	26 (21.1)	9 (15.5)
-SCA14	7 (5.7)	4 (6.9)
-SCA1	5 (4.1)	2 (3.4)
-SCA2	4 (3.3)	1 (1.7)
-SCA7	3 (2.4)	1 (1.7)
-SCA13	2 (1.6)	1 (1.7)
-SCA17	1 (0.8)	1 (1.7)

enrolled 123 patients (78% of responders) and 58 patients participated in the subsequent clinical evaluation (47% of enrolled patients). Baseline characteristics of the overall study population and the clinically evaluated subgroup are similar *(Table 1)*. The proportion of females (59.3%) is higher than males (40.7%): p=0.035. The mean age at examination is 57.9 years and the median disease duration 13 years. ADCA (SCA without identified gene mutation), SCA3 and SCA6 were the most frequent diagnoses.

Fatigue

The median FSS value in our patients was 5.8 (IQR 4.6-6.6), with 69% of the patients having a median FSS score of 5 or higher, indicating severe fatigue. The median FSS item scores and their score distributions are presented in *Table 2*. The largest proportion of scores ≥ 5 is seen in FSS item 2: "Exercise brings on my fatigue," one of the items relating fatigue to physical functioning. Nearly 70% of the patients classified fatigue among their three most disabling symptoms (scoring 5 or higher on FSS item 8, agreeing with this quote).

Table 2 Medians and score distribution of fatigue Severity Scale (FSS) item scores of 123 patients with spinocerebellar ataxia (SCA)

		Score distribution (%)		
FSS item	Median (IQR)	1-3	4	5-7
2. Exercise brings on my fatigue	6 (5-7)	6.5	7.3	86.2
1. My motivation is lower when I am fatigued	6 (5-7)	13.8	3.3	82.9
6. My fatigue prevents sustained physical functioning	6 (5-7)	14.6	3.3	82.1
4. Fatigue interferes with my physical function	6 (5-7)	13	5.7	81.3
7. Fatigue interferes with carrying out certain duties and responsibilities	6 (5-7)	14.6	8.1	77.2
3. I am easily fatigued	6 (5-7)	10.6	13	76.4
9. Fatigue interferes with my work, family or social life	6 (4-7)	22	7.3	70.7
8. Fatigue is among my three most disabling symptoms	6 (3-7)	26	4.1	69.9
5. Fatigue causes frequent problems for me	5 (3-6)	27.6	14.6	57.7

There was no significant relation between gender, age, disease duration, or SCA subtype and FSS value *(Table 3, Figure 1a)*. Medication use and co-morbidity were not significantly related to FSS value either *(Table 3)*. RHS and Nb-PCS, reflecting physical function, were significantly correlated with FSS value *(Table 3)*. However, SARA, indicating severity of ataxia, did not correlate with FSS in the clinically evaluated subgroup (p=0.94). In this subgroup, patients with visual symptoms (symptomatic nystagmus or diplopia) were more fatigued than patients without visual complaints (p=0.017,

Table 3 **Correlations (Spearman's rank correlation coefficient and corresponding p-value) between the Fatigue Severity Scale (FSS) and basic characteristics, physical and mental functioning and sleep in SCA patients, followed by p-values between FSS and categorical variables of gender, medication use and specific diagnosis.**

	Self Assessment group(n=123)
Age	0.077 (.397)
Disease duration	0.081 (.378)
Physical Functioning	
RHS	-0.398(< .001)
Nb-PCS	-0.437(< .001)
Physical Functioning (PF)	-0.370(< .001)
Role-Physical (RP)	-0.496(< .001)
Bodily Pain (BP)	-0.282(.002)
General Health (GH)	-0.388(< .001)
Mental functioning	
BDI	0.559(< .001)
Nb-MCS	-0.455(< .001)
Vitality (VT)	-0.705(< .001)
Social Functioning (SF)	-0.485(< .001)
Role-Emotional (RE)	-0.218(.017)
Mental Health (MH)	-0.450(< .001)
Sleep	
ESS	0.257(.004)
PSQI	0.229(.011)
Gender	(p=.107)
Specific diagnosis	(p=.379)
Medication use	(p=.058)
Co-morbidity	(p=.361)

Figure 1 **Boxplot of median FSS (Fatigue Severity Scale) values (95% CI) in (a) four diagnostic subgroups of cerebellar ataxia and (b) the clinically evaluated cerebellar ataxia patients, with or without visual symptoms.**

Figure 1a

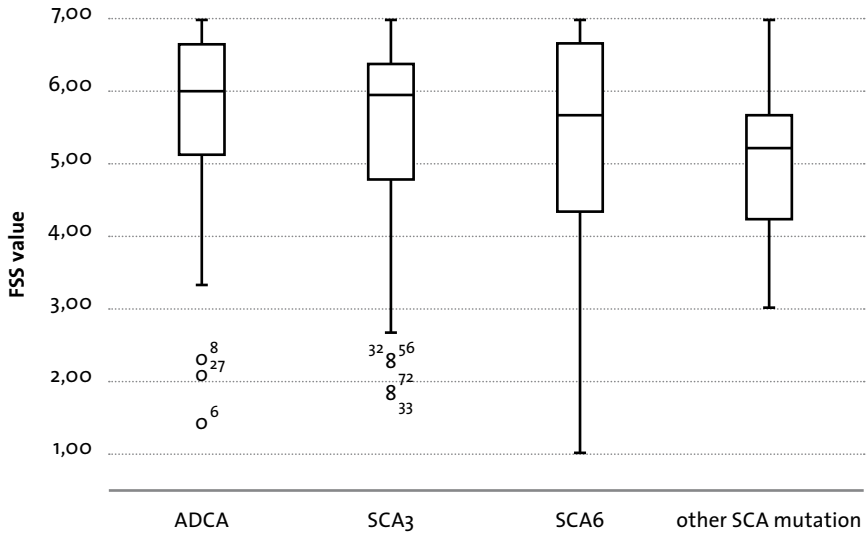

Figure 1b

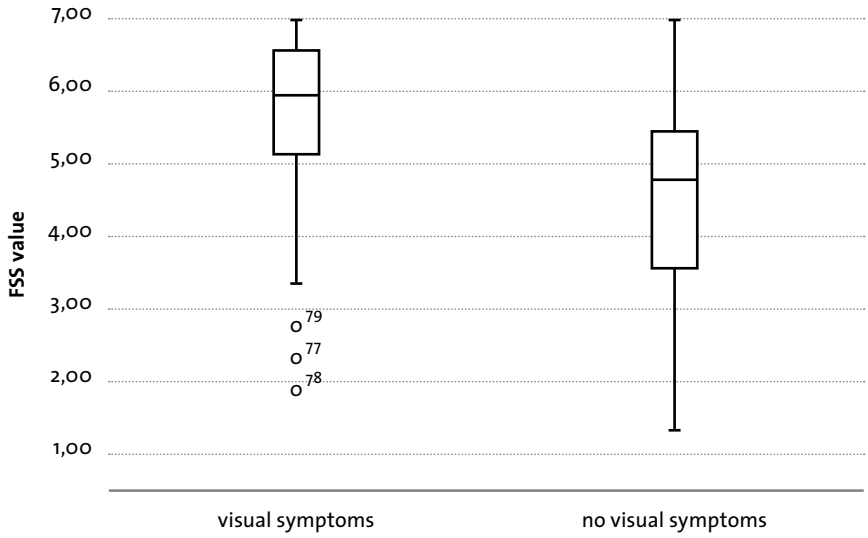

Figure 1b). BDI, reflecting depression, and both PSQI and ESS were significantly related to FSS value *(Table 3)* but not cognitive functioning on MSSE (p=0.94).

Predictors of fatigue

When our population was divided into patients with mildly to moderately fatigue (FSS < 5, n=38) and severely fatigued (FSS-value ≥ 5, n=85), again, physical functioning, mental functioning and sleep were significantly related to fatigue *(Table 4)*.

Table 4 Patient characteristics stratified by severe fatigue and mild-moderate fatigue.

*Variable entered in the multivariate logistic regression model based on p-value =< 0.15

	Severe fatigue: FSS ≥ 5 (n=85)	Mild-moderate fatigue: FSS<5 (n=38)	p-value
Gender (number of men (%))	32(38)	18(47)	.327
Mean age in years (SD)	58(12)	57(14)	.576
Median disease duration in years (IQR)	13(8-19)	12(6-19)	.376
Specific diagnosis (number (%))			.358
ADCA	24 (28)	7 (18)	
SCA3	31 (36)	13 (34)	
SCA6	18 (21)	8 (21)	
Other SCA mutation	12 (14)	10 (26)	
Medication use (number)			.254
None	16	11	
Antidepressants/ sedatives	16	4	
Cardiovascular	22	6	
Other	30	17	
Physical functioning			
RHS (mean (SD))	23.1(7.2)	27.2(7.5)	.004*
Nb-PCS (mean (SD))	32.61(9.48)	39.72(11.73)	.002*
Mental functioning			
BDI (median (IQR))	10(7-16)	5(3-8)	<.001*
Nb-MCS (mean)	48.85(11.33)	56.11(8.71)	(<.001)
Sleep			
ESS (mean (SD))	8.7(5.1)	6.3(3.9)	.013*
PSQI (median (IQR))	6(3-8.5)	4(2-8)	.038*

These variables were then entered in the multivariate logistic regression model, except for Nb-MCS, revealing that the Nb-PCS and BDI were the best independent predictors for severe fatigue (FSS ≥ 5). Each point increase (improvement) on the Nb-PCS score was associated with a 5% decrease (OR=0.95; 95% CI: 0.91 to 0.99, p=0.030) in risk of severe fatigue, whereas each point increase (deterioration) on the BDI score resulted in a 16% (OR=1.16; 95% CI: 1.06 to 1.27, p=0.001) increase in risk of severe fatigue.

Discussion

This study demonstrates that fatigue is a prominent and disabling symptom in adult patients with SCA, with 69% of our population displaying a FSS value of 5 and higher, indicating severe fatigue, and nearly 70% classifying fatigue among their three most disabling symptoms. The median FSS value of 5.8 in our population is evidently higher than the median FSS value of 2.9 in a previously described, healthy control group of 133 patients with similar age distribution (mean 54.2 years, SD 14.8) and percentage of females (47.8%).[20]

We are aware that the response rate of 41% may indicate a sampling bias, however, regarding the distribution of age, disease duration and SCA-subtypes, our study matches the population of a previous study on self-rated health status, representing 304 members of the Dutch ADCA patient federation.[30]

Physical functioning and depression appear to be the strongest predictive factors for the presence of severe fatigue. Strikingly, we did not find a significant correlation between FSS value and severity of ataxia (SARA), although the latter was significantly related to physical functioning scored by RHS and Nb-PCS (data not shown). This implies that physical functioning, related to fatigue, is not only determined by cerebellar ataxia. Indeed, subgroup analysis of clinically evaluated patients showed significantly more severe fatigue in patients with symptomatic nystagmus or diplopia, visual symptoms not addressed by the SARA.

We did not find a relation between fatigue and gender, age or disease duration, which means fatigue was also present in young patients and was an early symptom in some of the SCA patients. This early onset of fatigue may be related to visual symptoms as an early disease symptom. Furthermore, depression, predicting fatigue, is not related to duration or severity of disease. Notably, disease duration was not related to self-rated health status either in a recent European multicenter study in 526 patients with SCA 1,2,3, and 6, also displaying depression to be a stronger predictor of compromised health status than the severity of ataxia and noncerebellar symptoms.[9]

Depression (BDI ≥ 16), although a predictor of fatigue in our study, is present in a minority (22%) of our fatigued patients. Depression may cause fatigue, whereas the other way around depression may also result from an impaired quality of life. A compromised quality of life was established in the European SCA population;[9] the health report on 304 Dutch cerebellar ataxia patients also described significantly lower scores on the physical and social functional status, mental health and vitality items of the SF36.[30]
Furthermore, we found significant correlation of fatigue with sleep disturbances, with 47% of our population having a PSQI>5, indicating a poor sleep quality. Correlation with ESS implicates that fatigue results in excessive daytime sleepiness.
The high prevalence of severe fatigue is in line with the observations in two previous studies on fatigue in a small group of 28 SCA3 patients and in a group of 130 patients with Friedreich's ataxia (FA): in FA, both severity of disease and disease duration were related to fatigue. However, these studies did not investigate predicting factors of fatigue.[7, 8]

The present results show strong similarities with studies on fatigue in other neurological disorders like MS, demonstrating severe fatigue in 64-83% of patients[10, 17, 18] and like PD, with fatigue being an early symptom and a major complaint in about half of the patients[16, 31] and also various neuromuscular disorders.[20, 32] Furthermore, similar predictive factors are described in these disorders: depression in MS, PD and stroke and physical impairment or physical (in)activity in MS and neuromuscular disorders.[15, 17, 18, 20, 31, 33, 34]

Fatigue is defined by the difficulty in initiating or sustaining voluntary mental and physical activities. Peripheral fatigue is similar to muscle fatigability, resulting from disorders of the peripheral nervous system (PNS), whereas central fatigue is caused by lesions in the central nervous system (CNS), disturbing cognitive processing and limiting the ability to sustain concentration and mental tasks ("mental fatigue"). Psychiatric disorders like depression also cause central fatigue by loss of motivation.[10, 14]

Since the cerebellum plays a fundamental role in action control and motor learning and since the nonmotor functions of the cerebellum, related to executive control, attention, memory, learning, visuo-spatial abilities and language are increasingly recognized,[2] it may also play an important role in the model of central fatigue. Interestingly, several patients ascribed fatigue to mental slowness, especially in multi-tasking. This may well be due to mental fatigue. Further research is needed to analyze the role of the cerebellum in central fatigue. Specific neurophysiologic methods can be used to assess peripheral fatigue in combination with central fatigue.[14, 35] Mental fatigue can be evaluated by neuropsychological assessment. It would be of high interest to investigate both strategies in patients with cerebellar ataxia.

Like nycturia, nocturnal muscle cramps and periodic limb movements, described in SCA3,[36] further studies are needed to analyze causes of sleep disturbances in cerebellar ataxia.

Treatment strategies for fatigue in cerebellar ataxia should focus on treatable causes of sleep disturbances or underlying vital depression, and on minimizing visual symptoms. In addition, it will be important to optimize physical functioning and endurance, for which rehabilitation programs are essential. A prospective study on exercise programs, reducing fatigue in MS and immune-mediated polyneuropathy,[14, 37, 38] is necessary to evaluate efficacy in cerebellar ataxia. Finally, pharmacotherapeutics like amantadine and modafinil, showing variable effects on fatigue in other neurological disorders,[14] should be evaluated in cerebellar ataxia.

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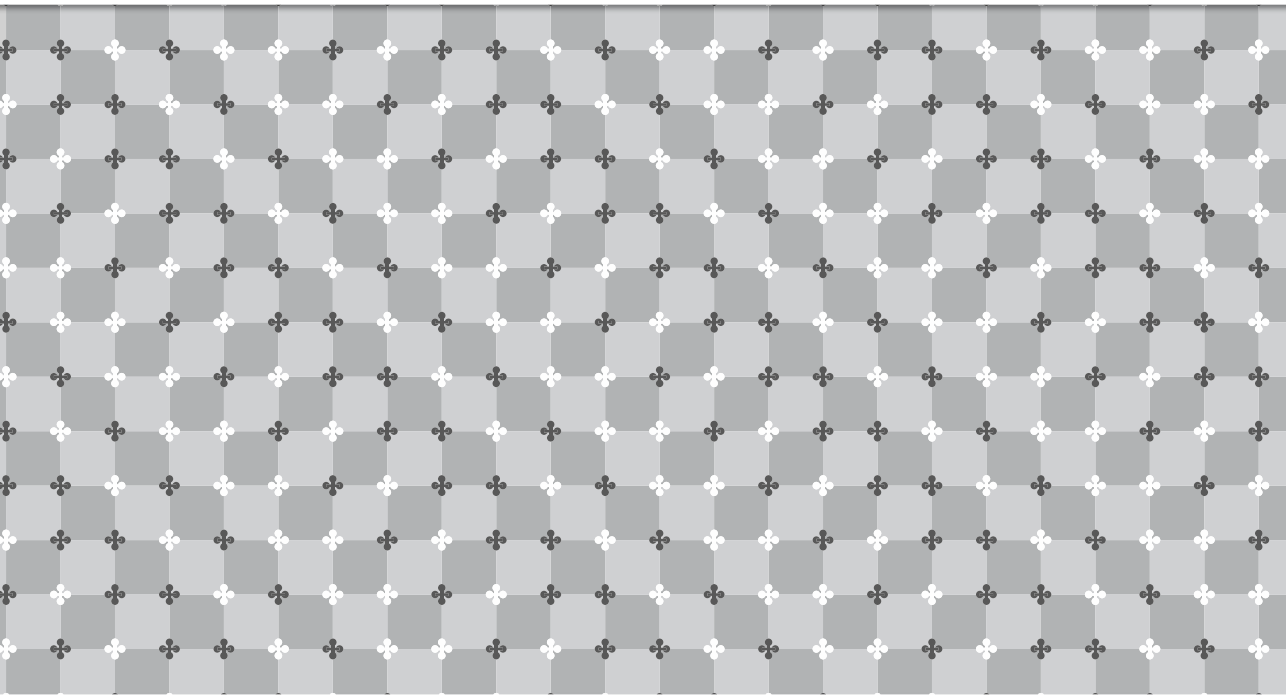
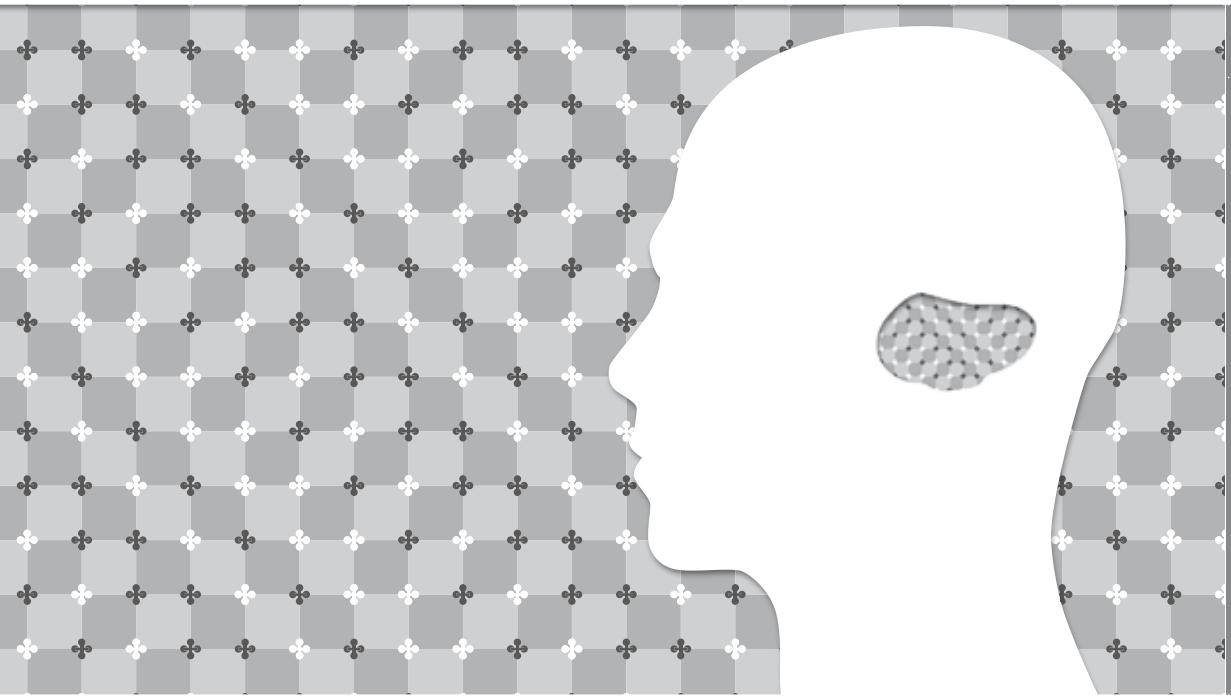
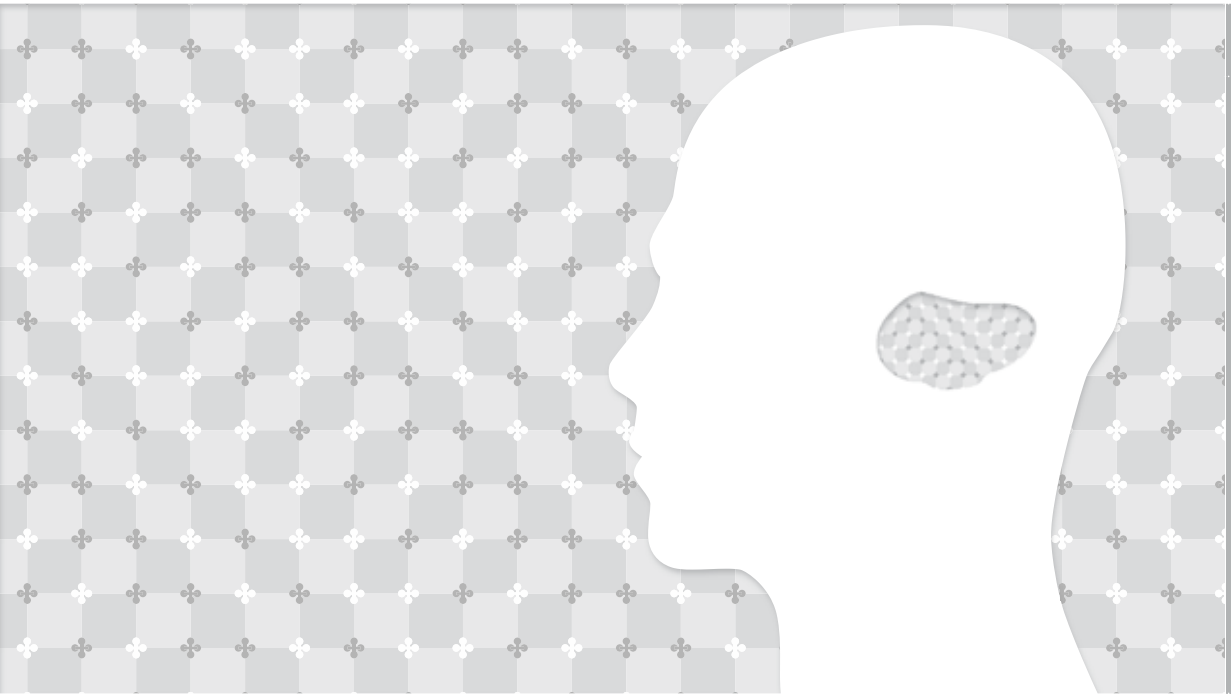


Chapter 3



Novel genotypes and phenotypes in cerebellar ataxia



Autosomal dominant cerebellar ataxia

A mutation in the fibroblast growth factor 14 gene is associated with autosomal dominant cerebellar ataxia

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Abstract

Hereditary spinocerebellar ataxia's (SCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders, for which ≥ 14 different genetic loci have been identified. In some SCA types, expanded tri- or pentanucleotide repeats have been identified, and the length of these expansions correlates with the age at onset and with the severity of the clinical phenotype. In several other SCA types, no genetic defect has yet been identified. We describe a large, three-generation family with early-onset tremor, dyskinesia, and slowly progressive cerebellar ataxia, not associated with any of the known SCA loci, and a mutation in the *fibroblast growth factor 14 (FGF14)* gene on chromosome 13q34. Our observations are in accordance with the occurrence of ataxia and paroxysmal dyskinesia in Fgf14 knockout mice. As indicated by protein modeling, the amino acid change from phenylalanine to serine at position 145 is predicted to reduce the stability of the protein. The present *FGF14* mutation represents a novel gene defect involved in the neurodegeneration of cerebellum and basal ganglia.

3.1 A mutation in the fibroblast growth factor 14 gene is associated with autosomal dominant cerebellar ataxia

Spinocerebellar ataxias (SCAs) are a growing group of hereditary neurodegenerative diseases for which ≥ 14 different genetic loci have been identified. An expansion of an unstable trinucleotide repeat in the coding or noncoding region has been found for nine different SCA Types,[1-4] whereas a pentanucleotide repeat expansion has been found in SCA10 (MIM 603516).[5] Anticipation has been described in several SCA types.[4] Although ataxia is the unifying clinical characteristic, other noncerebellar symptoms, such as dopamine-responsive parkinsonism, early-onset tremor, or epilepsy, may develop early or late in a specific SCA type.[6-8]

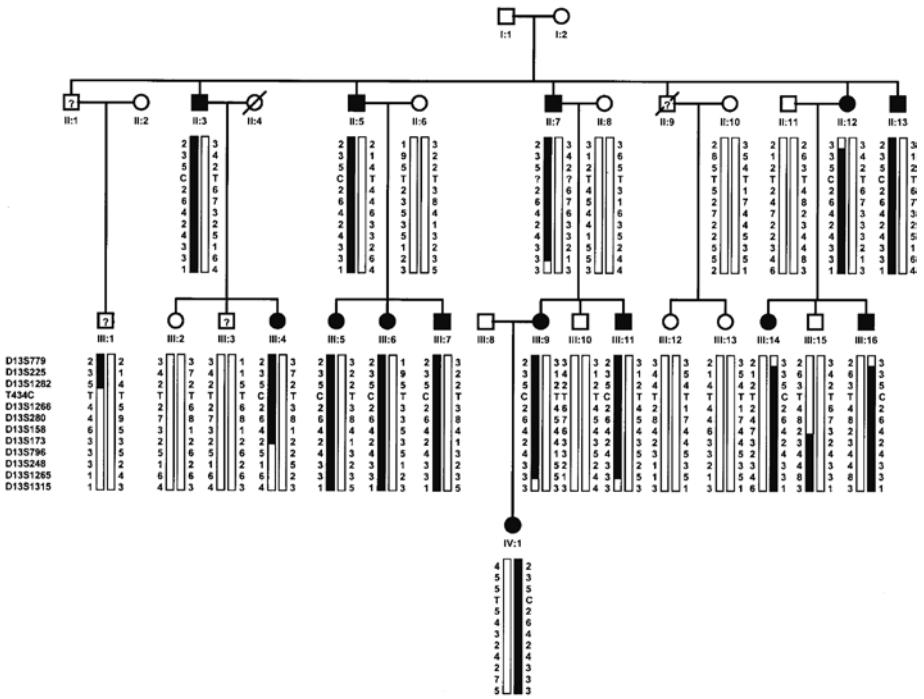
We describe a large, four-generation white family of Dutch descent with an autosomal dominant cerebellar ataxia slowly progressing over decades, in which the known candidate genes involved in other SCA types did not show a repeat expansion. The proband of this family was a 45-year-old woman (individual III:9 *in fig. 1*) who was referred for tremor and progressive ataxia. She reported that her 69-year-old father had severe ataxia, whereas her 24-year-old daughter complained of tremor and mild unsteadiness. Testing for trinucleotide-repeat expansions in SCA1, SCA2, SCA3, SCA6, SCA7, and DRPLA (MIM 164400, 183090, 109150, 183086, 164500, and 125370) of the proband gave results in the normal population range.

Twenty-one members of this three-generation family were examined by two neurologists. Ataxia, tremor, or both was seen in 14 members (7 men and 7 women) and transmitted as an autosomal dominant trait *(fig. 1)*. Individual III:1 was not willing to participate in the study, although by family report he was considered to be affected. We could neither perform neurological examination of this patient nor obtain blood for DNA isolation. For the 21 family members examined, the medical records and, if available, hard copies of structural brain imaging were reviewed. Two patients underwent nerve conduction studies, and five patients were evaluated by means of a battery of neuropsychological tests. Clinical data for the 14 affected family members are summarized in *table 1*. The age at examination was 48.8±17.9 years (range 24–79 years). All had noticed trembling of both hands since childhood; the trembling was exacerbated by emotional stress and physical exercise. Patients first experienced mild unsteadiness and ataxia of upper limbs, especially under unusual circumstances, at age 15–20 years.

The age at onset of gait ataxia was retrospectively determined by rough estimation based on the extremely slow progression of the disease process. Historical data showed no indication of anticipation in consecutive generations. Six patients did not complete primary education, and only 4 of the 14 patients attended secondary school. Aggressive outbursts were mentioned in five patients and depression in three patients.

Neurological examination showed dysmetric saccades, disrupted ocular pursuit movements, gaze-evoked nystagmus, cerebellar dysarthria, and a high-frequency, small-amplitude tremor in both hands in most of the patients. Six patients showed head tremor, and subtle orofacial dyskinesias were seen in eight patients. Severe limb and gait ataxia was present in the three oldest patients. One at-risk individual (III:1, age 48 years), with known chronic alcohol abuse, showed a gaze-evoked nystagmus, although he did not have tremor, dyskinesia, or ataxia. Further neurological examination was

Figure 1 **Pedigree structure of a family with autosomal dominant ataxia and haplotype reconstruction of chromosome 13q34 STR markers.**



Black symbols represent affected individuals; open symbols represent unaffected individuals. Question marks within symbols denote individuals with clinical status that is unknown because of either lack of data or uncertain diagnosis (see text). Black bars indicate a shared "risk" haplotype.

unremarkable except for brisk knee jerks and diminished vibration sense at ankles in several patients. Neuropsychological testing showed low IQ scores (four patients) and impairment of memory functions (three patients), abstract thinking (three patients), and word fluency (one patient).

Moderate cerebellar atrophy on structural imaging was seen in two patients *(fig. 2)*, whereas findings were normal in seven other patients. The individual with chronic alcoholism showed generalized cerebral atrophy. Imaging of dopamine-D2 receptors, using [123I]iodobenzamide single-photon-emission computed tomography (IBZM-SPECT), in

Table 1 Clinical phenotype of 14 affected family members with early-onset tremor and cerebellar ataxia

	Age (in years) at		Result of neurological examination[a]							
	Examination	Onset of ataxia[b]	W	P	N	D	T	LA	GA	Imaging Finding
II:3	79	30	WC	A	+	U	+	++	Unable	n.a.
II:5	71	34	WC	Dp	–	U	+	++	Unable	cerebellar atrophy
II:7	69	27	C	–	+	++	+	++	++	cerebellar atrophy
II:12	64	40	↓↓	–	–	++	+	+	++	normal[c]
II:13	61	30	↓	–	+	++	+	+	+	n.a.
III:4	54	40	↓	–	+	++	+	+	+	normal
III:5	31	30	↓	A	+	+	+	+	+	n.a.
III:6	30	–	normal	A	+	+	–	+	–	n.a.
III:7	27	–	normal	A	+	+	+	–	+	normal
III:9	48	28	↓	–	+	+	+	+	+	normal
III:11	42	37	↓	–	+	+	+	–	+	normal
III:14	43	40	↓	–	+	+	–	–	+	normal
III:16	39	–	↓	A	+	–	+	–	–	n.a.
IV:1	24	–	normal	Dp	+	–	+	+	+	normal

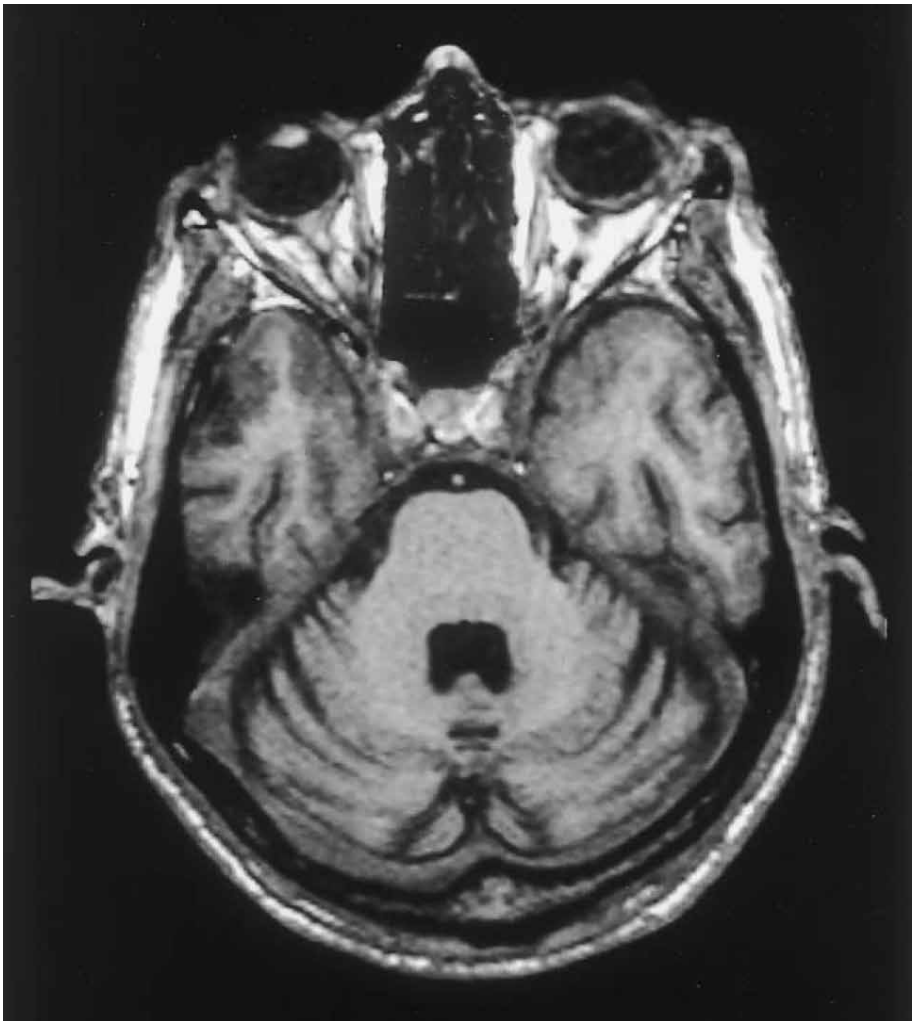
[a] A = agressive outbursts; C=walking with a cane; D = dysarthria; Dp = treated for depression; GA = gait ataxia; LA = limb ataxia; N = gaze-evoked nystagmus; n.a. = not available; P = psychiatric episodes; T = tremor; U = unintelligible speech; W = walking; WC = wheelchair; ↓ = mild unsteadiness; ↓↓ = impaired walking; – = absent; + = present or mild; ++ = moderate; Unable = unable to walk without assistance.

[b] Age at onset of gait ataxia was estimated retrospectively by patient

[c] CT scan of the brain at the age of 60 years.

one patient (II:7) showed a reduced dopamine D2-receptor binding of the left striatum. Normal binding ratios in striatum of the same patient were found on iodine-123-N-fluoropropyl-2bcarbomethoxy-3b-(4-iodophenyl) nortropane (FP-CIT) SPECT. Nerve conduction studies revealed mild axonal polyneuropathy on electromyography in both patients examined (II:7 and II:12).

Figure 2 **MRI of the brain of patient II:7 showed atrophy of cerebellar hemispheres (T1-weighted axial images).**



After approval by the medical ethics committee of the Erasmus Medical Center, Rotterdam, genomic DNA was isolated from peripheral blood from all 14 affecteds, 4 spouses, and 7 at-risk individuals, as described elsewhere.[9] We performed a systematic genome screen, using STRs from the ABI PRISM Linkage Mapping Set MD-10 (Applied Biosystems). Additional markers for fine mapping were obtained from Généthon and from the Center for Medical Genetics (Marshfield Medical Research Foundation) genetic marker sets. Marker order and distances were obtained from the Marshfield integrated linkage map. Markers were amplified according to methods specified by the manufacturers. PCR products were loaded on an ABI 3100 automated sequencer (filter set D), and the data were analyzed with ABI GeneMapper (version 2.0) software.

Two-point linkage analysis was performed with the MLINK program of the LINKAGE package (version 5.1).[10] Maximum LOD and location scores were calculated for each marker, assuming ataxia to be an autosomal dominant disorder with 90% penetrance and with a gene frequency of 1:10,000; no phenocopies were allowed, and equal allele frequencies of the genotyped markers were used in the calculations. Individuals with unknown clinical status *(question marks in fig. 1)* were regarded as unknown in the linkage analysis. We obtained positive LOD scores for adjacent markers on chromosome 13q34 (D13S158, D13S173, and D13S1265). Further refinement of this region by saturation with additional markers confirmed these findings, and two-point linkage analysis yielded a maximum LOD score of Z = 4.28 at recombination fraction 0 for marker D13S280. Changing allele frequencies of the polymorphic markers did not significantly alter LOD and location scores. Subsequently, haplotypes for 11 adjacent markers on chromosome 13q34 were then constructed by parsimony *(fig. 1)*, and several recombinants that defined the limits of the ataxia-susceptibility region were detected. The recombination events in affected individuals demonstrate that the region is limited by marker D13S779 on the centromeric side (individuals II:12, III:14, and III:16) and by marker D13S796 on the telomeric side (individual III:4), showing the critical region to be 10.6 cMon the sex-averaged linkage map. The SCA8 locus could be excluded by testing the following markers flanking the SCA8 locus (MIM 603680) on chromosome 13q21: D13S1309,D13S1268, D13S275, D13S1296, D13S279, and D13S18. SCA8 CTG-repeat length was determined by radioactive PCR essentially as described by Weber and May[11] and was within the normal range for affected and unaffected family members (data not shown).[2]

According to Ensembl and the Human Genome Browser, within this critical region, ≥ 10 genes with a known function and 58 predicted transcripts with unknown function are located, including *fibroblast growth factor 14 (FGF14)* or *fibroblast growth factor homologous factor 4 (FHF4)* (MIM 601515). A recently published mouse model lacking *Fgf14* showed ataxia and paroxysmal dyskinesia.[12] This led us to begin our

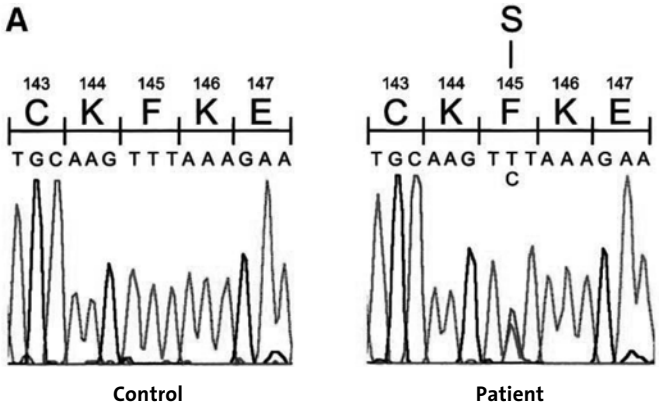
candidate-gene mutation analysis with the *FGF14* gene in this family. The *FGF14* gene encompasses ~200 kb on the genomic level. Two major mRNA transcripts are produced, FGF14a and FGF14b, which differ in the definition of exon 1.[13] The two transcripts encode protein isoforms of 247 and 252 amino acids, respectively. For mutation analysis, the genomic structure of *FGF14* was determined by aligning cDNA (GenBank accesssion number NM_004115) to genomic sequence (GenBank accesssion number NT_009952). Primers were designed to amplify DNA from coding regions, splice sites, and ≥ 50 bases of flanking intronic sequence on both sides of each exon. Amplification was performed with 10-min initial denaturation at 94°C; 35 cycles of 30-s denaturation at 94°C, 30-s annealing at 55°C (for exons 2, 3, and 4), at 57°C (for exon 5), or at 61°C (for exon 1), and 90-s extension at 72°C; and 5-min final extension at 72°C. Primer sequences and sizes of amplified fragments are listed in *table 2*. PCR reactions for all exons were performed in 50 ml containing 10× PCR buffer (Gibco/Invitrogen); 1.5 μM MgCl2; 200 μM dNTP; 0.8 μM forward primer; 0.8 μM reverse primer; 2.5 U *Taq* polymerase (Gibco/Invitrogen); and 50 ng genomic DNA. PCR products were purified with the Amersham GFX-PCR, DNA, and Gel Band Purification Kit, and their approximate concentration was determined by use of Low DNA Mass Ladder (Gibco BRL). Sequencing reactions were essentially performed as specified by the manufacturers, using BigDye Terminator chemistry, version 1 (Applied Biosystems). Products were loaded on an ABI 3100 automated sequencer and analyzed with SeqScape (version 1.1) for heterozygous base calls and sequence alignment.

We identified a T→C transition at position 434 of the FGF14a ORF in exon 4, resulting in an amino acid substitution of a serine for a phenylalanine at position 145 (F145S) *(fig. 3A)*. Testing of the base change in exon 4 on all available family members and 376 control chromosomes from the general Dutch population was done with allele-specific oligo hybridization; PCR products containing exon 4 were blotted onto Hybond-N+ (Amersham Biosciences). The blots were hybridized for 1 h at 37°C in 5× sodium chloride sodium phosphate EDTA, 1% SDS, and 0.05 mg/ml single-strand salmon sperm DNA

Table 2 Primer sequences and amplified product sizes

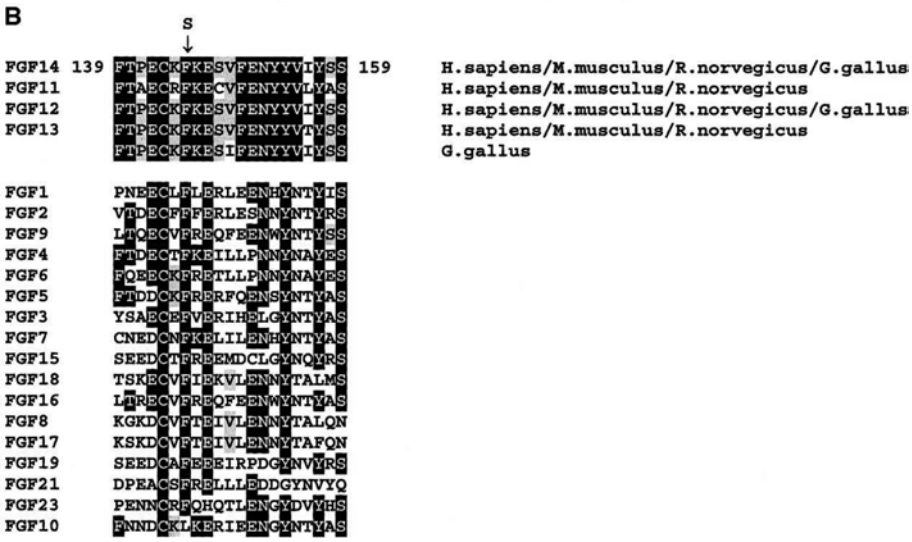
Exon	Forward Primer 5'→3'	Reverse Primer 5'→3'	size (bp)
1a	agggcgagccacggtctg	gagggaaggagcctggagaa	373
1b	aatcactgagaagtctcaaag	ctgcagatctagctcgatga	362
2	gcctgtttctgtggcttact	aactatgtaactggtggcctga	357
3	tattgtcgcatcagcccttc	ttgttgttgctgccattgtt	423
4	gtatatccggtcctccatgc	tccagcactttgtgaaggtt	428
5	ctctgtgggctggaaatga	agcaggaatgtctggtgagg	388

Figure 3 A Sequence electropherograms of mutations found in *FGF14*.



Both normal *(left)* and mutated *(right)* DNA and amino acid sequences are shown.

Figure 3 B Amino acid sequence comparisions

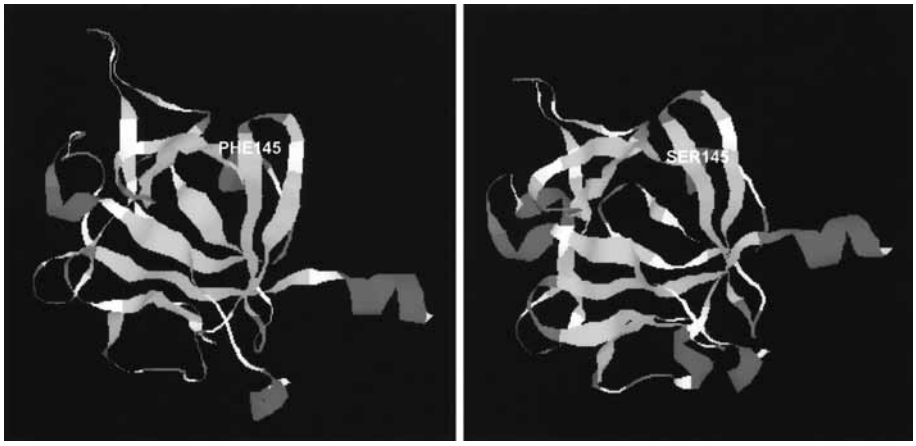


B (top), Amino acid sequence comparison of FGF11–FGF14 homologs in *Homo sapiens*, *Mus musculus*, and *Gallus gallus*, showing the conservation of the amino acid mutated in FGF14 (F145S) in the family with ataxia. The change of a phenylalanine to a serine in patients is indicated by an arrow. Only the alignment with amino acids 139–159 of FGF14 is shown. *B (bottom)*, Amino acid sequence comparison around the mutated F145S for all FGFs found in *Homo sapiens*. Amino acid alignments were performed with ClustalW.

with either the normal (tgcaagtttaaagaat) or mutated (tgcaagtctaaagaat) sequence primer. Filters were washed to a final stringency of 0.3 × SSC, 0.1% SDS, at 37°C. The base change in exon 4 was not found in the general Dutch population, and it segregates completely with the disease phenotype in the family. No other sequence changes segregating with the disease phenotype were identified. The individual (III:1) for whom diagnosis was uncertain did not share the disease-associated haplotype and mutation.

To investigate the structural consequences of the F145S mutation described here, the sequence of *FGF14* was submitted to the 3D-PSSM (position-specific scoring matrix) fold-recognition server,[14] which identified six homologues of known structure with E values ranging from 0.005 to 0.1. A molecular model of FGF14 was built on the basis of the known structure of human FGF9 (MIM 600921). The corresponding structure of FGF9, solved by X-ray crystallography at 2.6 Å resolution (Protein Data Bank entry 1G82), was therefore used as a modeling template.[15] Side chains were built, using WHAT IF,[16] and the model was then refined with YASARA.[17] Validation of the model with WHAT_CHECK[18] showed structure Z scores in normal ranges, slightly better than those of the template. The model coordinates are available on request. With 39% sequence identity and an almost gapless alignment spanning 144 residues, both proteins can be expected to adopt the same fold.[19] The highest scoring alignment, with 39% sequence identity, spanned residues Arg 63 to Tyr 206 and was gapless except for a single one-residue insertion. In this model, F145 is deeply buried and forms a central part of the hydrophobic core. An *in silico* mutation to serine showed that although its hydroxyl group can form a hydrogen bond with the backbone oxygen of V114, the now-missing

Figure 3 C Molecular models of wild-type and mutant FGF14.



The dark-grey and light-grey ribbons correspond to α-helix and β-sheet structures, respectively. Indicated is the position of the wild-type (F145) *(left panel)* and mutated (Ser145) residue mutated in the family.

bulky phenyl ring leaves a big space in the protein's core *(fig. 3C)*. It is well known that mutations leading to empty space in the core are very destabilizing,[20] especially if the structure is rigid and cannot easily accommodate the changes. We therefore conclude that the F145S mutation does not act directly, by influencing the interaction of FGF14 with other cellular factors, but most likely indirectly, by reducing its stability.

The present study demonstrates for the first time that a mutation in *FGF14* is associated with autosomal dominant cerebral ataxia with apparent complete penetrance. The FGF protein family currently consists of 23 known FGFs.[21-23] FGF11 through FGF14 share <30% amino acid identity with other FGFs but retain core conserved amino acid residues in exons 2, 3, and 4. However, FGF11 through FGF14 share 58%–71% amino acid sequence identity with each other, lack a secretory sequence as seen in the other FGFs, and are indeed not secreted in the medium of transfected cells but accumulate to high levels intracellularly instead.[13,21] Furthermore, no interaction with FGF receptors has been identified, making it likely that their mechanism of function is different from the other FGFs.

The alternatively spliced exon 1 appears to encode dominant protein-trafficking signals to target the proteins to different subcellular compartments;[13,21,24] FGF14a is mainly located in the nucleus, whereas FGF14b is located in the cytoplasm of transfected NIH3T3 cells.[13] In addition, in the primary sequence, two nuclear-localization domains can be predicted, (89-PSASRRR-95) and (122-KKRR-125). Other functional motifs are a peroxisomal domain (125-RLRRQDPQL-133) and an endoplasmatic reticulum membrane domain (308-KSKT-311).
The expression pattern of Fgf14 in mice suggests a role in both neuronal development and adult brain function. In embryonic mice at E12.5, the gene is expressed in the ventral lining of the third and fourth ventricles of the subventricular zone, which gives rise to neurons of several brain regions, including the cerebellum. The gene is also expressed in the supraoptic and septal areas, spinal cord, and several nonneuronal tissues.[13] Fgf14 is also expressed in the adult mouse brain, with highest levels of expression in the granular cells of the cerebellum and moderate to high levels in hippocampus in amygdala, cerebral cortex, striatum, and thalamus. A β-galactosidase reporter protein showed that Fgf14 is mainly present in axons and almost entirely absent in cell bodies.

Mutations in *FGF* genes are rare; only mutations in *FGF23* have been associated with autosomal dominant hypophosphataemic rickets.[22] The F145S mutation in the present family with cerebellar ataxia is the first genetic defect found in one of the *FGF11–FGF14* genes, encoding a subgroup of FGFs that do not interact with FGF-receptor proteins. The F145S mutation found in this family affects a highly conserved amino acid and

is located in a region of the protein where several other amino acids are conserved between FGFs (for example, C143 is conserved in all FGFs; *see fig. 3B*). The region of the protein is especially conserved in the subgroup of FGF11–FGF14 (MIM 601514, 601513, 300070, and 601515), indicating that it might encode a domain that is specifically involved in the function of this subgroup of FGFs. However, the exact effect of the mutation on the function is difficult to predict, since very little is known about the function of FGF14.

The clinical presentation of the F145S mutation — tremor in childhood years, ataxia slowly progressive over decades, with normal life expectancy in the family we describe — was quite characteristic for this SCA type. The absence of genetic anticipation and of variation in clinical phenotype in the present family already made an expansion of a trinucleotide repeat unlikely before identification of the F145S mutation. The *FGF14* gene is separated from the SCA8 locus by ≥ 30cM on the sex-averaged linkage map.[2] However, the controversy over the causal relation between the expanded CTG repeat and SCA8 justifies screening of the *FGF14* gene in patients with SCA8 for whom no linkage data excluding the current locus are available.[25-27]

The phenotype of the F145S mutation in the present family showed striking similarities with the findings of ataxic gait, widened stance, lack of forefoot-hindfoot correspondence, and paroxysmal hyperkinetic dyskinesia in homozygous Fgf14-knockout mice at age 3 wk.[12] The cerebellar ataxia in the present patients with the F145S mutation may be caused by dysfunction of cerebellar granule cells in the internal granular layer, since FGF14N β-galactosidase staining in transgenic mice was most intense in the molecular layer where axons of granule cells extend. The findings in patients with the *FGF14* mutation and in the Fgf14-knockout mice may suggest a dysfunction in the basal ganglia circuitry. Fgf14 is not expressed by dopaminergic neurons but in other neurons of the striatopallidal and striatonigral pathways.[12] The inability to complete primary education, low cognitive performances, and aggressive outbursts in several of our patients might reflect changes in the development and survival of neuronal populations in the cerebral cortex, amygdala, and basal ganglia. Fgf14 is localized in axonal projections, and it has been suggested that it plays a role in axonal function, synaptosomal function, or neurotransmission.[12] The question is whether and how Fgf14 dysfunction represents a novel pathway of neurodegeneration. It seems unlikely that intranuclear inclusions, which are the pathological hallmarks of polyglutamine disorders,[3] will be found in the brains of patients with the F145S mutation, and we need to wait for available postmortem tissue to investigate which cell type is primarily involved and to determine the subcellular distribution of mutant FGF protein. In addition, functional studies, using model systems, will be required to help solve these questions.

It is tempting to compare the available Fgf14 knockout model directly with the clinical phenotype in the family described here, although we need to be cautious since in the family we describe the trait segregates in an autosomal dominant fashion. Each patient carrying the mutant copy of the gene still has one fully functional copy of the gene as well. However, because the F145S mutation is located in the central core of the protein and is likely to result in a decreased stability of the protein, a loss of function of the protein is a more likely explanation than increased aggregation properties of the protein. Expression studies using the mutant protein in transgenic mice and cellular models may resolve this issue in the near future. Since an animal model lacking Fgf14 is available, it is therefore important to investigate whether a heterozygous knockout mouse, which appears to be normal at age 4 wk,[13] does show abnormalities when more-sensitive tests for motor and coordination are applied or when tests are performed at a later stage in life.

In conclusion, the pathogenic F145S mutation in the *FGF14* gene is a representative of a novel gene defect for cerebellar ataxia. Mutational screening in familial ataxia of unknown genetic cause, and in SCA8 in particular, will resolve the question of how frequent this gene defect is. Pathologic examination of brain tissue from ataxic patients with the F145S mutation and functional studies using model systems may shed more light on the normal function of FGF14 and characterize a new pathway to neurodegeneration.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ (for polymorphic STR markers)

ClustalW (European Bioinformatics Institute), http://www2.ebi.ac.uk/clustalw/ (for amino acid sequence comparisons)

Ensembl, http://www.ensembl.org/ (for identification of transcripts in the critical region)

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/Genbank Overview.html (for NM_004115 and NT_009952)

Généhon, http://www.genethon.fr/php/index_us.php (for polymorphic STR markers)

Human Genome Browser, http://genome.cse.ucsc.edu/ (for identification of transcripts in the critical region)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for SCA1 [MIM 164400], SCA2 [MIM 183090], SCA3 [MIM 109150], SCA6 [MIM 183086], SCA7 [MIM 164500], DRPLA [MIM 125370], SCA8 {MIM 603680], SCA10 [MIM 603516], FGF9 [MIM 600921], FGF11 [MIM 601514], FGF12 [MIM 604513], FGF13 [MIM 300070], and FGF14 [MIM 601515])

Protein Data Bank, http://www.rcsb.org/pdb/ (for 1G82)

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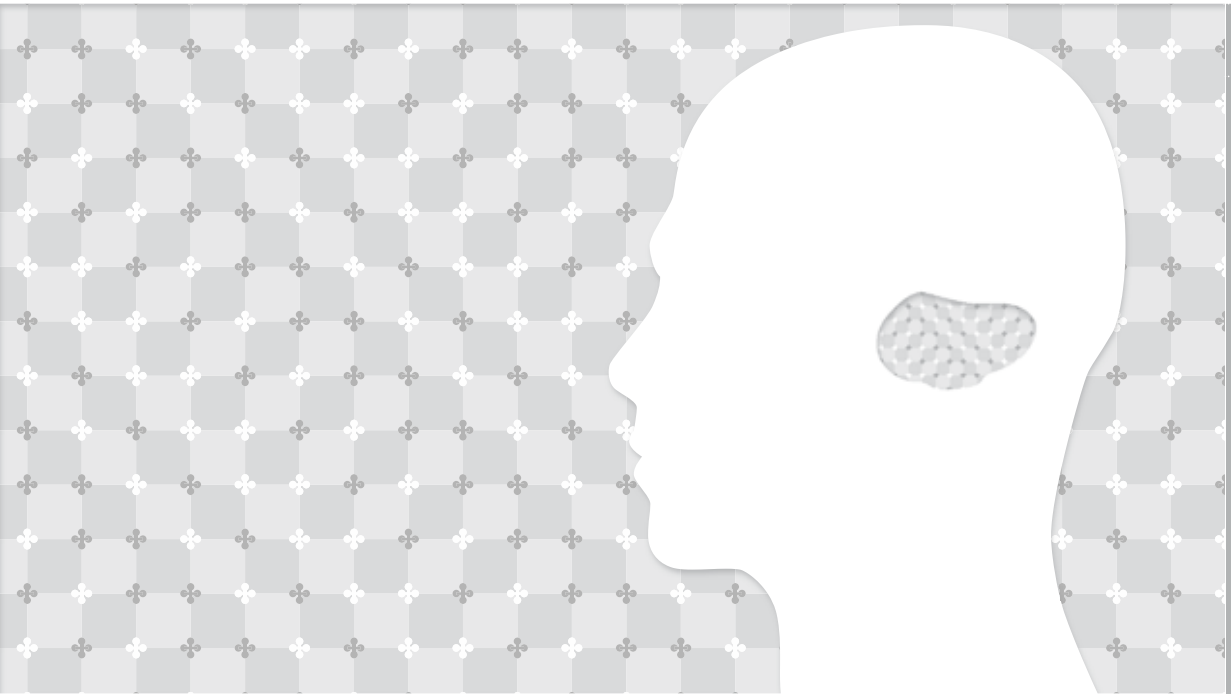

Autosomal dominant cerebellar ataxia

Spinocerebellar ataxia associated with a mutation in the fibroblast growth factor 14 gene (SCA27)

a new phenotype

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Abstract

Autosomal dominant cerebellar ataxias (ADCAs) are genetically classified into spinocerebellar ataxias (SCAs). We describe 14 patients of a Dutch pedigree displaying a distinct SCA-phenotype (SCA27) associated with a F145S mutation in the *fibroblast growth factor 14 (FGF14)* gene on chromosome 13q34. The patients showed a childhood-onset postural tremor and a slowly progressive ataxia evolving from young adulthood. Dyskinesia was often present, suggesting basal ganglia involvement, which was supported by functional imaging in 1 patient. Magnetic resonance imaging (MRI) of the brain showed only moderate cerebellar atrophy in the 2 eldest patients. Neuropsychological testing indicated low IQ and deficits in memory and executive functioning. Behavioral problems were also observed. Further investigations will have to determine the role of FGF14 in the pathogenesis of neurodegeneration and the frequency of this *FGF14* mutation in SCA.

3.2 Spinocerebellar ataxia associated with a mutation *in the fibroblast growth factor 14* gene (SCA27)

a new phenotype.

The genetic classification of the autosomal dominant cerebellar ataxias (ADCAs) into spinocerebellar ataxias (SCAs) has currently revealed more than 20 genetic loci and 12 of the corresponding genes. In approximately 30% of SCA patients, a genotype has not yet been identified. The worldwide prevalence of SCA is estimated at 0.3 to 2.0 per 100,000.[1]

The age at onset and severity of this disorder is partly type specific, but also correlates with the extent of tri- or pentanucleotide repeat expansions, arising from most SCA mutations: a phenomenon called anticipation. Other more-or-less distinguishing clinical signs are retinopathy in SCA7, seizures in SCA10, tremor in SCA12 and 21, and mental retardation in SCA13, but there is an evident overlap in physical findings.[1,2–6]

We describe the clinical features in a Dutch family displaying a distinctive SCA phenotype associated with a mutation in the *fibroblast growth factor 14 (FGF14)* gene.

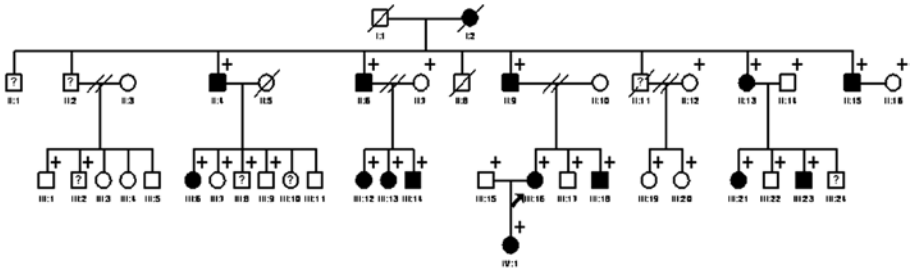
Patients and methods

The pedigree of the three-generation (II, III, and IV) Dutch family is demonstrated in Figure 1. Fourteen affected relatives (7 men, 7 women), 7 unaffected individuals, and 5 spouses were examined by two neurologists. The clinical status of 2 other examined relatives was considered unknown: Individual III:2, known with chronic alcohol abuse, showed an isolated gaze-evoked nystagmus without tremor or ataxia, and Individual III:8 mentioned complaints of unsteadiness from orthopedic origin. His neurological examination was normal. Testing for trinucleotide repeat expansions in SCA1, 2, 3, 6, 7, and dentatorubral-pallidoluysian atrophy (DRPLA) in the proband gave results in the normal population range.

Patient 1

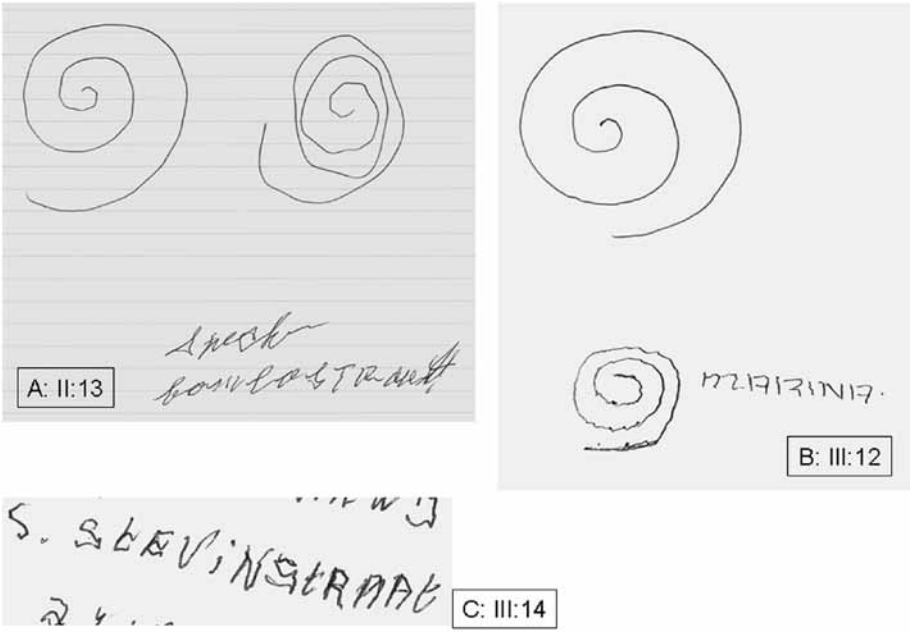
The proband (III:16) was evaluated at the age of 45 years. Since childhood, this house worker had noticed trembling of both hands that was increased by physical or emotional stress but was not decreased by consumption of alcohol. She complained about unsteadiness in sport lessons in her early teens with an unstable gait since the age of 30 years. These complaints began to interfere with her daily activities over the last

Figure 1 **Simplified pedigree of the Dutch family.**



Squares = male subjects;
circles = female subjects;
solid symbols = affected members;
open symbols = unaffected members;
question marks = phenotype unknown;
arrow = proband;
plus sign = clinically evaluated patients.

Figure 2 **Handwriting and circle-drawing of Patient II:13 (A), Patient III:12 (B), and Patient III:14 (C).**



Writing of II:13 is severely disturbed by limb ataxia and tremor. Influence of tremor is shown in III:12 and III:14, aged 31 and 27 years, both mentioning "bad handwriting" since early childhood.

few years, and her speech became slightly slurred. Neurological examination at age 48 showed mild cerebellar dysarthria, gaze-evoked nystagmus, slow saccades, and jerkiness of smooth-pursuit eye movements. Mild gait and limb ataxia were present, with inability to perform tandem walking. Tendon reflexes were brisk, although plantar responses were normal. The patient showed a mild tremor of the head and a prominent postural tremor of the arms, increasing on voluntary movement. Psychometric evaluation showed a low Raven-IQ of 79 (8th percentile) and mild memory impairment. Executive functioning and verbal and nonverbal abstraction were also impaired. Cranial magnetic resonance imaging (MRI), electromyography (EMG) of the extremities, and somatosensory evoked potentials (SSEP) studies all were normal.

Patient 2

The 69-year-old father of the proband (II:9) reported unsteadiness since his late twenties. His walking had deteriorated over the past 10 years and he required a cane. He had completed primary school without further education. Neurological examination revealed a severe scanning dysarthria and mild orofacial dyskinesia. The patient showed a prominent postural and action tremor of the hands with illegible handwriting. Severe limb and gait ataxia was present, with inability to turn without falling. Cranial MRI showed moderate cerebellar atrophy. Functional imaging with [123I]iodobenzamide SPECT (IBZM-SPECT) displayed a reduced dopamine D2 receptor binding of the left striatum, whereas normal binding ratios were found using iodine-123-*N*-fluoropropyl- 2β-carbomethoxy-3β-(4-iodophenyl) nortropane (FP-CIT) SPECT. Psychometric testing showed a Raven-IQ of 10 to 25th percentile, mild impairment of nonverbal memory function, and moderate executive dysfunction.

Table 1 summarizes the clinical findings of the 14 affected individuals. The mean age at examination was 49 years (range 24–79 years). Mean estimated onset was 11 years (range, 7–20 years). Postural tremor of both hands, and sometimes head titubation, was the first and well-recognized neurological symptom in all patients.
This tremor was aggravated by stress or fatigue and no beneficial effects were reported on using alcohol, propranolol, or dopaminergic medication. Ataxia typically started in the third or fourth decade in generation II and III (mean onset 34 years).
A remarkable finding was the clearly lower education level of all affected family members. In contrast, all but one of their unaffected relatives had attended secondary school. In 7 patients, relatives reported behavioral problems, resulting in aggressive outbursts, conflicts with partners and close relatives, and irregular working status. Two other affected individuals suffered from depression. The unaffected relatives had no history of psychiatric or behavioral problems.

Neurological examination of all affected family members showed signs of ataxia, nystagmus, dysarthria, or all of these *(Table 1)*. The severity of ataxia was related closely to the duration of the disease. In the youngest patients, writing was disturbed mainly by tremor, and in the elderly patients by a combination of tremor and limb ataxia *(Fig. 2)*. Seven patients showed titubation. Orofacial dyskinesia was observed in 8 patients, sometimes combined with slight choreatic movements of the trunk and shoulders. Impaired vibratory sense of the legs was present in 9 patients.
MRI or computed tomography (CT) of the brain was available in 8 patients. This showed moderate cerebellar atrophy in the 2 eldest individuals and normal findings in the

Table 1 Clinical features of the 14 affected patients

Individual	II:4	II:6	II:9	II:13	II:15	III:6
Age (yr)						
At examination	79	71	69	64	61	54
At tremor onset	10	10	20	10	10	12
At ataxia onset	30	36	27	50	30	40
Education	PS	< PS	PS	SE	< PS	SS
Social Status	Wi	Di	Di	M	M	M
Working status (age in yr when stopped)	UE (35)	UE (32)	?	HM	UE (41)	HM
Psychiatric history	A	Dp	-	-	-	-
Neurological examination						
Walking	WC	WC	C	↓↓	↓	↓
Writing	U	NA	U	↓↓	↓	↓
Dysarthria	++	++	++	++	++	+
Nystagmus	+	+	+	+	+	+
Postural tremor	+	+	+	+	+	+
Titubation	+	+	–	+	+	+
Dyskinesia	+	–	+	+	+	+
Limb ataxia	++	++	++	+	+	+
Gait ataxia	++	++	++	++	+	+
Vibratory sense	↓	↓	↓	↓	N	N
Brain MRI/CT (age in yr)	–	Cerebellar atrophy (66)	Cerebellar atrophy (69)	N (64)	–	N (54)

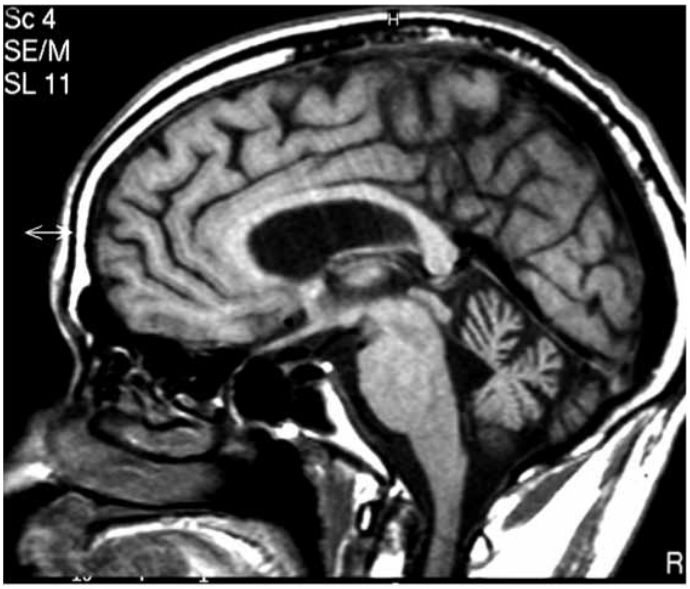
PS : primary school; SS : secondary school; Di : divorced; S : single; HM : homemaker;
SE : special education; Wi : widower; M : married/ partner; UE : unemployed; ? : unknown; SW : sheltered workplace;

other 6 patients *(Fig. 3)*. EMG studies of the extremities in four patients (II:9, 13, III:16, and IV:1) were normal. Neuropsychological evaluation was carried out in 4 affected individuals (II:9, II:13, III:14, and III:16) and 1 unaffected individual (III:22). Intelligence, estimated through Raven's progressive matrices, was below average in the three eldest patients (II:9, II:13, and III:16), as were the scores on the verbal and nonverbal memory tests. Executive functioning was diminished in all 4 patients. In contrast, the unaffected individual had a good performance on all tests with a Raven IQ of 92.
DNA analysis showed a F145S mutation in the *FGF14* gene on chromosome 13q34 resulting in an amino acid change of phenylalanine into serine in all affected family mem-

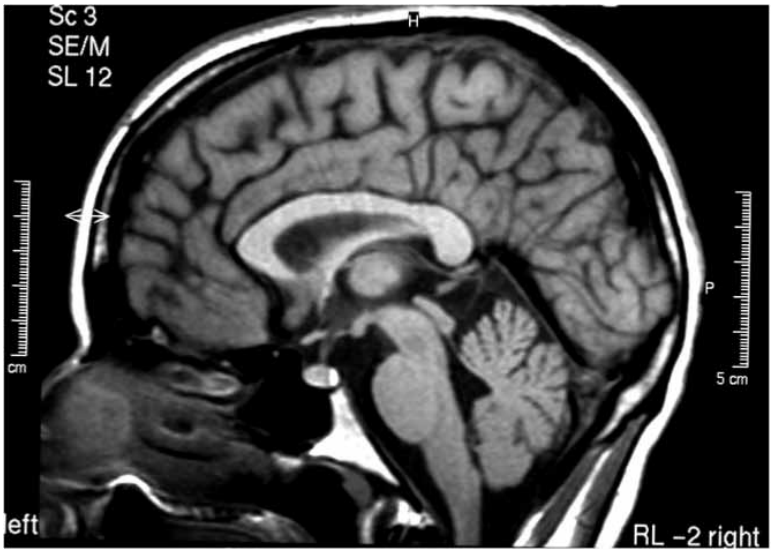
	III:12	III:13	III:14	III:16	III:18	III:21	III:23	IV:1
	31	30	27	48	42	43	39	24
	8	(20)	10	10	10	12	6	7
	31	(30)	-	30	35	40	-	18
	PS	SE	PS	SS	PS	SS	SE	SS
	S	S	S	M	M	M	Di	Di
	SW	SW	UE	HM	HM	W	W	HM
	A	A	A	-	-	-	A	Dp
	↓	N	N	↓	↓	↓	N	N
	↓	↓	↓↓	↓	N	N	↓	↓
	+	+	+	+	–	+	–	–
	+	+	+	+	+	+	+	+
	+	–	+	+	+	–	+	+
	–	–	+	–	–	–	+	–
	+	–	+	–	–	+	–	–
	+	–	+	+	+	–	+	+
	+	+	–	+	+	–	–	+
	↓	↓	↓	N	N	N	↓	↓
	–	–	N (27)	N (48)	N (42)	N (43)	–	N (24)

W : working; A : aggressive outbursts; Dp : depression; WC : wheelchair; C : cane; N : normal; U : unable; NA : not applicable; ↓↓ : severely impaired; ↓ : mildly-moderately impaired; ++ : severe; + : mild-moderate; – : absent

Figure 3 **Sagittal T1-weighted brain magnetic resonance imaging (MRI) of Patient II:9 (a) and Patient IV:1 (b)**



a:II:9



b:IV:1

Moderate cerebellar atrophy is seen in Patient II:9, aged 69 years; brain MRI of 24-year-old Patient IV:1 is normal.

bers. The unaffected relatives and 2 individuals with unknown phenotype (III:2 and III:8) did not share the disease-associated haplotype and mutation; apparently there was complete penetrance. This mutation was not found in 376 controls of the general Dutch population.

Discussion

The mutation in the *FGF14* gene, recently classified as SCA27 (MIM #609307), is one of three known point mutations related to SCA. All other SCA-mutations give a trinucleotide or pentanucleotide repeat expansion. The most striking clinical observation in this family is the early onset at teen ages with a tremor, even years before any signs of cerebellar disease are present. Additional remarkable observations are low performances in education and professional career and frequent behavioral problems. The ataxia differs from that in most other SCA types by the extremely slow progression. The combination of postural tremor with an ataxic intention tremor, as found in this family, has been reported in cerebellar disease and is defined as ataxic tremor in the literature.[7,8]

In our view, however, the consistent occurrence of postural tremor before the onset of ataxic symptoms might be suggestive for a noncerebellar origin of this tremor, probably from the basal ganglia circuitry (see below). A similar phenotype, with postural tremor as a presenting symptom, has also been found in SCA12,[3] whereas postural tremor later in the disease has been reported in SCA2, SCA7, SCA8, SCA19, SCA21, and DRPLA.[1,6,9–11] In SCA14, an early onset tremor of the neck caused by an axial myoclonus may precede ataxia.[12] Titubation, seen in 7 patients, can occur in combination with a postural tremor and has been described in midline cerebellar disease.[8]

The mean onset of ataxia in the present family (34 years; range 18–50 years) is similar to that in most other SCA types. The finding of moderate cerebellar atrophy only in the 2 eldest patients differs from the occurrence of cerebellar atrophy, sometimes with cortical or brainstem atrophy, early in the course of the disease in all known SCA types.[1] Dyskinesia, found in 8 affected individuals, has also been described in SCA17 and DRPLA.[1,13] The presence of diminished vibratory sense of the legs and hyperreflexia, as signs of involvement of the long spinal tracts, is seen in frequently SCA. No concomitant polyneuropathy was found in this family.

Neuropsychological evaluation of 4 patients indicates a lower cognitive functioning. Cognitive impairment and dementia has been reported in many other SCA subtypes, probably related to dysfunction of cerebrocerebellar circuits. Behavioural problems, as in this family, are infrequent, however, occurring only in SCA17 and DRPLA.[1,13]

The FGF14 protein is one of a family of 23 known FGF proteins. The novel F145S mutation in this family is the first known genetic defect in the FGF11-FGF14 subgroup associated with a clinical phenotype.[14] Animal studies show that FGF14 is highly expressed in the granular cells of the cerebellum and in the hippocampus, amygdala, cerebral cortex, and non dopaminergic neurons of the striatopallidal and striatonigral pathways. This expression pattern correlates with the symptoms of dyskinesia and the findings on the IBZM-SPECT scan seen in this family. In addition, the symptoms of ataxia and paroxysmal dyskinesia in FGF14-deficient mice, corresponding with the phenotype of this family, is striking.[15,16]

An interesting question is how often mutations in the *FGF14*-gene may be found in SCA families with unknown genotype. A German group has just identified a frameshift mutation in the *FGF14* gene in a patient with cerebellar ataxia and mild mental retardation.[17] No *FGF14* mutations were identified in a series of 53 Caucasian SCA patients from France,[18] however, in accordance with our own observations in 38 SCA patients, which revealed no *FGF14* mutations. The *FGF14* mutation seems to be an infrequent cause of SCA. Another issue is how and to what extent different *FGF14* mutations may explain phenotypical variations. The phenotype of the German patient is consistent with our family, except for the polyneuropathy with pes cavus. Both mutations result in an early onset of symptoms. In our family, the *F145S*-mutation is likely to result in a decreased stability of the protein; the frameshift mutation causes truncation of the protein.[14,17] Loss of function of the protein is therefore a more likely explanation than is increased protein aggregation. Little is known, however, about the molecular function of FGF14. It has been suggested that it plays a role in axonal or synaptosomal function or neurotransmission.[14–16] This may give rise to a broad spectrum of neurological symptoms also of the peripheral nervous system. Further clinicopathological studies and genetic studies may clarify the role of FGF14 in the pathogenesis of neurodegeneration. It thus seems important to screen families with related, more isolated cerebellar, extrapyramidal, psychiatric, or cognitive symptoms for mutations in the *FGF14* gene to reveal the variability of the phenotype.

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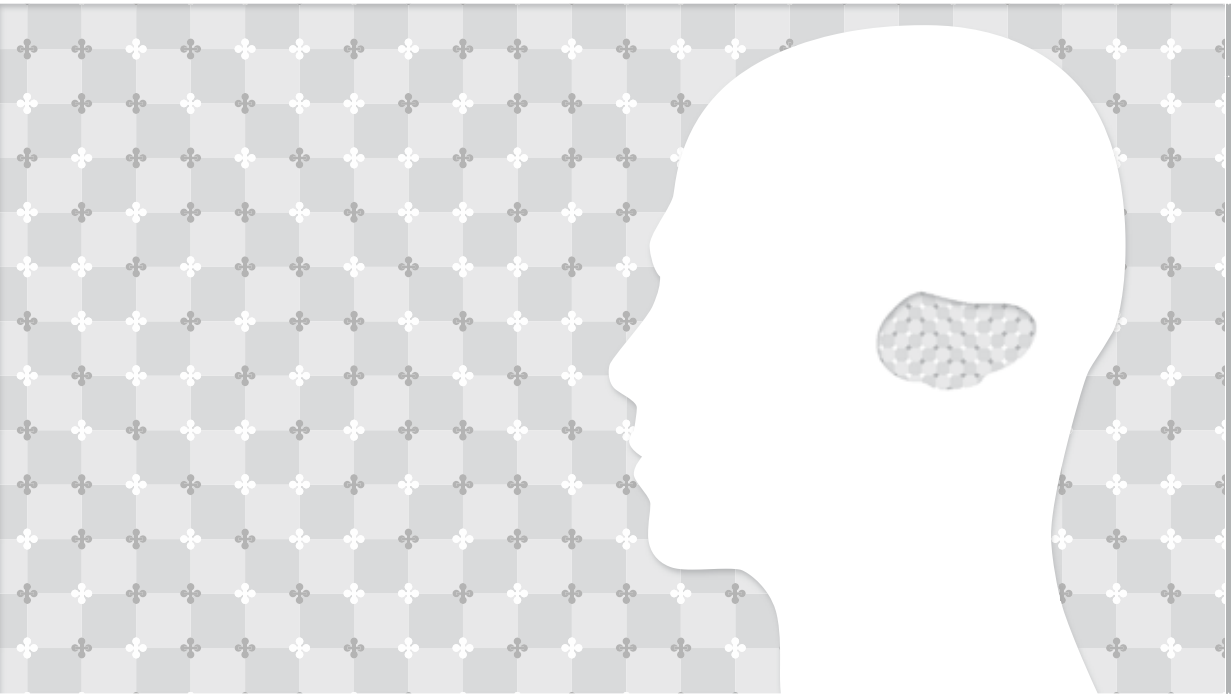

Autosomal recessive cerebellar ataxia

A new locus for a childhood-onset, slowly progressive, autosomal recessive spinocerebellar ataxia maps to chromosome 11p15

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Abstract

Key points

- **Here we report a non-consanguineous Dutch family with a pure spinocerebellar phenotype with cerebellar ataxia, pyramidal signs, posterior column involvement with deep sensory loss, a postural tremor and absence of other (non-) neurological features. Neuroimaging shows atrophy of the cerebellum, vermis, pons and medulla oblongata. Onset of symptoms is in early childhood but remarkably, the severity of symptoms and progression of the disease within this family is very variable.**
- **The clinical phenotype of the family is not consistent with any of the known autosomal recessive cerebellar ataxia's.**
- **Using a systematic genome wide scan we mapped the responsible gene for autosomal recessive ataxia in this family to a 5.9 cM interval on chromosome 11p15. A large number of genes and expressed sequence tags are identified in this critical region, but no obvious candidate gene can yet be assigned, as genes for ataxia mostly have different functions and features.**

3.3 A new locus for a childhood-onset, slowly progressive, autosomal recessive spinocerebellar ataxia maps to chromosome 11p15

The cerebellar ataxias are a heterogeneous group of neurodegenerative disorders, characterised by symptoms and signs of cerebellar degeneration and by pyramidal and extrapyramidal features as well as polyneuropathy in a variable extent. Prominent clinical features are signs of cerebellar ataxia such as uncoordinated gait, uncontrolled co-ordination of hand, speech and eye movements, whereas (extra) pyramidal signs, retinal, cardiac, muscle and/or neuronal involvement are less common. The clinical picture shows large variation in age at onset and disease progression.
Sporadic ataxias may be attributed to various toxic, inflammatory, paraneoplastic, metabolic, endocrinal or malabsorption conditions. Hereditary ataxias consists of a large number of autosomal dominant ataxias and ataxias with an autosomal recessive, and X-linked mode of inheritance. The remaining ataxia's of unknown cause are referred to as idiopathic sporadic cerebellar ataxias.

Most symptomatic ataxia's can be diagnosed on the basis of a typical history or by simple laboratory tests. In hereditary ataxias the family history is important. However, a negative family history cannot rule out autosomal recessive or X-linked ataxia and even autosomal dominantly inherited ataxia may be missed because of reduced penetrance, early death of gene carriers before onset of symptoms, imprinting effects, variable expression, adoption or non-paternity.[1]

Gene defects have been identified for several hereditary forms. Autosomal dominant ataxias are often associated with genes containing unstable expanded trinucleotide repeats, like polyglutamine-coding CAG repeat expansions in SCA 1, 2, 3, 6, 7, 17 and DRPLA or trinucleotide or pentanucleotide repeat expansions in (non-) coding regions in SCA 8, 10 and 12. Other mutations have been identified, like point mutations in FGF14 and SCA14 and several loci, such as SCA 4, 5, 11, 13, 15, 16, 18, 19, 21, 22, 23 and 25 have been mapped.[2,3] Some autosomal recessive ataxias have been clinically characterised, their genes localised or their genes and their proteins identified *(table 1)*.[2-4] The most common form (11- 38%) of autosomal recessive ataxia is Friedreich's ataxia (FRDA),[5-8] which shows a large variation in clinical presentation, that is age at onset and severity

Table 1 The Autosomal recessive ataxias, their loci, genes, proteins, onset and distinguishing features[2, 3, 4]

Autosomal recessive syndrome/s (references)	Locus	Gene	Protein
Friedreich's ataxia (FRDA1)/ 2 (FRDA2)[5, 6, 7, 40]	9q13/ 9p23	X25/ -	Frataxin/ -
Ataxia teleangiectasia (AT)/ AT-like[9, 10]	11q22-23/ 11q21	ATM/ hMRE11	Phosphatidylinositol 3-kinase/ hMRE11 protein
Vitamin E deficiency (AVED)[11]	8q13	α TTP	α-tocopherol transfer protein
Abetalipoproteinemia (ABL)[12]	4q22-24	MPT	Microsomal triglyceride transfer protein
Spastic ataxia Charlevoix-Saguenay (ARSACS)[13, 14]	13q12	SACS	Sacsin
Infantile onset spinocerebellar ataxia (IOSCA)[15]	10q24	-	-
Ataxia with oculomotor apraxia 1 (AOA1)/ 2 (AOA2)[16, 17]	9p13/ 9q34	APTX/ SETX	Aprataxin / Senataxin
Refsum disease (RD)[18, 19, 41]	10p11-pter, 6q22-24	PHYH, PEX7	Phytanoyl-CoA hydroxylase, Peroxin 7
Carbohydrate deficient glycoconjugate syndrome 1a (CDG 1a)[20, 21]	16p13	PMM2	Phosphomannomutase 2
Tay-Sachs disease (GM2 gangliosidosis)[22, 42]	15q23-24	HEXA	Hexosaminidase A
Krabbe[43]	14q31	GALC	Galactosylceramidase
Metachromatic leucodystrophy (MLD)[44]	22q13	ARSA	Arylsulfatase A
Wilson's disease[45]	13q14-21	ATP7B	ATPase Cu transporting β polypeptide
Cerebrotendinous xanthomatosis (CTX)[46]	2q33-ter	CYP27A1	Cytochrome 450, subfamily27A1
Hartnup[47]	5p15	-	-
Maple Syrup Urine disease (MSU)[48]	19q13	BCKDHA	Branched chain keto acid dehydrogenase E1 alpha
Biotinidase deficiency[49]	3p25	BTD	Biotinidase

	Onset (years)	Distinguishing features
	4-40	Hyporeflexia, positive Babinski sign, deep sensory loss, cardiomyopathy, diabetes mellitus, scoliosis
	0-20	Teleangiectasia, immune deficiency, cancer, chromosomal instability, elevated AFP
	2-52 (<20)	As FRDA but rare cardiomyopathy and diabetes, head titubation
	2-52	Steatorrhea, areflexia, retinitis pigmentosa, acanthocytosis, low cholesterol and β-lipoproteins
	Childhood	Spasticity, polyneuropathy, striated retina, mitral valve prolaps
	½-1 ½	Ophthalmoplegia, hypotonia, hypacusis, athethosis, peripheral neuropathy
	2-8 / 10-22	Oculomotor apraxia, chorea-athethosis, hypoalbuminemia, sensory neuropathy, elevated AFP, CPK, and immunoglobulins
	Childhood	Retinitis pigmentosa, deafness, polyneuropathy, cardiomyopathy, elevated phytanic acid
	Childhood	Hypotonia, mental retardation, failure to thrive, lipodystrophy, hepatic dysfunction, polyneuropathy, retinitis pigmentosa
	Child/ adulthood	Mental retardation, cherry red spot, blindness, epilepsy, hypotonia, startle response , low hexosaminidase A
	Child/ adulthood	Mental retardation, polyneuropathy, optic atrophy, epilepsy, low galactocerebroside
	Child/ adulthood	Mental retardation, polyneuropathy, spasticitiy, optic atrophy, epilepsy, psychiatric symptoms, low arylsulfatase
	10-30	Liver cirrhosis, Kayser-Fleischer rings, arthritis, nephrocalcinosis,high calcium, low ceruloplasmin, high copper
	10-20	Cataract, premature atherosclerosis, spasticity, xanthoma, xanthelasmata, cholesterol and cholestanol elevated
	Child/ adulthood	Pellagra, emotional instability, aminoaciduria
	Newborn/ child	Feeding problems, epilepsy, mental retardation, hypoglycaemia, ketosis
	1-2	Hypotonia, epilepsy, optic atrophy, hearing loss, skin rash, alopecia, ketoacidosis, organic aciduria

Table 1 **The Autosomal recessive ataxias, their loci, genes, proteins, onset and distinguishing features[2, 3, 4]** *continued*

Autosomal recessive syndrome/s (references)	Locus	Gene	Protein	
Carnitine acetyltransferase deficiency[50]	9q34	CRAT	Carnitineacetyltransferase	
Gamma-glutamyl cysteine synthetase[51]	6p21	GCLC	Gammaglutamylcysteine synthetase	
L-2 Hydroxyglutaric acidemia[52]	-	-	-	
Niemann-Pick C[53]	18q11-12	NPC1	NPC1 protein	
Progressive myoclonus epilepsy (Baltic or Unverricht-Lundborg)[23]	21q22	CSTB	Cystatin B	
Marinesco-Sjogren syndrome[24]	5q31	MSS	-	
Posterior column ataxia with retinitis pigmentosa (PCARP)[25]	1q31-32	AXPC1	-	
Boucher Neuhauser syndrome[54]	-	-	-	
Holmes syndrome[26, 27, 55]	-	-	-	
Ataxia with neuronal migration defect[56]	16q12-22	BFPP	-	
Ataxia with deafness and mental retardation[57]	-	-	-	
Ataxia with saccadic intrusions[58]	-	-	-	
Ataxia with optic atrophy and deafness[8]	6p21-23	-	-	
Leukoencephalopathy with vanishing white matter[59]	12/14q24/ 1//2p23/3q27	EIF2B1, B2, B3 B4, B5	Translocation initiation factor eIF2B 5 subunits	
Ataxia with axonal neuropathy (SCAN1)[60]	14q31-32	TDP1	Tyrosyl-DNA phosphodiesterase 1	
Ataxia with laryngeal abductor paralysis and motor neuropathy[61]	-	-	-	
Ataxia adult onset with thalamic lesions[62]	-	-	-	

	Onset (years)	Distinguishing features
	Childhood	Hypotonia, mental disturbances, oculomotor palsy, failure to thrive
	Adult	Haemolytic anaemia, myopathy, polyneuropathy
	Childhood	Mental retardation, short stature, leucodystrophy, macrocephaly
	Child/ adulthood	Epilepsy, spasticity, hepatosplenomegaly, dementia, psychiatric symptoms
	6-13	Epilepsy, myoclonus, mental deterioration
	Childhood	Cataract, myopathy, hypotonia, short stature, microcephaly, mental retardation, hypergonadotropic hypogonadism
	Childhood	Deep sensory loss, retinits pigmentosa, areflexia
	10-20	Hypogonadotropic hypogonadism, chorioretinal dystrophy
	10-30	Hypogonadotropic/ hypergonadotropic hypogonadism
	Congenital	Bilateral frontoparietal polymicrogyria, mental retardation, epilepsy
	Congenital	Deafness, mental retardation
	>30	Saccadic intrusions, myoclonic jerks, spasticity, deep sensory loss, fasciculations, pes cavus
	Childhood	Deafness, optic atrophy
	child/ adulthood	Leukodystrophy, ovarian failure, optic atrophy, motor deterioration, epilepsy
	Child/ adulthood	Axonal polyneuropathy, pes cavus
	adult	Dysphonia, motor neuropathy
	>30	Hyporeflexia, deep sensory loss, axonal polyneuropathy, mild cognitive impairment, bilateral thalamus lesions

Table 1 **The Autosomal recessive ataxias, their loci, genes, proteins, onset and distinguishing features[2, 3, 4]** *continued*

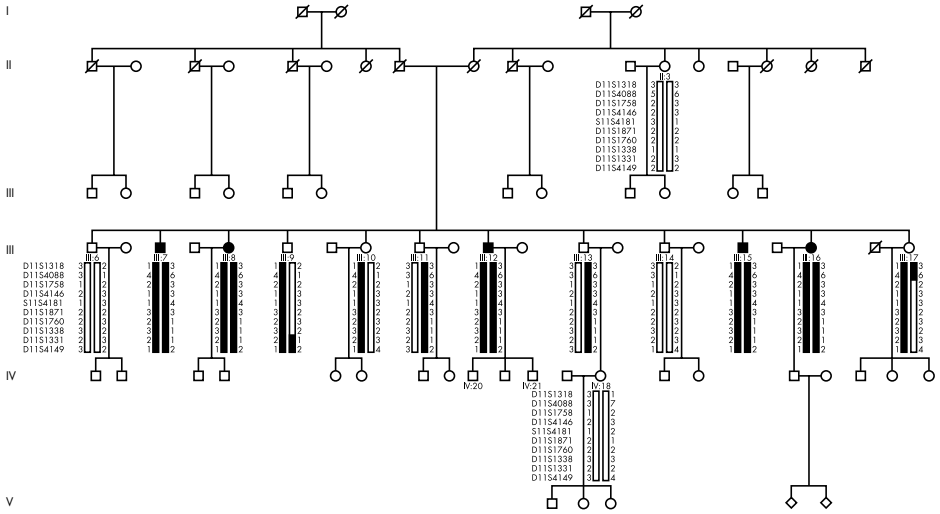
Autosomal recessive syndrome/s (references)	Locus	Gene	Protein	
Xeroderma pigmentosum A-G[63]	9q22, 2q21, 3p25, 19q13, 11p11-12, 16p13, 13q32-33	XPA, XPB, XPC, XPD, XPE, XPF, XPG	-	
Nijmegen breakage syndrome[64]	8q21	NBS1	-	
Cockayne syndrome a[65]	5	CKN1	-	
Cerebelloparenchymal disorder II[66]	-	-	-	
Ataxic cerebral palsy[67]	9p12-q12	-	-	
Joubert syndrome 1/2[68/69]	9q34/ 11p12-q13	JBTS1/ CORS2	-/-	
Behr syndrome[70]	-	-	-	
Gillespie syndrome[71]	-	-	-	
Chorea-acanthocytosis[28]	9q21	CHAC	Chorein	
Cayman Island ataxia[29]	19p13	ATCAY	Caytaxin	
Cerebelloparenchymal disorder III[8, 30, 37]	9q34-ter	CLA1	-	
Ataxia, mental retardation, optic atrophy, skin abnormalities (CAMOS)[31]	15q24-26	CAMOS	-	
Norwegian infantile onset ataxia[32]	20q11-13	CLA3	-	

	Onset (years)	Distinguishing features
	Child/ adulthood	Defective DNA repair, skin atrophy, teleangiestasia, skin cancer, mental retardation
	Childhood	Microcephaly, short stature, no mental retardation, immunodeficiency, cancer, chromosome instability
	Childhood	Retinitis pigmentosa, optic atrophy, short stature, presenile appearance, photosensitivity, deafness
	>40	Ataxia and dysarthria
	Congenital	Nonprogressive ataxic cerebral palsy
	Congenital	Vermis hypoplasia, mental retardation, hypotonia, episodic hyperpnea, retinal dystrophy, renal cysts
	Childhood	Optic atrophy, mental retardation
	Congenital	Aniridia, mental retardation
	25-45	Chorea, acanthocytes, epilepsy, peripheral neuropathy, myopathy, self-mutilation, basal ganglia atrophy
	Childhood	Hypotonia, mental retardation, nonprogressive ataxia
	Congenital	Short stature, mental retardation
	Congenital	Mental retardation, microcephaly, optic atrophy, short stature, abnormal osmiophilic pattern of skin vessels
	congenital	No mental retardation, pes planus, short stature, spasticity, nonprogressive ataxia

of clinical symptoms. Other forms of autosomal recessive ataxia are ataxia teleangiectasia (AT)[9] or AT-like disorders,[10] primary vitamin E deficiency (AVED),[11] abetalipoproteinemia (ABL),[12] spastic ataxia of Charlevoix-Saguenay (ARSACS),[13,14] infantile onset spinocerebellar ataxia (IOSCA),[15] ataxia with oculomotor apraxia (AOA1 and 2),[16,17] and Refsum's disease (RD).[18,19] Furthermore, inherited metabolic disorders can cause ataxia like carbohydrate deficient glycoconjugate syndrome,[20,21] GM2 gangliosidosis[22] and other disorders *(table 1)*.[2-4]

Some forms of autosomal recessive ataxia have a specific geographical distribution: the southern Mediterranean area in AVED, Canadian families of French origin in ARSACS, and Finland for IOSCA, while AOA is frequent in Portugal and in Japan and RD is more frequent in the Scandinavian population. The genes and their cognate proteins are known in these autosomal recessive ataxia's, except for IOSCA, which has only been localised (10q23-q24).[15] In other forms pathognomonic clinical features may sometimes help to establish the clinical diagnosis: myoclonus and/or epilepsy in progressive myoclonus epilepsy,[23] cataract in Marinesco-Sjogren syndrome,[24] retinitis pigmentosa in dor-

Figure 1 **Pedigree of the family with autosomal recessive spinocerebellar ataxia.**



Fourteen family members participated in the study, 5 affected (III.7, III.8, III.12, III.15 and III.16, represented as black symbols in the pedigree) and 9 unaffected individuals (II.3, III.6, III.9, III.10, III.11, III.13, III.14, III.17, IV.18, represented as open symbols in the pedigree). Haplotypes from chromosome 11p15 are shown below the genotyped individuals (black bars indicate risk haplotype and open bars indicate unaffected haplotype). A slash line indicates deceased status.

sal column ataxia with retinitis pigmentosa,[25] hypogonadism in Holmes syndrome[26,27] and chorea in chorea-acanthocytosis[28] *(table 1)*. Also there are some inbred families with autosomal recessive ataxia, like Cayman Island ataxia,[29] two Lebanese families with non-progressive congenital cerebellar ataxias[30,31] and a Norwegian family with an infantile autosomal recessive inherited ataxia[32] *(table 1)*.
We here describe, using a systematic genome scan, the assignment of a new disease locus to a 5.9 cM interval on chromosome 11p15 in a non-consanguineous Dutch family with a childhood onset, slowly progressive autosomal recessive spinocerebellar ataxia without involvement of other (neurological) systems and with a variable severity.

Methods

Patients

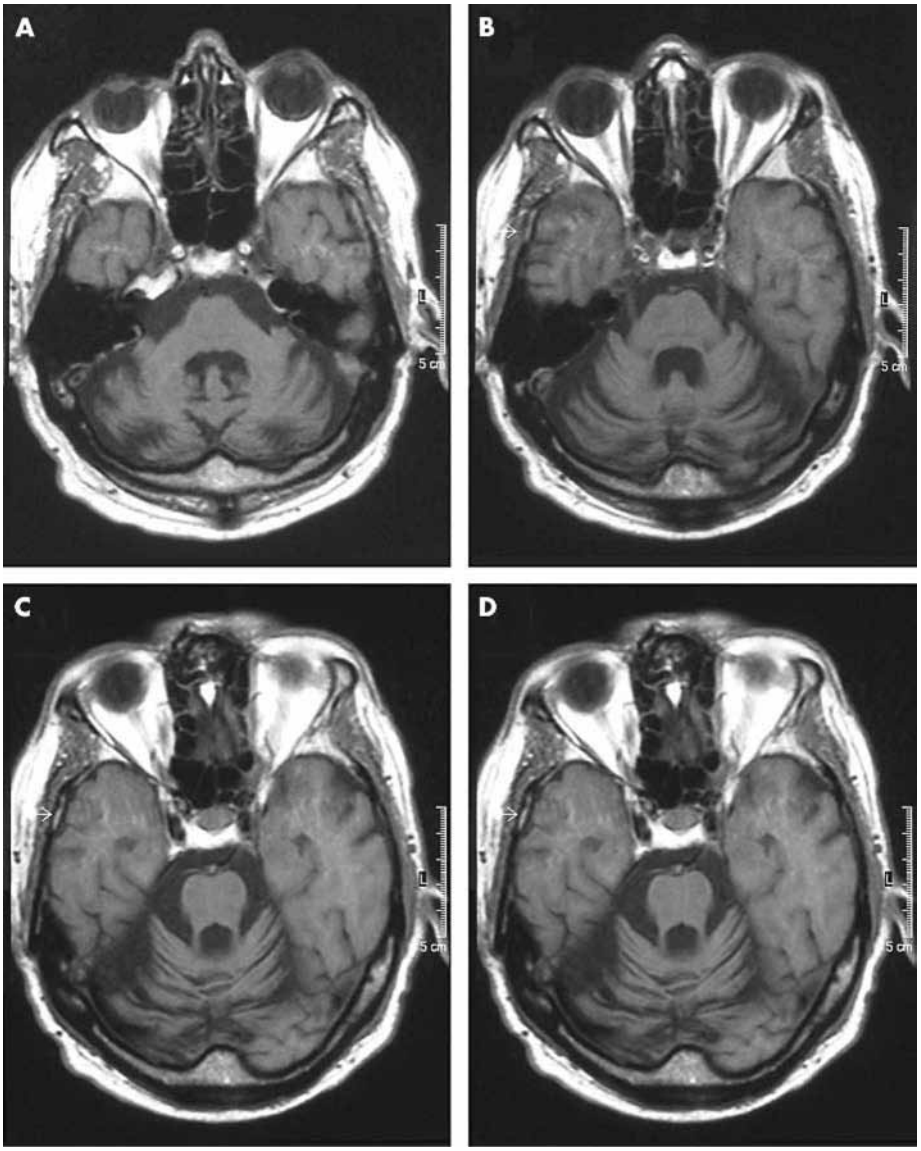
A sibship of 12 individuals from a non-consanguineous couple of Dutch descent *(fig. 1)* came to our attention because of the presence of ataxia in 5 siblings. Their mother, suffering from maturity onset diabetes, died at the age of 86 years and their father at the age of 56 years because of a head trauma. Neither they nor their relatives showed ataxia. One of the healthy sibs (III.13) and his daughter (IV.18) were seen for genetic counselling, because they wanted to be informed about their own risk for ataxia and the risk for their children.
Three neurologists (BvW, EB and JCvS) neurologically investigated all siblings (eight men and four women) and four other closely related family members *(fig 1)*. In addition to clinical evaluation, available medical records and hard copies of neuroimaging were reviewed. All affected individuals except one and one unaffected individual underwent Magnetic Resonance Imaging (MRI) of the cerebrum. An electromyography (EMG) was performed in two patients, and an ophthalmological- and neuropsychological examination was performed in one affected individual.

After clinical evaluation five affected individuals (III.7, III.8, III.12, III.15 and III.16) and seven unaffected individuals (III.6, III.9, III.10, III.11, III.13, III.14, III.17) within the same sibship were defined. The four closely related individuals (IV.18, IV.20, IV.21 and II.3) were found to be without neurological symptoms. Blood samples were collected from 14 individuals *(fig. 1)* of this family. All signed an informed consent under a protocol approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam, The Netherlands.

Genomic testing

Genomic DNA was isolated from peripheral leukocytes as described.[33] For the systematic genome scan 381 markers from the ABI Prism Linkage Mapping Set MD 10 (Version 2.5) were tested. Additional markers (STR's) for fine mapping were obtained from the

Figure 2 **MRI scan of the cerebrum of individual III.15 showing atrophy of the cerebellum, vermis, medulla oblongata and pons.**



Marshfield genetic maps 9 and 11[34] or new developed as described.[35] STR polymorphisms were amplified using 25 ng genomic DNA in 7.5 µl PCR reactions containing 1X PCR Gold Buffer; 2.5 mM $MgCl_2$; 10 µM primer pair mix and 0.4 units AmpliTaq Gold DNA polymerase (Applied Biosystems). Amplification conditions were 10' at 95°C followed by 35 cycles of 30" at 95°C; 30" at 55°C; 1'30" at 72°C; after a final extension for 5' at 72°C. PCR products were pooled in panels and loaded on an ABI3100 automated sequencer. Data were analysed using Gene Mapper Version 2.1 software (Applied Biosystems).

Linkage analysis

Two-point and multipoint linkage analyses were performed using MLINK and LINKMAP.[36] Marker order and genetic distances were used from the Marshfield genetic map. LOD scores were calculated assuming ataxia in this family being an autosomal recessive disorder with 100% penetrance with a gene frequency of 1:2500. No phenocopies were allowed and equal allele frequencies were used because of the limited number of available independent family members.

Results

Clinical data

The first patient (III.15), who was examined at the age of 60 years, had childhood onset ataxia, causing problems with fine motor movements, tremor of the hands and slurred speech. His gait became progressively unstable from childhood, and started to interfere with his daily activities after the age of 45. He was able to carry out his work until the age of 48 and became wheelchair dependent at the age of 55. There were no complaints of cognitive impairment or of visual, hearing, or swallowing problems nor did he have weakness or sensory disturbances in his extremities. Neurological examination showed normal mental status, an upbeat nystagmus, saccadic pursuit eye movements and cerebellar dysarthria. At inspection fasciculations were present in the forearms, hands and legs. Neurological examination showed normal strength in all extremities, hypertonia in his legs, hyperreflexia with Babinski signs bilaterally. Limb ataxia and severe gait ataxia was present with an inability to walk. A low frequency tremor was seen in the hands at rest, increasing with action and intention. Sensory examination showed decreased vibration sense in the legs, indicating impaired posterior column function. Extensive laboratory investigations of acanthocytes, cholesterol, triglycerid, low density lipoproteins, very low density lipoproteins, lactate, pyruvate, thyroid function, vitamin E, lysosomal enzymes, copper and ceruloplasmin, alpha-fetoprotein and immunoglobulins did not show any abnormalities. An EMG showed a mild decrease of the motor nerve conduction velocity (NCV) of the posterior tibial nerve and compound muscle action potential (CMAP) of the peroneal and median nerve, with absence of

Table 2 **Clinical findings in the affected individuals of the family with autosomal recessive spinocerebellar ataxia** *(fig.1)*

Patient	III.7	III.8	III.12	III.15	III.16
Sex	m	f	m	m	f
Age at onset	childhood	childhood	childhood	childhood	childhood
Age at examination (years)	46	50	56	60	64
Walking	↓↓	↓↓	(↓)	↓↓↓	↓↓↓
Writing	↓↓	↓↓	↓	↓↓↓	↓↓↓
Nystagmus	–	+	–	+	+
Saccadic pursuit	+	+	–	+	+
Postural tremor	–	–	+	+	–
Dysarthria	+	+	+	+	+
Gait ataxia	+	+	–	+++	+++
Limb ataxia	+	+	+	++	++
Vibration sense	↓	↓	n	↓	n
Hyperreflexia	+	–	+	+	+
Babinski sign	–	–	–	+	+
EMG	n	o	o	mild axonal neuropathy	o
Neuropsychological examination	cognitive slowness, mild concentration disturbance	o	o	o	o
MRI scan cerebrum	atrophy cerebellum and pons	o	mild atrophy cerebellum and pons	atrophy cerebellum and pons	atrophy cerebellum and pons
Ophthalmological examination	o	o	o	n	o

+ = symptom present – = symptom absent ↓ = decreased

n = normal o = not examined

the sensible nerve action potential of the sural nerve indicating a very mild axonal polyneuropathy. There were no signs of denervation or reinnervation in the musculus tibialis anterior, vastus medialis, rectus femoris or first interosseus. There was a normal H-reflex of the musculus soleus and a normal F-response, stimulating the medial and peroneal nerve.

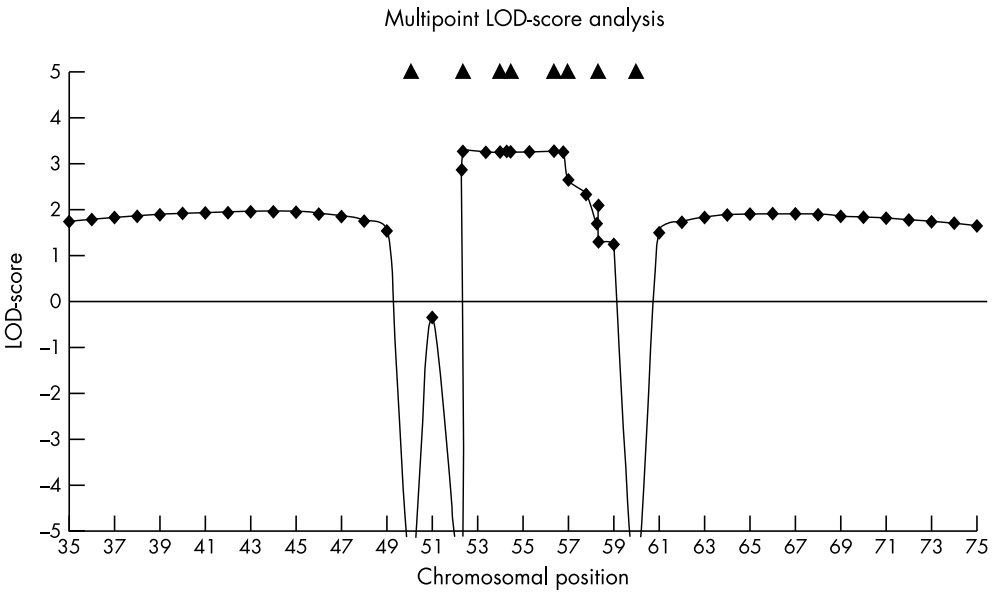
Ophthalmological examination was normal. MRI of the cerebrum showed atrophy of the cerebellum, vermis, pons and medulla oblongata *(fig. 2)*.
Table 2 summarises the clinical characteristics of the five affected individuals of the pedigree, three males and two females. Mean age at examination was 55.2 years, ranging from 46 to 64 years. The onset of the disease was in childhood. The severity of clinical symptoms (cerebellar ataxia, pyramidal signs and deep sensory loss) varied from mild to severe. The unaffected family members showed none of the symptoms described in the affected family members. The MRI of the cerebrum in the unaffected sibling (III.13) showed no abnormalities. An autosomal recessive mode of inheritance was assumed as no other affected individuals were found in the extended family. The clinical characteristics of the patients in this family, the variability in severity and the absence of other (non-) neurological features were not consistent with a known autosomal recessive syndrome.

Molecular data
DNA testing for known ataxia genes (SCA 1, 2, 3, 6, 7, DRPLA, FRDA 1) was carried out, but no mutations were found. Several autosomal recessive ataxia loci, such as FRDA 2, ATM, α-TTP, SCAN 1, ARSACS, IOSCA and AOA 1 and 2 *(table 1)* were ruled out by testing polymorphic STR markers surrounding these loci (data not shown). A systematic genome scan was started to identify the locus for autosomal recessive ataxia in this family. Markers with a positive LOD scores (>1.5) were identified on several chromosomal regions. To exclude or confirm linkage in these regions haplotype analysis was performed and if necessary additional markers were tested from the Marshfield genetic map. Except for loci on chromosome 9q34 and 11p15 all other chromosomal regions could be excluded. On chromosome 9q34 the region could not be excluded between the markers D9S1856 and D9S279 (0 cM distance of about 500kb) because markers in this region were uninformative. Also new STR markers, developed from BAC sequences in between, did not confirm or exclude linkage in this region. Testing of additional markers on chromosome 11p15 resulted in the identification of significant linkage of autosomal recessive ataxia to chromosome 11p15. Both the markers D11S1758 and D11S1871 showed a lod score of 3.2 *(table 3)*. Multipoint linkage analysis using eight additional markers confirmed the position of the gene defect to chromosome 11p15 with a lod score higher than 3 in the interval D11S1758-D11S1871 *(fig. 3)*. Haplotype analysis on this region, suggesting no phenocopies and 100% penetrance, determined the

Table 3 **Two-point LOD score table of selected chromosome 11 markers**

	Recombination fraction (θ)						
Marker	0	0.01	0.05	0.1	0.2	0.3	0.4
D11S1318	−∞	1.52	1.962	1.931	1.532	0.958	0.334
D11S4088	−∞	1.521	1.962	1.931	1.532	0.958	0.334
D11S1758	3.283	3.218	2.953	2.612	1.9	1.145	0.393
D11S4146	1.175	1.157	1.083	0.982	0.746	0.466	0.165
D11S4181	1.476	1.452	1.352	1.22	0.923	0.581	0.212
D11S1871	3.284	3.218	2.953	2.612	1.9	1.146	0.396
D11S1760	1.477	1.453	1.353	1.22	0.922	0.58	0.212
D11S1338	1.651	1.624	1.509	1.357	1.021	0.643	0.238
D11S1331	1.175	1.158	1.083	0.982	0.746	0.466	0.165
D11S4149	−∞	1.512	1.922	1.858	1.416	0.835	0.261

Figure 3 **Multipoint lod-score analysis of autosomal recessive spinocerebellar ataxia with several markers on chromosome 11p15.**



The x-axis represents the chromosomal position of the markers, indicated as black triangles. Markers from left to right: D11S1318-D11S1758/D11S4088-D11S4146-D11S4181-D11S1871-D11S1760-D11S1331/D11S1338-D11S4149. The y-axis indicates the multipoint lod score.

distal boundary of the critical region by a recombination event in subject III.17 between D11S4088 and D11S1758. The proximal boundary is determined by a recombination event in subject III.9 between D11S1338 and D11S1331. These recombination events *(fig. 1)* thus map the gene for autosomal recessive ataxia in this family between D11S4088 and D11S1331, a region of 5.9 cM.

Discussion

The present study describes a new locus on chromosome 11p15 for an autosomal recessive spinocerebellar ataxia in a Dutch family. An autosomal recessive mode of inheritance is assumed as no other affected individuals were found in the extended family. The number of affected siblings in generation III (5 out of 12) in this family could suggest an autosomal dominant mode of inheritance with reduced penetrance and/or variable expression and/or an imprinting effect. However, none of the family members in the extended pedigree showed symptoms described in affected family members, making this mode of inheritance most unlikely. The clinical characteristics of the patients in this family represent a pure spinocerebellar phenotype with cerebellar ataxia, pyramidal signs and posterior column involvement with deep sensory loss. In two patients (III.12 and III.15) the cerebellar signs were accompanied by a tremor of the hands, which can be described as a postural tremor. The combination of postural tremor plus limb ataxia has been referred to as ataxic tremor and is part of cerebellar disease. No other features of neurodegeneration were present. Onset of symptoms was in early childhood, with complaints of clumsiness, awkward writing and sometimes dysarthria and tremor. Remarkably, the severity of symptoms and progression of the disease within this family are very variable. The presenting symptoms in childhood were very subtle in patient III.7, III.8 and III.12, not interfering with their daily activities until around the age of around 40. In patient III.15 and III.16, a more progressive course was present since childhood, leading to wheelchair dependence. The early onset and the slow progression are not found in most autosomal dominant ataxias or in Friedreich's ataxia. Also the absence of (non-) neurological symptoms like optic atrophy, deafness, scoliosis, diabetes, fundus abnormalities or cardiac involvement argues against Friedreich's ataxia or other known ataxias. The clinical phenotype of the family is not consistent with any of the known autosomal recessive syndromes, found in the updated genetic classification of autosomal recessive ataxia's given in *table 1*:[2-4] this fact motivated us to look for a new gene for autosomal recessive ataxia in this family.

Before starting a genome wide screen several known loci which can cause autosomal cerebellar ataxia were excluded. First, repeat expansions in the SCA 1, 2, 3, 6, 7, DRPLA and FRDA 1 genes were excluded. Second, autosomal recessive ataxia loci, such as

FRDA 2, ATM, α-TTP, SCAN 1, ARSACS, IOSCA and AOA 1 and 2 *(table 1)* were ruled out by testing polymorphic STR markers surrounding these loci. Finally, a genome wide screen was initiated which identified several chromosomal regions. Two regions, chromosome 9q34 and chromosome 11p15 could not be excluded after haplotyping and additional markers were tested. A possible region for autosomal ataxia between D9S1856 and D9S279 (0 cM) could not be excluded due to uninformative markers. However, the region does not overlap with the region for nonprogressive autosomal recessive ataxia on 9q34-qter described as cerebelloparenchymal disorder type III[8,30,37] and AOA 2[17] *(table 1)*. Testing of additional markers on the 11p15 region revealed two fully informative markers (D11S1758 and D11S1871) which showed no recombination with autosomal recessive ataxia in this family. In a multipoint analysis significant linkage was found in the region between these markers (LOD > 3). Haplotype analyses located the disease locus between D11S4088 and D11S1331, a region of 5.9 cM. Except for D11S1871 no markers were homozygous in all patients. Testing of newly developed markers on closely linked sequences showed that this homozygosity was not enlarged, so it is probably caused by inheritance by state rather then by descent (data not shown). In this region no other locus for autosomal recessive ataxia has been described proving further evidence for locus heterogeneity.

Many genes and expressed sequence tags are identified in the critical region of 5.9 cM (about 5 Mb),[38] but no clear obvious candidate gene can be assigned. Molecular genetic research over the last decade has led to a new classification of autosomal dominant ataxia's,[39] consisting of 25 different types of SCA.[2,3] In the last few years autosomal recessive ataxia's have increasingly been investigated and proven to be genetically heterogeneous *(table 1)*. These genes play a role in metabolic homeostasis, cell cycling or in DNA repair systems. Also chaperone genes involved in recessive ataxias have been described.[2-4] It is difficult to assign a likely candidate gene in this critical region because these genes mostly have different functions and features. As Friedreich's ataxia, a very common form of autosomal recessive ataxia, is mainly caused by a trinucleotide (GAA) repeat in intron 1 of the *FRDA* gene[5], it might be interesting to look for genes with a trinucleotide repeat in their genomic sequences. Determination of the repeat length in patients versus controls in this family might suggest the pathogenic effect leading to autosomal recessive ataxia in this family. Differences in expanded repeat sizes between the affected siblings, due to instability of the expanded repeat when transmitted, could well explain the intrafamilial clinical variability of the ataxia.[7] Also, testing of additional autosomal recessive families with similar clinical features might reduce the size of the critical region and therefore simplify identification of the genetic defect. Finding the responsible mutation for autosomal recessive ataxia in this family will provide new tools towards diagnosis, understanding of the underlying pathophysiology of this disease and finally and hopefully, better strategies for therapeutic intervention.

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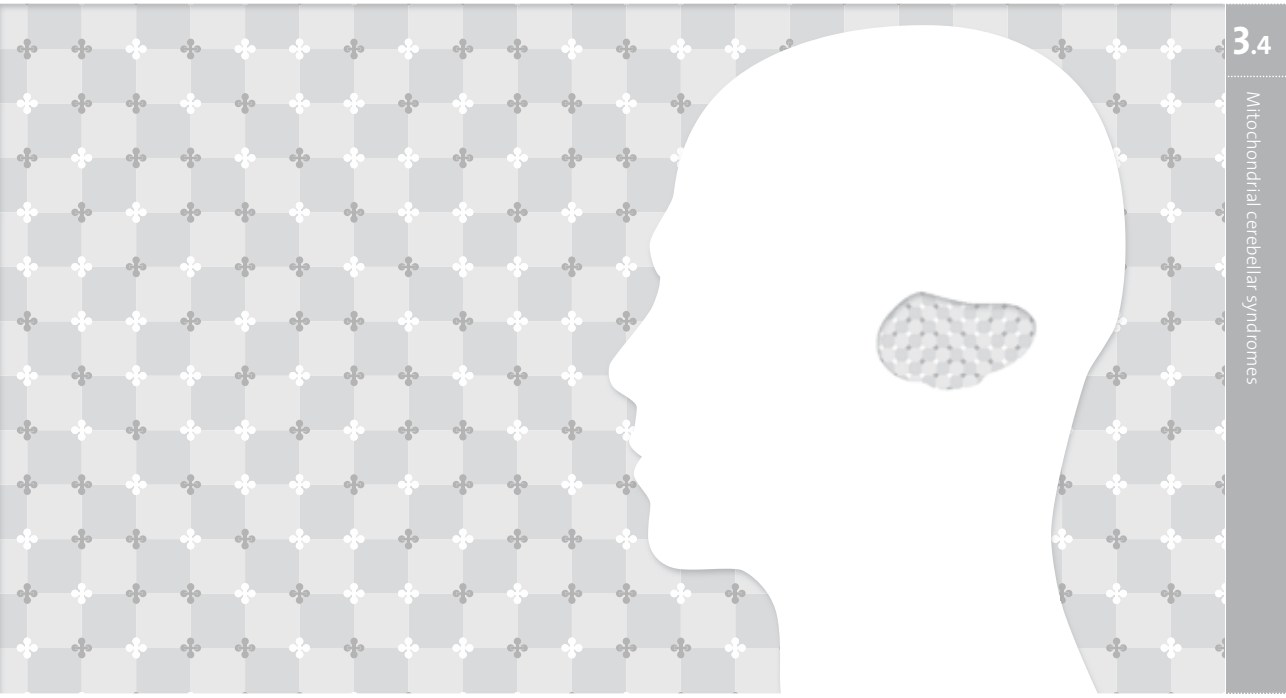

Mitochondrial cerebellar syndromes

Mitochondrial G8363A tRNA (Lys) gene mutation presenting as cerebellar ataxia or myopathy with axonal neuropathy

a clinical and molecular study in a three-generation Dutch family

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Abstract

We describe ten relatives of a three-generation family with a mitochondrial G8363A tRNA(Lys) mutation We distinguish two phenotypes of disease-onset, both evolving in the second or third decade: one presenting with myopathy and axonal neuropathy, followed by cerebellar ataxia; the other presenting with cerebellar ataxia, often also developing myopathy. Earlier disease-onset was correlated to a higher mutation load in urine, which was also indicative for a cerebellar onset phenotype. Severity of ataxia correlated with disease-duration, so earlier onset (higher mutation load) predicts a more severe clinical outcome. Analyzing mutation load in urine is preferred to blood in molecular correlation studies.

3.4 Mitochondrial G8363A tRNA (Lys) gene mutation presenting as cerebellar ataxia or myopathy with axonal neuropathy

a clinical and molecular study in a three-generation Dutch family

The rare mitochondrial G8363A tRNA(Lys) mutation has first been identified in families with cardiomyopathy and hearing loss.[1] This transfer RNA gene mutation has also been related to the classical Leigh and MERFF (myoclonic epilepsy and ragged red fibers) syndromes, to myoclonus epilepsy and to autism.[2,3,4,5] Also, cerebellar ataxia has been described as predominant feature in this mutation, with onset ranging from childhood to young adulthood and co-existence of variable clinical features like hearing loss, lipomatosis, external ophtalmoplegia, mental retardation but also peripheral neuropathy and myopathy.[6,7,8] The pathogenicity of the G8363A mutation is due to a change in the conformation of the tRNA that severely impairs aminoacylation of tRNA.[9]

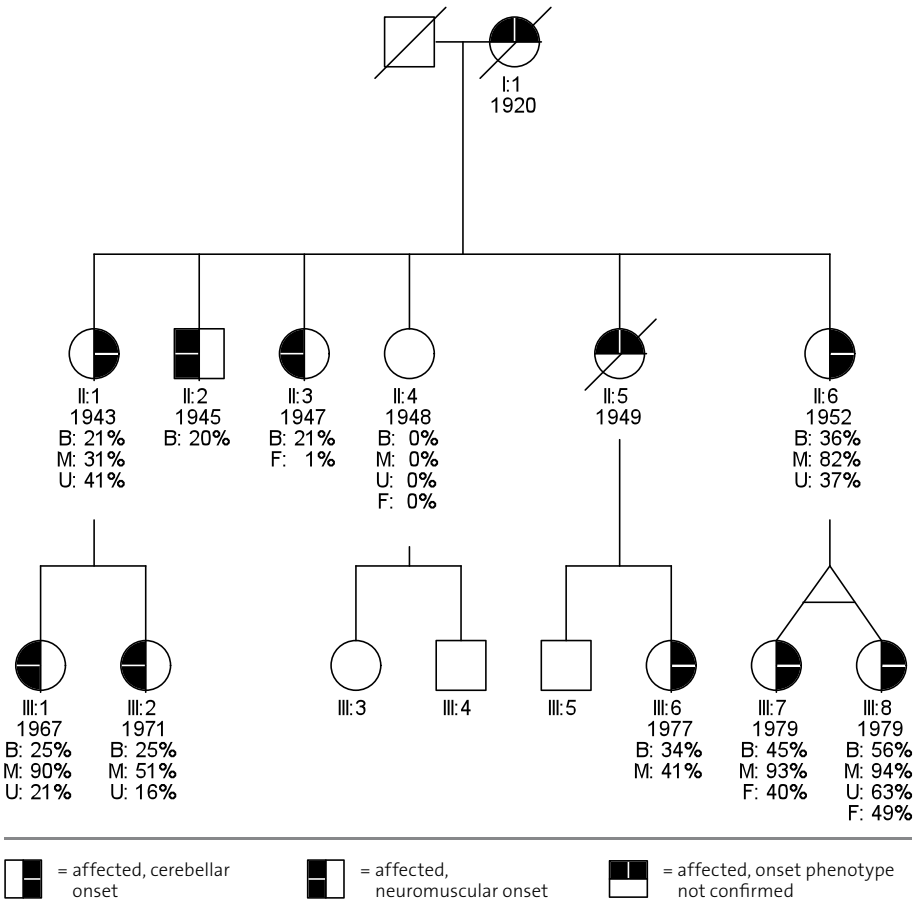
Different hypotheses are used to explain the phenotypic variability resulting from a specific tRNA mutation, based on genetic and tissue-specific factors influencing expression and translation of the gene and differences in tissue-toxicity. Also, modifiers may play a role. In part, the variability is related to the level of heteroplasmy within the affected tissue, that may act like a threshold for expression of the phenotype.[10] Level of heteroplasmy in affected tissue corresponds more accurate with severity of the phenotype than heteroplasmic state in blood. However, a recent evaluation of genotype-phenotype correlation in all previously reported cases of m.G8363A mutations, showed significant correlation of higher (>90%) mutational load in blood with a more severe, life-threatening multisystem phenotype, compared to lower (<60%) levels. Various exceptions on this finding prevent mutation load to be used as a clinical parameter so far, especially in genetic counselling.[8]

We present the clinical, molecular and pathological findings in ten patients of a three-generation Dutch family with a m.G8363A-mutation, displaying two different adult-onset phenotypes and showing the importance of scrutinizing clinical and molecular data.

Case report

The pedigree of the presented family is depicted in *figure 1*. *Table 1a* provides a summary of clinical and genetic findings in the affected relatives. Four patients presented with myopathy, two of them also displaying an axonal neuropathy. Cerebellar ataxia, developing later in the course of disease in these cases, appeared to be the presenting symptom in the remaining six patients, four of them also displayed muscle weakness. To study genotype-phenotype correlations, mutation load was analyzed in blood (n=9), muscle (n=7), urine (n=5) and in fibroblasts (n=2) and related to disease onset, phenotype and severity of cerebellar ataxia. Respiratory chain enzyme activities

Figure 1 **Family pedigree, indicating date of birth and mutation load in blood (B), muscle (M), urine (U) and fibroblasts (F)**



were measured in muscle and fibroblasts. Three muscle biopsies were available for morphological studies (in patient II:6, III:1, III:2). All showed mild myopathic changes with multiple ragged red fibers in Gomori and oxidative stainings. In III:1 and III:2 also sporadic cytochrome oxidase (Cox)-negative fibers were present. Electron microscopy in patient II:6 showed an aberrant structure of the mitochondrial cristae.

The proband (III:1), first evaluated at the age of 37 years, reported muscle weakness of her thighs from the age of 30 years. In the following year, this was followed by clumsiness of the hands and slurring of speech. She mentioned fatigue as a major complaint. On examination at age 41, we found cerebellar dysarthria, mild dysmetria of the upper and lower extremities, and disturbed tandem walking. Furthermore, moderate muscle weakness of the proximal limbs (Medical Research Council (MRC) grade 4) was present and mild disturbance of vibratory sense at the feet.
MRI-scan displayed a subtle atrophy of the vermis, nerve conduction studies and myography were normal.

The proband's mother (II:1) experienced slowly progressive gait instability from the age of 28 years, on evaluation at age 64, she could hardly walk without support. Onset of dysarthria and difficulties in swallowing had been in her forties. She had reported muscle weakness of the since the last decade. Painful muscle cramps in the lower legs and, more recently, paresthesias of the hands and feet were present. Neurological examination showed moderate dysarthria and gait ataxia, and severe dysmetria of the limbs. Moderate limb girdle weakness was found and mild disturbance of vibratory sense in the feet. MRI displayed subtle atrophy of the vermis and EMG at the age of 61 years was normal. Ophthalmologic and cardiologic evaluation were unremarkable.
The younger sister of the proband (III:2) had complained about fatigue from the age of 30 years but medical records describe a normal neurological examination at age 32. From the age of 35 years, muscle pain of the lower limbs evolved and also mild disturbance of gait and speech. Examination at age 38 displays a mild gait- and limb ataxia, without evident muscle weakness.

A maternal aunt of the proband (II:6), examined at age 55, reported gait disturbance and dysarthria from the age of 30 years, followed by muscle weakness of the limbs ten years later. She is still able to mobilize without support. Recently, she also noticed paresthesias of the feet and hands and fatigue and was diagnosed with a type 2 diabetes. On examination, we found moderate cerebellar dysarthria, gait- and limb ataxia and evident limb girdle weakness (MRC4-). Vibratory sense is diminished at the feet. EMG at age 56 years displayed a mild, mainly sensory axonal neuropathy. Her 29 years old twin daughters (III:7 and III:8), both reported complaints of imbalance from the age of 20 years. Neurological examination displayed evident gait- and limb ataxia but

also presence of subtle limb girdle weakness. Brain MRI in III:7 was normal, in III:8 here was subtle atrophy of the vermis. Another niece of the proband (III:6), aged 31 years, displayed a similar phenotype of mild cerebellar ataxia and dysarthria, with later onset at age 27 and without muscle weakness.

A different phenotype was noticed in a maternal uncle and aunt of the proband (II:2 and II:3). In both patients, onset has been less clear: patient II:3 mentioned poor performance in gymnastics at primary school and had never been able to ride a bike.

Table 1A Summary of clinical characteristics of affected relatives

	Age at examination (yrs)	Age at onset (yrs)	Duration	First symptom	Cerebellar symptoms			
					Dysarthria	Gait ataxia	Limb ataxia	SARA (max 40)
I:1				*ataxia*				
II:1	*64*	*28*	*36*	*ataxia*	++	++	++	*16*
II:2	*n.a.*	*?*	*?*	*muscle weakness*	++	++	++	*na*
II:3	*61*	*26*	*35*	*muscle weakness*	++	++	++	*20*
II:6	*56*	*30*	*26*	*ataxia*	++	++	++	*15*
III:1	*41*	*31*	*10*	*muscle weakness*	+	+	+	*6*
III:2	*37*	*35*	*2*	*muscle weakness*	+	+	+	*6*
III:6	*31*	*27*	*4*	*ataxia*	+	+	+	*6*
III:7	*29*	*20*	*9*	*ataxia*	+	+	+	*8.5*
III:8	*29*	*20*	*9*	*ataxia*	+	+	+	*10*

Yrs = years; FSS = Fatigue Severity Scale; - = absent;
SARA = Scale for assessment and rating of ataxia; EMG = electromyography; +/- = dubious;

From the age of 26 years, fatigue, difficulties in walking and weakness of the upper limbs have evolved. Medical record at age 31 mention cerebellar dysarhtria, gait ataxia, dysmetria of the hands with intention tremor and muscle weakness of the arms with scapulohumeral atrophy, atrophy of the hypothenar muscle, clawing of fingers and pes cavus, Sensory deficits evolved in her early thirties. At the age of 58 years, she was diagnosed with diabetes. Neurological examination at age 61 displayed severe cerebellar dysarthria and gait ataxia, severe intention tremor of the limbs and a gaze-evoked nystagmus. Weakness of the neck- and limb girdle muscles, but also weakness and

	Neuromuscular signs					
	Distal neuropathy	Muscle weakness	Vibratory sense	Mean FSS (max 7)	EMG (age in yrs)	MRI (age in yrs)
	+	++	↓	*5.7*	*n (61)*	*atrophy vermis (61)*
	+ *distal atrophy, pes cavus*	+	↓	*na*	*myopathy (30) + axonal neuropathy (44)*	
	++ *distal atrophy, pes cavus*	+	*n*	*6.2*	*myopathy (31) + axonal neuropathy (56)*	
	+	++	↓	*3.1*	*axonal neuropathy (57)*	
	-	++	↓	*5.6*	*n (36)*	*atrophy vermis (36)*
	-	+/-	↓	*2.2*		
	-	-	*n*	*3.6*		
	-	+/-	*n*	*3.6*		*n (29)*
	-	+	*n*	*4.7*		*atrophy vermis (29)*

+ = moderate; ↓ = diminished; na = not applicable.
++ = severe; n = nomal;

sensory deficits of the distal limbs were present, with *pes cavus*. Electrophysiological data from medical history reported normal nerve conduction studies and myopathic changes on myography and at the age of 31. At age 56, an axonal sensomotor neuropathy has been described (no responses on sensory conduction studies and mild slowing of motor conduction velocities in the arms). Patient II:2 did not participate in this study but allowed us to take a history by telephone, to study his medical records, and obtain DNA. Onset of the disease was most likely at his late teens or early twenties, presenting with muscle weakness of the hand and feet. Around the age of 26 years, signs of cerebellar ataxia developed. Medical records at age 30 describe muscle weakness of the face, neck, shoulders and upper arms, with proximal muscular atrophy, clawing of the fingers and *pes cavus*. Hypesthesia of the feet was noted, with normal vibratory sense, suggesting a combination of myopathy and peripheral neuropathy. Furthermore, cerebellar dysarthria and moderate gait-and limb ataxia were described. Electrophysiological studies at this age describe normal conduction studies at the arms and myopathic changes in proximal muscles on myography. At the age of 44 years, a sensomotor neuropathy has been reported, but without corresponding EMG data.
No medical records of the probands grandmother (I.1) could be obtained. Heteroanamnesis displayed that she had shown a similar phenotype to one of her daughters (II.1) with onset of cerebellar ataxia in her thirties, followed by muscle weakness and muscle cramps.

Table 1B **Spearman's correlations (significance level) for onset age related to level of heteroplasmy in blood, muscle and urine and for disease duration related to symptoms of ataxia and fatigue**

	Heteroplasmy of the m.G8363A tRNA(lys) mutation (%)				
	blood	muscle	urine	SARA	Mean FSS
Onset age	*-0.461*	*-0.414*	*-1.000** (p <0.001)*		
Disease duration				*0.822* (p 0.012)*	*0.717*(p 0.045)*

SARA = Scale for assessment and rating of ataxia; * correlation is significant at the 0.05 level (2-tailed)

FSS = Fatigue Severity Scale **correlation is significant at the 0.01 level (2-tailed)

Discussion

We describe a large family with cerebellar ataxia, myopathy and, in some cases, axonal neuropathy, with onset in the second or third decade, related to a m.G8363A-mutation. No cardiomyopathy, hearing loss or epilepsy, previously described in relation to this mutation, was seen in this family.[7,11] One patient (II:6) displayed a lipoma of the neck, which has been associated with this mutation before.[6] In two females (II:3 and II:6), type 2 diabetes was diagnosed in their mid fifties, which we consider as an age-related coincidence, diabetes has never been reported in association to the mutation.[8] Interestingly, we could distinguish two onset- phenotypes: one presenting with neuromuscular signs (myopathy and peripheral neuropathy) and the other with a cerebellar onse. *(Table 1a)*. *Figure 2a* displays the mean and range of mutation load in blood and urine related to onset phenotype. This strongly suggest a higher mutation load in blood and urine to be related to the cerebellar onset phenotype, although patient numbers are too small to quantify significance. Unexpectedly, we could not relate onset phenotype to the mutation load in muscle. We assume this is due to the fact that we were not able to obtain muscle from II:2 and II:3, both displaying a pronounced neuromuscular phenotype.

Table 1b and corresponding scatterplots in *Figure 2b* display the relation between mutational load in blood, muscle and urine and onset age. The scatterplots suggest a higher percentage of heteroplasmy to predict earlier onset of disease. A significant correlation of onset age and mutation load in urine was confirmed by analyzing Spearman's rank correlations. Furthermore, severity of ataxia, evaluated with SARA (Scale for Assessment and Rating of Ataxia),[12] was significantly correlated to disease duration *(Table 1b)*. These data predict a more severe clinical outcome in patients with earlier disease onset, which is related to a higher mutational load. No corresponding progression of cerebellar atrophy was found on brain MRI: cerebellum was normal in most patients, only two (III:1 and III:8) displayed subtle atrophy of the vermis *(Table 1a)*. Our study confirms the findings in a meta-analysis by Virgilio et al, describing the m.G8363A mutation load in blood to correlate with disease-onset and disease-severity.[8] Urine samples are expected to be more representative than blood in analyzing mutational load. Indeed, the significant correlation of mutational load in urine and disease onset add to this premise.

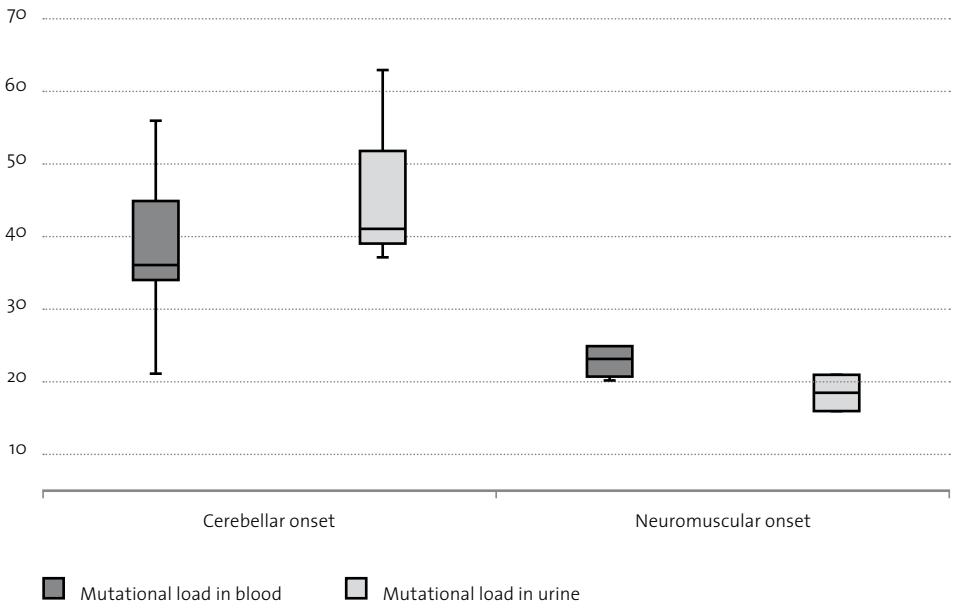
Unexpectedly, we could not confirm the correlation between onset age and mutation load in muscle samples, although the scatterplot is suggestive *(Figure 2b)*. Representation of some muscle samples, obtained with a thin needle biopsy, may have been inadequate due to sample errors and replacement of affected muscle tissue by fat and connective tissue. This may give rise to a false low percentage of hetero-

plasmy. However, to avoid larger and more invasive muscle biopsies, and urine samples obviously being more accessible than muscle biopsies, we suggest further studies to confirm the reliability of urine samples to study mutation load in genotype-phenotype studies. Based on our results, we think mutation load (in urine) has a modest value in predicting phenotype within families with a m. G8363A-mutation, although the clinical use of these correlation studies in genetic counselling is still limited.

Biochemical analysis displayed various combinations of deficiencies in enzyme activity of complex I, III, IV and V, which is a known biochemical footprint in this mutation.[10] No relation with clinical phenotype was seen.

Fatigue and exercise intolerance has been a predominant symptom in nearly all relatives in our family. We have evaluated fatigue in eight relatives, using the Fatigue Severity Scale *(Table 1a)*, with a mean score ranging from 1 to 7.[13] Only one patient had a mean FSS score in the range found in healthy controls (mean FSS 2.7 or lower).[14] Four of the remaining seven relatives had a mean FSS score of over 4, reflecting moderate to severe fatigue. Mean FSS score significantly correlated to disease duration *(table 1b)*, suggesting symptoms of fatigue to be progressive during the course of disease. This

Figure 2A Onset phenotype related to mutation load in blood and urine



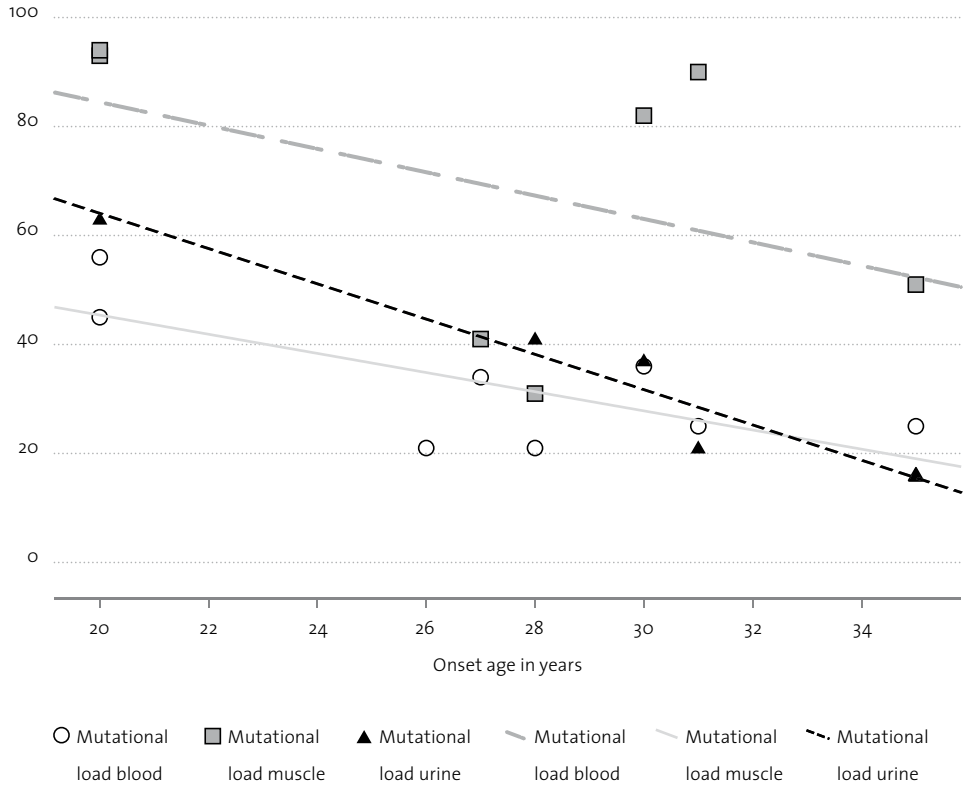
Mutational load in blood: cerebellar onset n=5, neuromuscular onset n=4;

Mutational load in urine: cerebellar onset n=3, neuromuscular onset n=2

indicates that "peripheral" fatigue, related to neuromuscular signs, accounts for a substantial part of the symptoms of fatigue: peripheral fatigue, related to severity of neuromuscular signs, increases with progression of disease. This is contrast with "central" fatigue, related to central motor control mechanisms and motivational input.[15,16] In our experience, in cerebellar syndromes, fatigue is an early disease symptom without relation with disease duration or severity of ataxia (SARA), and may probably be related to central fatigue. (Brusse E., unpublished data).

In conclusion, this case reports adds to the phenotypic description of the mG8363>A-mutation, presenting in adulthood with either a neuromuscular phenotype or cerebellar ataxia. Ataxia is the most prominent symptom in our family; fatigue is an early and disabling symptom. Onset phenotype is was indicated by mutation load in urine, which was significantly correlated to onset age, leading us to prefer the use of urine samples in further genotype-phenotype studies. Other genetic and tissue-specific factors will have to be elicited to explain inter- and intra-familial heterogeneity.

Figure 2B Onset age related to mutation load in blood, muscle and urine



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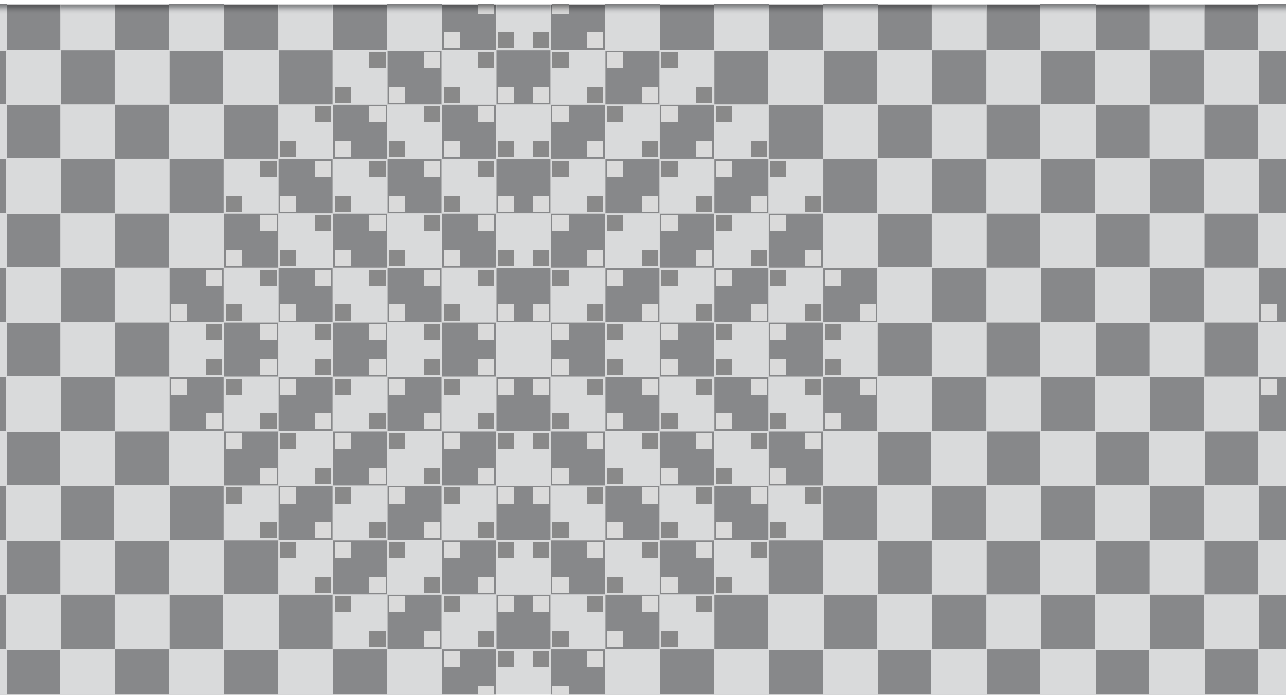
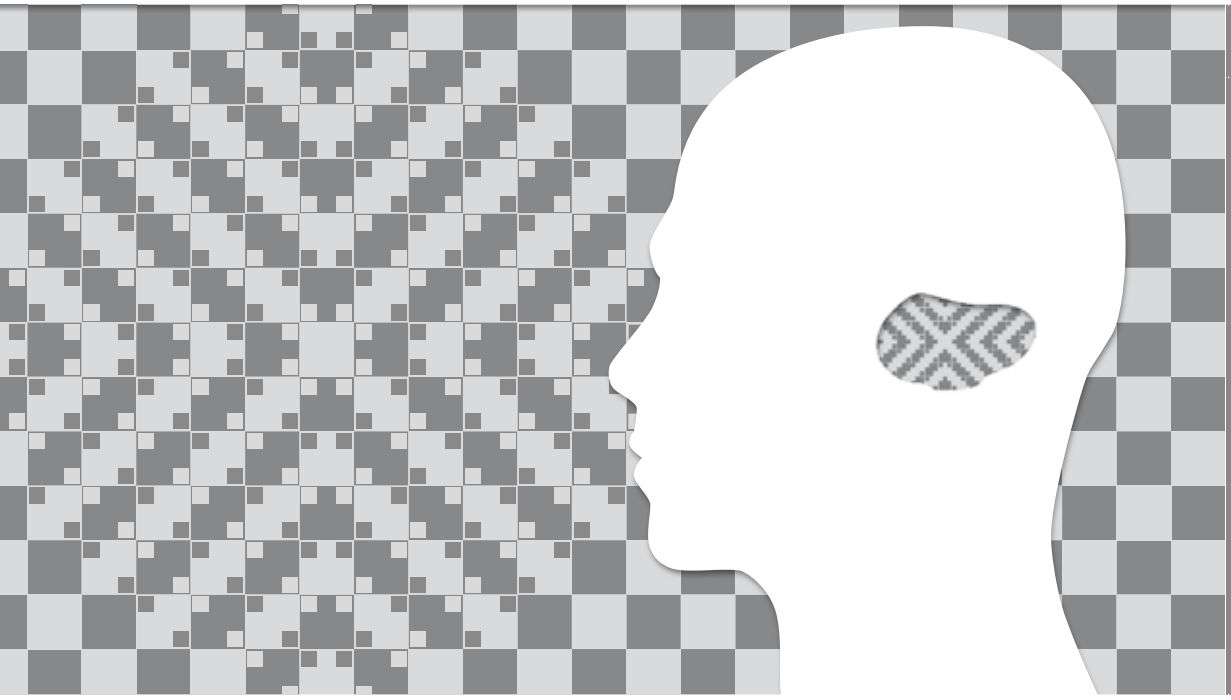


Chapter 4



General discussion and future perspectives

4 General discussion and future perspectives

During its first decade, the multidisciplinary neurogenetic consultancy, effectuating the evaluation of patients with cerebellar ataxia by neurologists and clinical and molecular geneticists, has been fruitful, resulting in a standardization of the diagnostic process in cerebellar ataxia and expansion of the clinical phenotype as well as the genotype in adult-onset hereditary and sporadic cerebellar ataxias. These results are reflected in a number of studies, presented in this thesis. Beyond these scientific results, the consultancy enables us to profit from our mutual expertise in the diagnostic process and provide adequate genetic counseling for patients and their relatives. These factors, combined with a growing experience in paramedic attendance and (mainly symptomatic) therapeutic strategies, have added to the care for patients with cerebellar ataxias and other hereditary neurological disorders.

I Diagnostic process in cerebellar ataxia

Main findings and implications for clinical practice

The development of the expert-opinion and consensus-based diagnostic algorithm for early and late adult-onset cerebellar ataxia, presented in **chapter 1:2**, is an important step in the clinical practice of these patients, standardizing the diagnostic evaluation in this heterogeneous group of disorders. To evaluate the actual improvement in diagnostic output by introducing this algorithm, it would have been wanted to retrospectively compare the diagnostic output with the effectuated diagnostic output in our clinic before using the algorithm, or to prospectively relate our results to the diagnostic output in an independent cohort, for instance from another clinic that does not use the algorithm. Unfortunately, interpretation of this comparison will be hindered due to the limited options for genetic testing and the subsequent change from a clinical to a genetic classification of cerebellar ataxias in the pre-algorithm era and also due to the effects of population bias when comparing the results of two clinics. However, we have documented the diagnostic output of the algorithm in adult patients with cerebellar ataxia, referred to our clinic from the introduction in January 2004. *Table 1* depicts the diagnostic output of the algorithm in 89 patients with cerebellar ataxia that we evaluated during the first four years after its introduction (to December 2007). In 31 patients (34.8%), a genetic deficit was found; in 7 patients (7.9%) another specific diagnosis was present and 52 patients (58.4%) displayed a describing diagnosis. Only four patients (4.5%) had a sporadic ataxia of unknown cause, not fitting one of the specific describing diagnoses. We therefore conclude that the algorithm succeeds in defining a specific

diagnosis in patients with cerebella ataxia, either a genetic, metabolic or describing diagnosis. An autosomal dominant cerebellar ataxia was found in 35 patients (39.3%). In 21 (62%) of the ADCA patients, a genetic cause was determined (including a patient with the new SCA27 mutation), therefore in 14 (38%) of the ADCA patients, no gene deficit was found. This is in accordance with a previous nationwide study in 137 families with 382 affected individuals, displaying an unknown genotype in 30% of ADCA patients.[1] In our population of 89 patients, 13 cases (14.6%) of autosomal recessive cerebellar ataxia were identified, 34 (38.2%) sporadic cases and 7 (7.9%) patients with a mitochondrial cause of cerebellar ataxia.

The algorithm is easily accessible for updates related to new genotypes. Since its publication in 2007, for instance, it has been shown that autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a frequent cause of early onset (<25 years) cerebellar ataxia in the Dutch population.[2] Furthermore, adult onset cerebellar ataxia is increasingly recognized as one of the phenotypes in the clinical spectrum related to *Polymerase gamma (POLG)* gene mutations.[3] POLG is the most important enzyme involved in the replication of mitochondrial DNA (mtDNA) and thus far, over 100 different autosomal dominant or recessive mutations have been described throughout the entire gene. These mutations account for a broad range of clinical features, often presenting as multisystem disorders. Blok et al described genotype-phenotype correlations in a Dutch cohort of 232 patients with *POLG* mutations, including two families with cerebellar ataxia that we have identified in our clinic. Both families displayed a complex phenotype, indicative of mitochondrial disease: one family presented with cerebellar ataxia, juvenile cataract, myoclonus epilepsy and cognitive deterioration, related to a novel, probably autosomal recessive, p.G426S *POLG* mutation. The other family, presenting with adult-onset cerebellar ataxia, occipital lobe epilepsy, polyneuropathy and cognitive deterioration, appeared to be compound heterozygotes of a p.A467T and p.W748S *POLG* mutation, which has been associated with this phenotype before. Meanwhile, we have identified another compound heterozygote of the p.A467T and p.W748S *POLG* mutations: a young woman displaying cerebellar ataxia, epilepsia and myoclonus from the age of 15 years, who deceased from a status epilepticus at age 36. Therefore, cerebellar ataxia related to *POLG* mutations or ARSACS should be included in an updated version of the algorithm.

Indications for further use of the algorithm in clinical practice

As was described before, a diagnostic flowchart enables a uniform procedure in the challenging diagnostic process of the heterogeneous group of rare disorders, representing cerebellar ataxia. A correct and early diagnosis is increasingly warranted to start adequate therapy (thus far mostly symptomatic and sometimes curative, as in some metabolic or immune-mediated ataxias) and to enable proper counseling and patient

Table 1 **diagnostic output in 89 patients with cerebellar ataxia, evaluated according to the algorithm from January 2004 to December 2007.**

	Number of patients			
	Genetic diagnosis	Specific diagnosis otherwise	Describing diagnosis	Total (%)
ADCA	**21**		**14**	**35** (39.3%)
SCA1	*3*			
SCA2	*1*			
SCA3	*7*			
SCA6	*2*			
SCA7	*3*			
SCA17	*1*			
SCA27	*1*			
EA2	*3*			
AR-CA	**6**	**1**	**6**	**13** (14.6%)
FRDA	*3*			
AT	*2*			
Locus 11p15	*1*			
AOA2		*1*		
X-linked				**0**
Sporadic	**1**	**5**	**29**	**35** (39.3%)
Toxic/ischemic		*2*		
MSA		*1*		
Immune-mediated		*2*		
ILOCA			*17*	
EOSCA			*8*	
Ceruloplasmin deficiency	*1*			
Unknown			*4*	
Mitochondrial	**2**	**1**	**4**	**6** (6,7%)
Total	**31**	**7**	**52**	**89** (100%)
percentage	*34.8%*	*7.9%*	*58.4%*	

ADCA = Autosomal Dominant Cerebellar Ataxia.
SCA = spinocerebellar ataxia.
EA = Episodic Ataxia.
AR-CA = Autosomal Recessive Cerebellar Ataxia.
FRDA = Friedreich's Ataxia.
AT = Ataxia Teleangiectasia.
AOA = Ataxia Oculomotor Apraxia.
MSA = Multiple System Atrophy.
ILOCA = Idiopathic Late-Onset Cerebellar Ataxia.
EOSCA = Early-Onset Spinocerebellar Ataxia

care. This algorithm will be used as one of the starting points in the development of a nationwide directive on diagnosis and treatment of subacute, adult-onset cerebellar ataxias; we have recently taken off this project in a multidisciplinary setting, representing the Dutch federation of Neurology (Nederlandse Vereniging voor Neurologie, NVN), the Dutch federation of Clinical Geneticists (Vereniging Klinische Genetica Nederland, VKGN), the federation of Clinical Genetic Laboratory studies (vereniging Klinisch Genetische Laboratoriumdiagnostiek, VKGL) and the Dutch federation of rehabilitation specialists (Nederlandse Vereniging voor Revalidatieartsen, VRA). We hope this will meet the goals of improving patient care by providing a reliable diagnosis with efficient use of diagnostic tools, improving knowledge and initiating further research.

To further evaluate the yield of our diagnostic algorithm, also related to the efficiency and costs of the used diagnostic tests, we are currently analyzing the diagnostic results in an extended cohort of patients with cerebellar ataxia, referred to our outpatient clinic from January 2004 to December 2009. In this way we will be able to inform our patients about their a-priory chances to get an exact diagnosis. Furthermore, specific tests in the flowchart may have to be reconsidered. It will be interesting for instance, to find out how many sporadic patients eventually were diagnosed with one of the SCAs and what was the corresponding onset age. In our cohort of 89 patients, genetic analysis appeared to be negative in all sporadic cases, irrespective of their onset age. Furthermore, the current flowchart advises to perform an extensive metabolic screening in all assumed autosomal recessive and sporadic patients with onset-age up to 25 years, and it will be important to study the efficiency of this metabolic screening in relation to onset age. Also, the exclusion of Fragile-X-associated tremor ataxia syndrome (FXTAS) in all males patients with an onset of over 50 years will have to be reconsidered. Although after the identification of this syndrome, the first screening studies suggested that > 5% of sporadic late onset cerebellar ataxia in males could be attributed to FXTAS,[4] consecutive studies indicated FXTAS to be a rare cause of late onset cerebellar ataxia[5,6] and we did not find any case of FXTAS as a cause of late onset cerebellar ataxia in our population thus far. It might be that FXTAS should only be analyzed in specific late onset ataxia phenotypes, for instance when tremor is an initial symptom. Finally, we will analyze whether specific clinical characteristics may be defined to be indicative for a specific genetic diagnosis. This information will be implemented in the development of the nationwide directive.

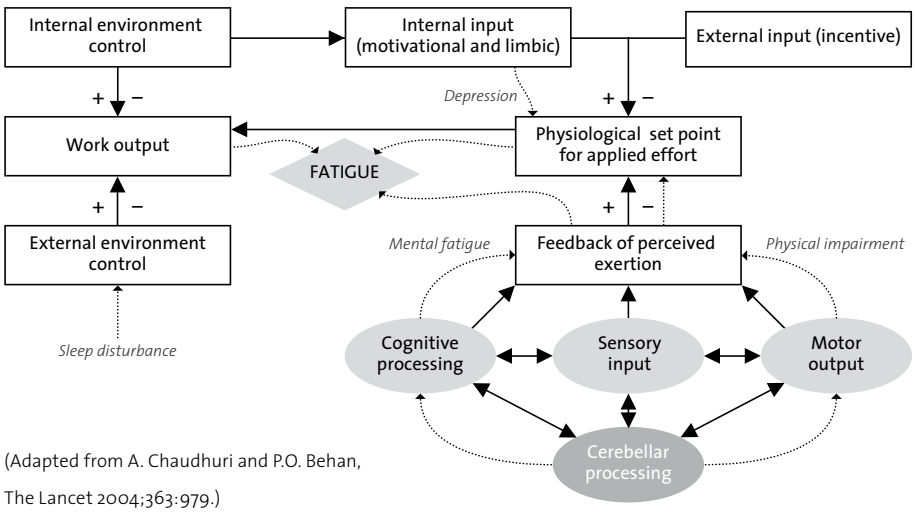
II Fatigue

Introduction, concept of fatigue and design of the study on fatigue

A substantial number of our patients with ataxia pointed to the limiting effects of fatigue in their daily life. One of them described the following analogy: "The effects of alcohol are similar to the effects of fatigue: too much alcohol makes you drunk, a higher level of fatigue increases my disabilities. Reversely, due to this increasing disability, I need more energy to fulfill my daily activities. This results in chronic fatigue and mental distress. Mental distress also increases my disabilities. Experimentally, I found a balanced combination of physical training and reposing to be effective in overcoming this perpetuating circle."

This observation has lead us to design a study to determine the frequency, severity and predictive factors of fatigue in patients with cerebellar ataxia, described in **chapter 2**, as a first step in developing a founded treatment strategy for fatigue in these patients. To our knowledge, fatigue has been studied in two small series of Friedreich ataxia and SCA3 patients, but without data about predictors of fatigue.[7,8] Fatigue has already been recognized as an important cause of disability in neurological disorders like multiple sclerosis (MS), Parkinson's disease (PD), head trauma, stroke or neuromuscular disorders.[9-14]

Figure 1 **Central governor model, with interplay of physiological variables that control the level of applied effort and work output. Possible deregulation of these pathways, related to fatigue in cerebellar ataxia, are depicted by dotted arrows.**



(Adapted from A. Chaudhuri and P.O. Behan, The Lancet 2004;363:979.)

Fatigue is a subjective perception; for clinical studies, a useful definition of fatigue is the difficulty in initiating or sustaining voluntary activities. This voluntary effort is influenced by many control systems: internal and external motivational input and level of perceived exertion, determined by feedback from motor, sensory and cognitive systems. This control system regulating voluntary effort is called the central governor model, and protects the body against damage due to excessive exercise *(Figure 1)*. A disruption at any level in this regulatory chain, leading to dissociation between the level of internal input and that of perceived exertion could lead to pathological fatigue.[9,13]

Although fatigue is a complex and subjective symptom, it is increasingly recognized that fatigue can be analyzed and quantified with clinimetric and neurophysiologic techniques.[15] A large number of (self-assessment)-scales have been developed attempting to quantify severity an impact of fatigue in different patient populations. A useful assessment needs to reflect the complaints of the patients, not only to make an inventory of its severity and causes but also to verify the effects of management strategies.[16]

We designed a self-assessment study in patients with autosomal dominant cerebellar ataxia (ADCA), as a more or less homogeneous ataxia population. We used the Fatigue Severity Scale (FSS) to evaluate the level of experienced fatigue using an unidimensional scale. The FSS has been validated in several peripheral and central nervous disorders, and showed good internal consistency, test-retest reliability and discriminative validity.[13,16,17] In the self-assessment, we also collected data on disease-specific factors (disease duration, severity of ataxia, physical impairment, SCA subtype) as well as factors known to be related to fatigue (medication use, sleep disturbances, depression, quality of life). These data include factors predicting fatigue in other neurological disorders and candidate factors to predict the severity of fatigue regarding the central governor model. Therefore, the self-assessment included the Beck Depression Inventory (BDI); the Rotterdam Handicap Scale (RHS), to assess the level of physical impairment; evaluation of the quality of life, using the short form SF-36 health survey, distinguishing a norm-based physical and mental component score (Nb-PCS and Nb-MCS); and furthermore the Pittsburgh Sleep Quality Index (PSQI) and the Epworth Sleepiness Scale (ESS), assessing sleep disorders. These are all validated self-assessment scales that were previously used in studies on fatigue in neurological disorders. A subset of 58 patients was clinically evaluated, measuring severity of ataxia with the Scale for the Assessment and Rating of Ataxia (SARA) and cognitive functioning with the Mini Mental State Examination (MMSE)

Main findings and implications for clinical practice

In **chapter 2,** we demonstrate the results of the study on fatigue in 123 patients with ADCA, with 69% of the patients having a median FSS score of 5 or higher, indicating

Table 2 **Summary of Fatigue Severity Scale (FSS) values and associated patient characteristics in our study and in several studies regarding other neurological disorders**

Disorder [ref, number of patients]	Mean/median FSS value(SD/IQR)	Patient characteristics, associated to severe fatigue
ADCA/SCA		
[this study, n=123]	*5.8(4.6-6.6)*	Physical functioning, depression, medication use, visual symptoms
SCA3		
[Friedman 2008,[7] n=28]	5.1 (1.3)	Excessive daytime sleepiness (no other factors studied).
Multiple Sclerosis		
[Lerdal 2007,[12] n=267]	n.a.*	Physical impairment, depression
[Penner 2007,[18] n=41]	4.93(1.69)	Physical impairment, depression, action control (attention, motivation)
Parkinson's Disease		
[Herlofson 2002,[19] n=66]	4.1(1.4)	Disease-severity
[Shulman 2001,[57] n=99]	3.6(1.5)	n.a.
Poststroke fatigue		
[Schepers 2006,[14] n= 167]	4.7 (1.3)	Depression, age, female gender, personality structure
Immune-mediated neuropathies		
[Merkies 1999,[17] n=113]	5.6 (1.4)	Quality of life (SF-36): physical functioning in CIDP and mental functioning in GBS
Pompe Disease		
[Hagemans 2007,[20] n=225]	5.2 (1.5)	Disability, respiratory failure, sleeping difficulties

*longitudinal study: three FSS categories were distinguished: both categories "persistent fatigue" and "sporadic fatigue" made up 75% of all patients, 25% had no fatigue.

n.a. = not applicable;
CIDP = chronic inflammatory demyelinating polyneuropathy;
GBS = Guillain-Barré syndrome;
SD = standard deviation;
IQR = inter-quartile range.

severe fatigue, and nearly 70% classifying fatigue among their three most disabling symptoms. The median FSS value of 5.8 in our population is evidently higher than the median FSS value of 2.9 in a previously described, healthy control group of 133 patients with similar age distribution and percentage of females. These results confirm our hypothesis of fatigue being a severe and disabling symptom in patients with cerebellar ataxia. The two previously published small series of patients with Friedreich ataxia (FA, n=130) and with SCA3 (n=28), also displayed a higher prevalence of fatigue in patients compared to healthy controls.[7,8] In FA, severity of disease but also disease duration were related to fatigue, however, these studies did not analyze predictors of fatigue.
We found physical functioning and depression to be the strongest predictors of severe fatigue in our ADCA patients, and this is in line with findings in other neurological disorders. *Table 2* summarizes data on severity and predictive factors of fatigue in other neurological disorders, related to our findings in cerebellar ataxia.[7,12,17-20] The median FSS of 5.8 in our patients is even higher than the FSS values described in other neurological disorders. In our study as well as in MS, Parkinson's disease (PD), immune-mediated neuropathies and Pompe's disease, but not in post-stroke fatigue, severity of fatigue has been related to severity of physical impairment. In the clinical evaluated subgroup however, we did not find a significant correlation between FSS value and severity of ataxia (quantified in the SARA), although the latter was significantly related to physical functioning. This implies that physical functioning, related to fatigue, is not only determined by cerebellar ataxia. Indeed, subgroup analysis of clinically evaluated patients showed significantly more severe fatigue in patients with visual symptoms like nystagmus or diplopia, symptoms not addressed by using the SARA.

We did not find a relation between fatigue and gender, age or disease duration, which means fatigue was also present in young patients and was an early symptom in some of the SCA patients. This may in part be due to the fact that depression, predicting fatigue, is not related to duration or severity of disease. Furthermore, an early onset of fatigue may be related to visual symptoms as an early disease symptom. Globas et al described that one-third of patients with SCA1, 2, 3 and 6 initially complained of other symptoms than gait ataxia, especially double vision, dysarthria, impaired hand writing and episodic vertigo. This could precede gait ataxia for a period up to 15 years, also in patients with short disease duration and low SARA scores.[21] Furthermore, carriers of SCA2 mutations without ataxia were found to display eye movement disturbances (reduced saccade velocity).[22] This indicates that visual symptoms like oscillations and diplopia may be an early disease symptom in cerebellar ataxia, related to early onset of fatigue.
We also found significant correlation of fatigue with sleep disturbances, with 47% of our population having a PSQI>5, indicating a poor sleep quality. Correlation with ESS implicates that fatigue results in excessive daytime sleepiness.

Our study indicates that fatigue should be taken seriously in all SCA patients and treatable causes of fatigue should be addressed: this includes vital depression and sleep disturbances like restless legs and nocturnal muscle cramps. Physical functioning should be optimalized, including visual symptoms. Again, this emphasizes the benefit of multidisciplinary care for patients with ataxia, carried out by a neurologist, clinical geneticist, rehabilitation doctor and other specialists, for instance an ophthalmologist.

Indications for further research

Although we have tried to perform a cross-sectional study, the response rate of 41% does not exclude a sampling bias, therefore, we suggest to confirm the results in a prospective multicenter study, including all SCA patients, referred to the tertiary centers for patients with cerebellar ataxia in the Netherlands. This will also enable us to perform a longitudinal study to evaluate the development of fatigue during the course of disease, also related to physical functioning. To evaluate both ataxia and non-ataxia symptoms in relation to fatigue, patients should be evaluated with the SARA as well as the INAS (the Inventory of Non-Ataxia symptoms), which has previously been proven to be a reliable assessment.[23]

Furthermore, the presence and severity of fatigue should also be evaluated in other subgroups of cerebellar ataxia.

Our findings support the multidimensional central governor model as a general concept of fatigue, being a common symptom in so many unrelated (neurological) disorders. According to this model, one could distinguish peripheral fatigue, similar to muscle fatigability, resulting from disorders of the peripheral nervous system (PNS), and central fatigue, the subjective sense of fatigue, perceived at the central nervous system (CNS). CNS-lesions that interrupt the pathways interconnecting the basal ganglia, thalamus, limbic system and higher cortical centers are thought to be responsible for central fatigue.[9,13] Central fatigue is also caused by disturbance of cognitive processing and the ability to sustain concentration and mental tasks ("mental fatigue"); by endocrine disturbances (downregulation of the hypothalamic-pituitairy-adrenal axis is hypothesized to cause fatigue in chronic stress) and by reduction of the internal input from the CNS, leading to loss of motivation (in psychiatric disorders like depression). Environmental factors like sleep disturbances and medication use may also cause fatigue *(Figure 1)*.[9,13]

We suggest to include the cerebellum in this model of central fatigue, as is delineated with dotted arrows in *Figure 1*, since the cerebellum plays a fundamental role in action control and motor learning and displays extensive connections with the motor- and prefrontal cortex, thalamus , brainstem and spinal cord. Furthermore, the non-motor functions of the cerebellum, related to executive control, attention, memory and learn-

ing, visuo-spatial abilities and language are increasingly recognized,[24] and disturbances in these domains may well contribute to symptoms of mental fatigue. Depression (reduction of internal input) and sleep disturbances (external environmental factor) have also been shown to be related to fatigue in ADCA patients. Finally, peripheral fatigue will play a role in several cerebellar ataxia subtypes, due to the coexistence of neuromuscular symptoms and signs, especially peripheral neuropathy.[25,26]

By using objective neurophysiologic methods, it is possible to assess the combination of peripheral fatigue and central fatigue in sustained voluntary muscle contraction by analyzing the level of central activation failure (CAF): suboptimal input from the CNS to activate the muscle.[13,27] Applying this analysis in patients with cerebellar ataxia may support our hypothesis on the role of the cerebellum in central fatigue. Also, neuropsychological assessment may elicit the role of cerebellar dysfunction in mental fatigue.

According to the pathways of the central governor model, it might be that altering the feedback of the motor system, by applying compensatory mechanisms to adapt to cerebellar and pontine deficits, will also result in fatigue. It is expected that the motor system relies on these compensatory mechanisms from the early onset of cerebellar dysfunction. This may initially mask motor dysfunction but nevertheless could lead to complaints of fatigue early in the course of disease. Therefore, in further studies it will be important to determine early, even subclinical signs of cerebellar or pontine dysfunction, including non-ataxia disease symptoms, like the previously described visual symptoms, also in relation to fatigue. This will be of major importance when therapeutic strategies or disease modifiers will become available. A structured interview of early symptoms in SCAs was developed by Globas et al.[21] Another method to evaluate early, even subclinical cerebellar pathology is to study the motor learning capacities of the cerebellum, that are thought to be sensitive to early cerebellar dysfunction. The department of neurosciences of the Erasmus MC University Medical Center is preparing a longitudinal study on motor performance and motor learning in presymptomatic and symptomatic SCA6 patients, using eye blink conditioning and prepulse inhibition. This may lead to an objective method to come to an early diagnosis of SCA. We will participate in this study to evaluate clinical onset of cerebellar ataxia and non-ataxia symptoms with a standardized clinical evaluation, using the above mentioned inventories. This will also facilitate a longitudinal evaluation of fatigue, using the FSS.

To come to therapeutic strategies for fatigue in cerebellar ataxia, it will be important to elucidate the precise role of the cerebellum in the central governor model. Given the positive results in other (neurological) conditions,[9,13,28] treatment strategies for fatigue using pharmacotherapeutics like amantadine or modafinil, and exercise programs should be evaluated in patients with cerebellar ataxia.

III Expansion of the genetic and phenotypical classification of cerebellar ataxias and current concept of pathophysiology

Despite the genetic and pathophysiological developments in cerebellar ataxia and the introduction of diagnostic algorithms, a substantial number of patients with hereditary ataxia is lacking a specific DNA diagnosis. In the Dutch population of ADCA patients, no genotype was identified in 30% of all families.[1] The implementation of the combined neurogenetic consultancy was also aiming to identify families without DNA diagnosis, for the identification of novel SCA genotypes, with a proper phenotypic correlation. This search for "SCA-negative" families was simultaneously performed in a nationwide cooperation of Dutch ataxia-experts, united in the SCA-Netherlands (SCAN) association.
We were able to find a novel SCA genotype and a novel locus for autosomal recessive spinocerebellar ataxia and to perform a detailed study on the associated phenotype, as we discuss in the following part.

The FGF14 mutation, related to SCA27

Main findings and implications for clinical practice

In **chapter 3.1**, we describe the identification by linkage analysis of a novel SCA genotype in a three-generation Dutch family, associated to the F145S missense mutation in the *FGF14* gene. The family showed a distinct phenotype, with a childhood-onset postural tremor and a slowly progressive ataxia, cognitive deficits and behavioral problems (**chapter 3.2**). After the publication of the *FGF14 gene* defect in our family, it has become clear that this gene defect is rare, and only a few families with a mutant *FGF14* gene have been described. Dalski et al evaluated a group of 208 German familial ataxia cases for mutations in the *FGF14* gene. They identified one 18 year-old male patient with cerebellar ataxia (onset-age of 12 years), tremor, pes cavus , memory loss and depression, showing a frameshift mutation in the *FGF14* gene, created by a single base pair deletion in exon 4 (c.487delA).[29] More recently, Misceo et al described a daughter and her mother carrying a translocation between chromosomes 5 and 13, disrupting the *FGF14-1b* gene. The 5- years-old daughter was the most affected, showing cerebellar ataxia and tremor from the age of 1 year, microcephaly, severe mental retardation and also pyramidal signs.[30] No *FGF14* mutations were identified either in a series of 53 Caucasian SCA patients from France or in a Chinese cohort of 90 SCA patients and 15 patients with childhood-onset postural tremor.[31,32] Until now, we have screened 175 Dutch families for the *FGF14* mutation but we did not identify any new Dutch SCA27 patients either (R. van Minkelen, personal communication).
The identification of the *FGF 14* mutation did play an important role in elucidating the clinical characteristics and pathopfysiological mechanisms underlying spinocerebel-

lar ataxia. The expanding genetic classification of ADCA nowadays distinguishes 28 genetic loci and 19 genes have been identified. Eleven of these 18 genes are caused by repeat expansions: 7 polyglutamine expansions (SCA 1,2,3,6,7,17 and DRPLA) and 4 non-coding expansions (SCA 8,10,12,31). Nine conventional SCA mutations are identified (SCA27, as well as SCA 5,11,13,14,15/16, 20, 23 and 28), but these are less frequent.[33,34] The overall phenotype of patients with conventional mutations differs from that of patients with polyglutamine expansions. The mean age at onset of polyglutamine expansion SCAs is in the third or fourth decade and is mainly determined by the CAG repeat length. Genetic anticipation may lead to an increased CAG expansion with younger age at onset, but also rapid disease progression and early death. All conventional mutations display a frequent childhood onset, and a very slow progression with normal life expectancy. Intranuclear neuronal inclusions, characteristic for polyglutamine expansion SCAs, are not seen in patients with conventional mutations and predominant Purkinje cell loss is seen, opposite to the widespread neuronal loss in polyglutamine expansion SCAs. Brain imaging in conventional mutation SCAs displays pure and global cerebellar atrophy without additional brainstem atrophy as seen in polyglutamine expansions SCAs.[33]

The *FGF14* mutation in the Dutch SCA-family has been the second genetic defect found in one of the 22 human fibroblast growth factor genes associated with human disease. In our original study, we predicted the mutation to destabilize the core of the FGF14 protein and speculated a loss of function of the protein due to this destabilization. Meanwhile, seven FGF's have been associated with human disorders[35] and studies from the *Fgf14* knockout mouse models have elucidated more details of the pathophysiological mechanism resulting from *FGF14* mutations. FGF14 in adult mice displays the highest levels of expression in cerebellar granual neurons and is also prominently expressed in Purkinje cells, hippocampus, amygdala, striatum, thalamus and cerebellar cortex and the *Fgf14*$^{-/-}$ mice display ataxia, dystonia and cognitive impairment.[36,37] This corresponds with the disease symptoms originating from multiple neurological systems in SCA27. FGF14 is one of four intracellular homologous factor family (iFGF11-14), that are not secreted and do not activate tyrosine kinase receptors. FGF14 co-localizes with voltage-gated Na-channels (Na_v) in the soma and initial segments of neurons, interacting with the cytoplasmic C-terminal tail of the channel's pore-forming α-subunit ($Na_v\alpha$), necessary for proper functioning of this channel. In rat hippocampal neurons, the FGF14^{F145S} reduces $Na_v\alpha$ subuntit expression at the axon initial segment, weakens Nav channel currents and reduces excitability of the neurons.[38,39] The SCA27-associated FGF14^{F145S} mutant seems to have a dominant-negative effect on wild type FGF14 function: it does not interact with $Na_v\alpha$ but rather binds wild type FGF14, preventing its interaction with $Na_v\alpha$.[35,38,40] Channel function nowadays is assumed to be one of the underlying mechanisms giving rise to cerebellar ataxia.[33] Ataxia can result directly from mutations in ion

channels (SCA 6,13,15 and episodic ataxia 1,2,5) or from perturbations in ion channel physiology in the absence of a primary channel defect (SCA 27, SCA5, Dentatorubral-Palliduluysian Atrophy (DRPLA), episodic ataxia 5, paraneoplastic cerebellar ataxia in Lambert-Eaton myastenic syndrome).[41]

Indications for further research

First of all, it is needed to extrapolate the growing pathophysiologic knowledge, generated by the *Fgf14* knockout mouse models, to the human brain. Post mortem brain tissue, available in one patient of the SCA27 family showed atrophy of the cerebellar vermis with a subtotal loss of Purkinje cells. Cerebellar hemispheres and inferior olivary nuclei were not affected. There was no pontine atrophy and thalamus, nucleus caudatus and striatum were also normal. As expected, there were no intranuclear or cytoplasmic inclusion bodies. Apparently, neurodegeneration induced by FGF14 results in isolated cerebellar atrophy.

The next step is to compare the expression of voltage-gated Na-channels in key-regions of the SCA27 brain (e.g. cerebellum, hippocampus and striatum, regions highly expressing FGF14 in the mouse model and linking to the human phenotype) with cell culture models of the *Fgf14*$^{-/-}$mouse. Furthermore, expression of wild type FGF14 in the SCA27 brain should be compared with FGF14^{F145S}. At this moment, we are planning a cooperation with dr. Fernanda Laezza from the University of Texas, who is working on the *Fgf14* knockout mouse models, to study the neurotoxic mechanisms induced by the *FGF14*^{F145S} mutation.

Ultimately, unraveling the mechanisms underlying neurodegeneration in the *FGF14* mutation will advance the therapeutic strategies for neurodegenerative disorders related to perturbed channel activity. Both Purkinje and granule cells in FGF14 null mice fail to fire repetitively in response to a depolarizing current. This would decrease inhibition of the deep cerebellar nucleus (DCN), the sole excitatory output from the cerebellum. Finding agents correcting this aberrant physiology may have therapeutic effects.[41] At this moment, therapeutic experience is present in other Na-channelopathies, like migraine, epilepsy, periodic paralysis, chroninc pain and cardiac arrythmias.[42] However, these disorders are related to abnormal hyperexcitability, in contrast to the presumably reduced excitability of the neurons related to FGF14. Opposite to voltage gated sodium channel (VGSC) blockers like anti-epileptic drugs and local anesthetics, it would be interesting to investigate the effect of a selective VGSC opener or compounds able to selectively increase $Na_v\alpha$ expression levels to the expression and functioning of VGSC, first in vitro in FGF14 cell cultures, then in vivo the mouse model and subsequently in our patients. This may eventually lead to therapeutic options in Na-channel hypoexcitability disorders.

A new autosomal recessive spinocerebellar ataxia locus on chromosome 11p15

Main findings and implications for clinical practice

In **chapter 3.3** we describe a Dutch family with an autosomal recessive, slowly progressive cerebellar ataxia, linked to chromosome 11p15. Although onset was in childhood, it was only around the fourth decade that gait- and limb ataxia became disabling in most patients, due to the slow progression. Two patients also displayed a postural tremor. MRI showed atrophy of cerebellum and pons. Although most autosomal recessive (AR) cerebellar ataxias display a childhood onset, the phenotype in our family did not correspond with the wide variety of AR cerebellar ataxia phenotypes. As was described in our algorithm and in several very useful reviews,[26,43] the presence of specific clinical signs, like peripheral neuropathy, areflexia, oculomotor apraxia but also the presence of cerebellar atrophy is useful as a diagnostic clue in autosomal recessive ataxia. One can clinically distinguish a Friedreich's ataxia (FA), or FA-like phenotype with normal brain MRI or mild atrophy of the vermis; FA-like cerebellar ataxia with cerebellar atrophy and early onset ataxia with cerebellar atrophy.[26] Our family fits the last category of this classification and the 11p15 locus should be considered as a diagnosis in patients within this category of autosomal recessive ataxia.

Indications for further research

Since our publication of the family with autosomal recessive early onset cerebellar ataxia related to chromosome 11p15, no other cases of cerebellar ataxia have been mapped to this locus, so it remains difficult to assign candidate genes in this critical region, because a large number of genes and expressed sequence tags are identified in this critical region on chromosome 11p15. The following candidate genes in the linkage region were sequenced: *PRKCDB, FXC1, TAF10, TRIM3, TRIM33, HPX* and *RRMI*, but unfortunately no mutation has been detected. Whole exome capture sequencing for targeted analysis has been performed and the variants are being checked and analyzed now. Hopefully this will generate the gene responsible for this autosomal recessive spinocerebellar ataxia on chromosome11p15, now termed SCAR7 (OMIM 609270).

Extending the phenotype of the mitochondrial m.8363G>A tRNA(Lys) gene mutation

Main findings and implications for clinical practice

The mitochondrial genome (mtDNA) encodes for 13 polypeptides, all structural components of the respiratory chain complexes, along with two ribosomal RNAs and 22 transfer RNAs. These tRNAs are needed for translation of the 13 mtDNA specified proteins, accounting for only a small part of the mitochondrial proteins. The majority of mitochondrial proteins are encoded by the nuclear DNA.[44] Mitochondrial tRNA gene mutations are related to a wide spectrum of disorders, ranging from common diseases like sensorineural deafness and cardiomyopathy to rare syndromic phenotypes like MERFF (Myoclonus Epilepsy with Ragged Red Fibers), MELAS (Mitochondrial Encephalopathy with Lactic acidosis and Stroke-like episodes) and CPEO (Chronic Progressive External Ophtalmoplegia). However, the disease phenotype to a certain extent is correlated to the specific tRNA gene that is affected: tRNA(Lys) mutations, for example are often related to a MERFF-like phenotype, whereas tRNA(Ile) defects result in cardiomyopathy.[45] But still there is a high level of phenotypic variability, even between patients with the same mitochondrial DNA mutation. This variability is partly attributable to the level of heteroplasmy within an individual. A given tissue may or may not be affected during life depending on reaching a critical threshold for expression. The threshold for biochemical expression is believed to be around 60% mutant for mtDNA deletions and >90% mutant for tRNA mutations.[46] Factors contributing to this difference in segregation of the mtDNA genotype between various tissues are not elucidated yet; however, age, nuclear background, sex and environmental factors are playing a role.[45,46] Due to the wide variation in presentation and course, diagnosis in mitochondrial disorders is challenging, apart from some well-recognized syndromes. As was described in our algorithm, a mitochondriopathy as a cause for cerebellar ataxia should be considered in the presence of a multisystem disorder. Systems frequently affected in mitochondriopathies are the peripheral nervous system, the central nervous system apart from ataxia (epilepsy, dementia, extrapiramidal disorders), the endocrine system, heart, eyes, ears and guts.[46]

In the family described in **chapter 3.4**, likewise, the multisystem phenotype with neuromuscular signs as well as cerebellar ataxia was leading us to the diagnosis of a mitochondrondrial disorder. This family also displayed a maternal pattern of inheritance, suggesting a mitochondrial gene mutation. However, as was stated before, most mitochondrial proteins are encoded in the nuclear genes, so autosomal inherited gene mutations may also lead to a mitochondrial phenotype. Indeed, mitochondrial dysfunction is an important pathophysiological mechanism in Friedreich ataxia and SCAs, as will be described in more detail below.

In our family, we describe two different phenotypes at onset, a neuromuscular and a cerebellar phenotype, which has been a new phenotypical observation in the m.G8363A tRNA(Lys) mutation. We found that cerebellar onset was related to a higher mutation load in urine, which also correlated to earlier disease-onset. Furthermore we predicted a more severe clinical outcome in patients with earlier onset (higher mutational load) since severity of ataxia correlated with disease-duration. Apart from predicting the phenotype, data on mutational load are also used in genetic counselling, since it is known that in mitochondrial point mutations, the chance of transmitting the disorder increases with the rate of heteroplasmy. However, there is no degree of heteroplasmy at which the risk is low enough to be ingnored.[46] Therefore, genetic counselling in our family and as well as other families with mitochondrial disease mutations is still very difficult.

Indications for further research

The expanding genetic classification of cerebellar ataxia evidently enables the unraveling of the autosomal and mtDNA-induced pathways of mitochondrial dysfunction, involved in cerebellar neurodegeneration, and their mutual involvement. The recognition and thorough, even standardized clinical and biochemical analysis of sporadic patients and families with "mitochondrial" cerebellar ataxia is the basis for proper genotype-phenotype correlations, necessary to interpret the effects of these disease-pathways in different tissues. This accounts for patients with new or unknown genotypes as well as known gene mutations. A database of clinical and molecular findings, derived from standardized patient evaluations, facilitates research in large populations, aiming to identify the most reliable and less invasive molecular tests to analyze mutational load related to phenotype and to improve clinical outcome predictions and genetic counseling. In the future this may hopefully lead to preventive and tissue-targeted therapeutic strategies.

Current concept of pathophysiology in cerebellar ataxia

It appears that various neurodegenerative disorders display common pathogenic mechanisms. For example, polyglutamine expansion mutations are also involved in other neurological disorders like Huntington's disease.[47] More recently, mutations in the gene encoding tau tubulin kinase 2 were found to cause SCA11, classifying SCA11 as a tauopathy.[33,48] Protein Phophatase 2 (PP2), which activity is deregulated in SCA12, dephosphorylates a diversity of kinases including protein kinase B/Akt and C, related to SCA1 and SCA14 respectively. Dysregulation of PP2 also plays a role in Alzheimer's disease.[33,49,50] Furthermore, the ubiquitin proteasome pathway is playing a role in SCA 1,2 and 3 as well as Parkinson disease and autosomal recessive ALS for instance.[51,52]

Table 3 Pathophysiological mechanisms involved in autosomal dominant cerebellar ataxias

Pathophysiological mechanisms	Polyglutamine expansion SCAs (genes)	Non-coding expansion SCAs (genes)	Conventional mutation SCAs (genes)
Toxic accumulation of aggregates and intranuclear inclusions	SCA 1 *(ATXN1)*, SCA2 (*ATXN2*), SCA3(*ATXN3*), SCA7(*ATXN7*), SCA17(*TBP)*, DRPLA(*ATN1*)	SCA8(*ATXN8*)	
Dysregulation of transcription and gene expression	SCA 1 (*ATXN1)*, SCA3(*ATXN3*), SCA7(*ATXN7*), SCA17(*TBP*), DRPLA(*ATN1*)		
- RNA-processing	SCA2 (*ATXN2*)	SCA8(*ATXN8)*, SCA31(*BEAN-TK2*)	
Dysfunction in synaptic transmission: glutamate transmission	SCA1(*ATXN1*), DRPLA (*ATN1*), perhaps also SCA3(*ATXN3*), SCA7(*ATXN7*), SCA17(*TBP*)		SCA5(*SPBN2*), SCA20(*DAGLA*) SCA23(*PDYN*)
Dysfunction in calcium homeostasis	SCA1(*ATXN1*), SCA2(*ATXN2*),	SCA12(*PPP2R2B*)	SCA14(*PRKCG*), SCA15/16(*ITPR1*)
- Ca-channel dysfunction	SCA6(*CACNA1A)*		
Mitochondrial stress and apoptosis	SCA2 (*ATXN2*), SCA6(*CACNA1A*), SCA7(*ATXN7*), SCA17(*TBP*)	SCA12(*PPP2R2B*)	SCA28(*AFG3L2)*
Other mechanisms			
- Sodium channel dysfunction			SCA27(*FGF14*)
- Potassium channel dysfunction			SCA13(*KCNC3*)
- Tau-phosphorylation			SCA11(*TTBK2*)
- Mediating intracellular signaling			SCA10(ATXN10)

However, most pathways leading to neuronal toxicity by the disease proteins in SCA have to be elucidated.[33,53]
Nevertheless, the increasing insight in the role of SCA proteins, encoded by the various SCA mutations, has lead to the identification of some common pathways to cerebellar ataxia, related to dysfunction in gene expression, synaptic transmission and other intracellular signaling pathways. *Table 3* gives an overview of these various disease mechanisms, which are described in more detail below. A specific SCA mutation may also induce combinations of these pathophysiological mechanisms. Furthermore, different mechanisms may have a common final pathway in the cerebellar neurodegeneration.

Dysregulation of transcription and gene expression

The polyglutamine expansions result in abnormally long tracts of glutamine residues, leading to a toxic gain of function: aggregation and deposition of misfolded proteins lead to neuronal dysfunction and eventually cell death. The aggregates result in the characteristic nuclear or cytoplasmic inclusions. Furthermore, SCA proteins containing stretches of polyglutamines give rise to transcriptional dysregulation, due to interaction with several transcription factors or chromatin remodelling. This interaction leads to neuronal dysfunction and accounts for the cell-type specific degeneration, seen in polyglutamine SCAs. Non-coding expansions might influence the level of transcription of the gene or produce toxic RNA transcripts.[50,54]

Dysfunction in synaptic transmission

Glutamate stimulation of ionotropic AMPA-type glutamate receptors and metabotropic glutamate receptors mediate the afferent input of Purkinje cells. The AMPA receptors cause local depolarization of dendritic spikes, leading to activation of voltage gated calcium channels. So defects in glutamate transmission are related to calcium homeostatis, and induce mechanisms leading to neuronal degeneration in a various number of SCAs. The β-III spectrin, related to SCA5 for instance, may be involved in stabilizing the Purkinje cell specific glutamate transporter EAAT4 in the cell membrane. This gene is also downregulated by mutant ataxin-1 in the SCA1 mouse model.[33,50,53]
Very recently, missense mutations in prodynorphin (PDYN) were identified to cause SCA23. PDYN is the precursor protein for the opiod neuropeptides and the mutations resulted in altered expression of enzymes regulating glutamate cycling and of EAAT4, also suggesting glutamate neurotoxicity.[34] A recently developed mouse model in DRPLA shows electrophysiological changes in various brain areas, with decreased currents through AMPA and GABA receptors in hippocampal neurons.[41]

Dysfunction in calcium homeostasis

Cerebellar Purkinje cells appear to be particularly sensitive to fluxes in intracellular calcium levels. These variations may result from reduction of chaperone activity, necessary for degeneration of misfolded proteins, and endoplasmic reticulum (ER) stress, induced by the presence of unfolded proteins. In SCA 2, disturbed neuronal calcium-signaling plays a role. In SCA6, a polyglutamine expansion is present in the *CACNA1A* gene, encoding an alpha subunit of the $Ca_v2.1$ voltage- gated calcium channel (VGCC). This is assumed to cause altered channel function or a toxic gain of function effect, due to the production of an aberrant C-terminal fragment. SCA 6 is therefore defined as a channelopathy. The mutant *protein kinase C gamma (PKC gamma)* gene, causing SCA14 , fails to phosphorylate transient receptor potential (TRP) channels, resulting in sustained Ca2+ entry. Impaired phosphorlylation of Ca2+ channels may also play a role in SCA12.[55] Ca2+ release from the ER is disturbed in SCA 15.[33,41,50,53]

Mitochondrial stress and apoptosis

Mitochondrial oxidative phosphorylation provides the main source of energy in the cell. Mitochondria also play a crucial role in other metabolic processes like mediating amino acid synthesis, fatty acid oxidation, calcium homeostasis and free radical scavenging. In the past decade, mitochondrial dysfunction has been elucidated as one of the common pathogenic mechanisms of autosomal as well as mitochondrial inherited neurodegenerative disorders. Mutant proteins may be localized in mitochondria, likely to directly cause mitochondrial defects; however, mitochondrial dysfunction may also be a secondary effect due to degenerative events in other cell organelles. Frataxin, the protein involved in Friedreich's ataxia, for instance, is a primary mitochondrial localized protein, involved in heme biosynthesis and detoxification of iron. Frataxin defects lead to accumulation of mitochondrial iron, which may result in oxidative stress through the production of free radicals. The antioxidant idebenone can reduce myocardial hypertrophy in Friedreich ataxia.[44] It has been shown that mitochondrial dysfunction is also common in the polyglutamine expansion as well as non-polyglutamine expansion SCAs. This may be clinically evident as in SCA7, displaying a combination of retinopathy, hearing loss and cerebellar ataxia. Mitochondria are also involved in the calcium homeostasis, one of the pathophysiological pathways in SCA. Neuronal death related to polyglutamine stretches is induced by oxidative stress and mitochondrial apoptosis pathways. Recently, SCA28 was related to mutations in *AFG3L2*, a mitochondrial metalloprotease, part of a protein complex in the mitochondrial inner membrane, ensuring protein quality control and displaying a chaperone-like activity on the respiratory chain complexes. Impaired mitochondrial proteolysis may therefore be a novel pathway in cerebellar neurodegeneration.[33,50,56] Ataxia due to mitochondrial dysfunction is also seen in other autosomal recessive disorders, for example related to the POLG mutations, and obviously in neurodegenerative disorders related to mitochondrial DNA deletions and -mutations.

IV Final remarks and conclusions

This thesis reflects the result of our work regarding both objectives outlining the startup of the multidisciplinary neurogenetic consultation: to improve clinical care and to expand the genotype and phenotype in cerebellar ataxias. The diagnostic process in cerebellar ataxia has been facilitated and standardized using the diagnostic algorithm. This will be continued and improved in the development of a nationwide, multidisciplinary diagnostic and therapeutic directive in the coming years, using the evaluation of the diagnostic output of our algorithm and a new search of the recent literature to come to an updated, evidence-based and efficient directive. Fatigue has been recorded as a severe and disabling complaint in cerebellar ataxia, and suggestions are made for therapeutic strategies to challenge fatigue. The finding of the *fibroblast growth factor 14* gene to be related to SCA27 has given new input in the unraveling of the pathophysiological mechanisms of ataxia and we soon hope to translate the knowledge generated in the $fgf14^{-/-}$ in vivo and in vitro mouse models to our patients. The description of the new locus in autosomal recessive ataxia, and novel genotype-phenotype correlations in the m.G8363A tRNA(Lys) mutation further added to the genetic and phenotypic classification of cerebellar ataxia.

Apart from adequate genetic counseling, an early and appropriate clinical diagnosis is crucial when curative and hopefully even preventive therapies become available. The basis for the development of curative therapies in patients with cerebellar ataxia lies in translational research. However, mutual inspiration between basic sciences and clinicians is necessary to make this translation possible. In the end, clinicians can only define and treat symptoms as indicated by their patients. The dedicated evaluation of patients with cerebellar ataxia in our multidisciplinary setting will hopefully continue to add to their benefit.

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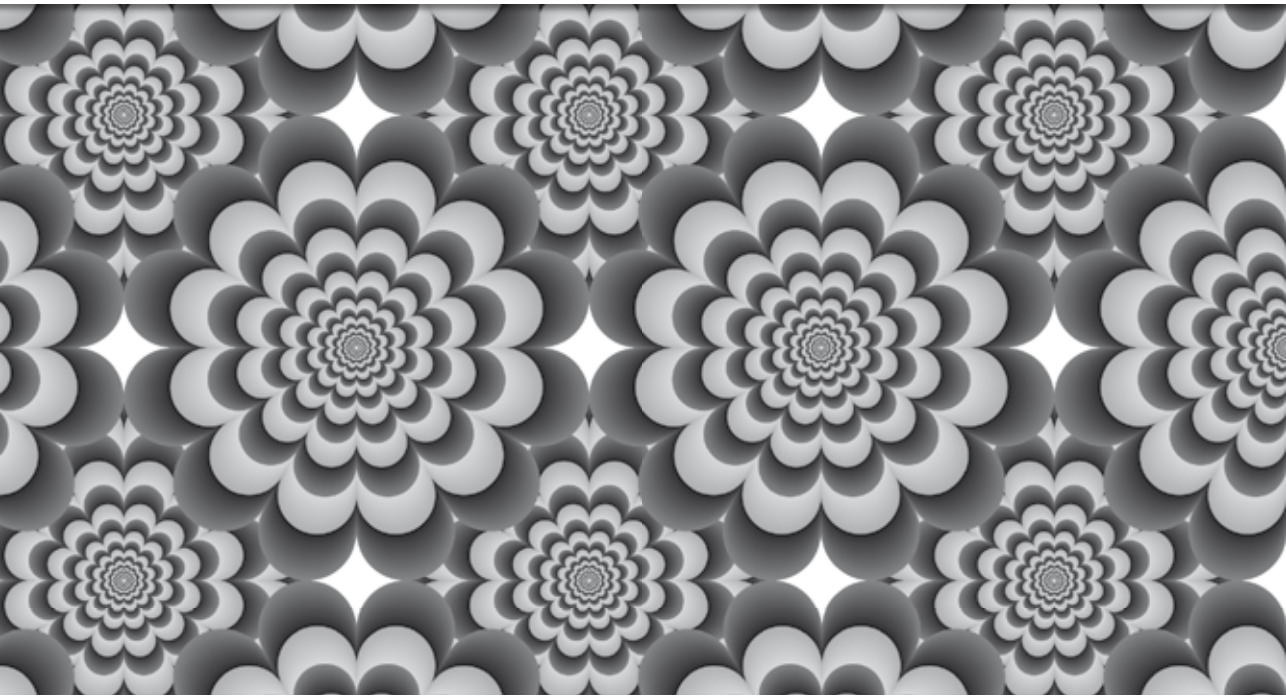
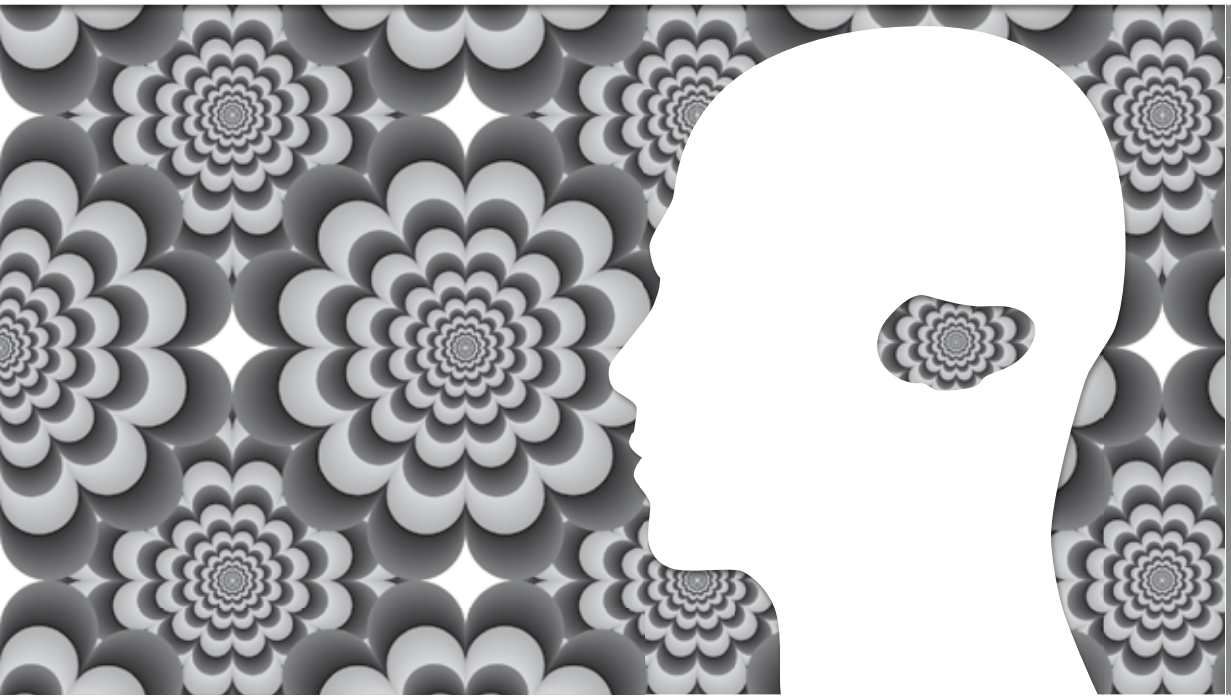




Summary

Chapter 1.1 starts with a general introduction, briefly describing the role of the cerebellum in action control and addressing the terminology that is used to classify the heterogeneous group of cerebellar ataxias. Furthermore, the realization of the multidisciplinary neurogenetic consultancy is described, that could be marked as a starting point of this thesis.

In **Chapter 1.2** we present a diagnostic algorithm, standardizing the diagnostic evaluation of patients displaying subacute cerebellar ataxia in (early) adulthood. Two main categories of subacute cerebellar ataxia are distinguished: hereditary and sporadic cerebellar ataxia. In the hereditary ataxias, an expanding genotype-based classification is used. According to this classification, the algorithm is based on family history and distinguishes four apparent modes of inheritance: apparent autosomal dominant, autosomal recessive, X-linked and sporadic cerebellar ataxias. The evaluation is further directed by clinical characteristics, when applicable. In apparently dominant cerebellar ataxias, episodic ataxias (EAs) are distinguished from non episodic spinocerebellar ataxias (SCAs). In apparently recessive cerebellar ataxias, after exluding Friedreichs ataxia (FA), further analysis is based on disease onset and specific clinical signs like oculomotor apraxia, polyneuropathy, ophthalmologic signs and cerebral white matter changes on brain MRI. In apparent X-linked inheritance, again, onset age determines further analysis, with adrenoleukodystrophy and fragile-X-associated tremor ataxia syndrome (FXTAS) as the main candidates. In apparently sporadic ataxias, symptomatic and paraneoplastic cerebellar syndromes, as well as multiple system atrophy (MSA) should be considered. Furthermore, a patient with apparent sporadic ataxia may well represent a hereditary cause of disease, especially when disease onset is before the age of 50 years: in this case the algorithm suggests further genetic testing. Apparent mitochondrial inheritance has not been provided in the algorithm, because mitochondrial disease may represent different modes of inheritance. A mitochondropathy should be considered based on clinical phenotype.
An overview of the hereditary, symptomatic and idiopathic sporadic cerebellar ataxias is also presented in this chapter. Finally, we discuss therapeutic and caring strategies, emphasizing the value of a proper diagnosis and adequate genetic counseling.

In **Chapter 2,** we report a study on fatigue in a cohort of 123 patients with autosomal dominant spinocerebellar ataxia (SCA), with or without genetic confirmation. All patients provided a self-assessment evaluation, 58 patients were clinically evaluated. Severity of fatigue was quantified with the Fatigue Severity Scale (FSS), and data were collected on disease duration, severity of ataxia (Severity of Ataxia Rating scale, SARA), physical impairment, SCA subtype, medication use, sleep disturbances, depression and

quality of life. We found that fatigue is a prominent and disabling disease symptom in adult patients with SCA, with 69% of our population displaying a FSS value of 5 and higher, indicating severe fatigue, and nearly 70% classifying fatigue among their three most disabling symptoms. Physical functioning and depression appeared to be the strongest predictive factors for the presence of severe fatigue. We did not find a significant correlation between FSS value and severity of ataxia (SARA), implying that physical functioning, related to fatigue, is not only determined by cerebellar ataxia. Indeed, in the clinically evaluated subgroup the presence of visual symptoms (not addressed by the SARA) was related to FSS value. Furthermore, fatigue appeared to be significantly correlated with sleep disturbances, but not with gender, age, disease duration, SCA subtype, medication use or co-morbidity. We therefore conclude that fatigue can be early symptom in SCA patients and all treatable factors for fatigue should be addressed, especially depression, visual symptoms and sleeping disorders.

Chapter 3 is focused on the identification of families with hereditary ataxias and unknown genetic deficits from our neurogenetic consultation, and the subsequent genotype-phenotype correlations that we performed in these families.

Chapter 3.1 and **3.2** describes a family with a new subtype of autosomal dominant cerebellar ataxia (ADCA). In **chapter 3.1**, we report the identification of a *fibroblast growth factor 14* gene mutation, related to ADCA, in a large three-generation Caucasian family of Dutch descent with 14 affected relatives displaying a childhood onset tremor, followed by dyskinesia and cerebellar ataxia slowly progressing over decades. A systematic genome screen provided positive LOD scores for adjacent markers on chromosome 13q34. Subsequent haplotype analysis defined a critical region, encoding ≥ 10 genes with a known function, including *fibroblast growth factor 14 (FGF14)*. Since a mouse model lacking Fgf14 was published, showing ataxia and paroxysmal dyskinesia, we analyzed *FGF14* as the first candidate gene in this family. We identified a substitution of a serine for a phenylalanine at position 145 (F145S) in the *FGF14* gene in this family, segregating with the disease phenotype. We predicted the *F145S* mutation to destabilize the Fgf14 protein, leading to a loss of function of Fgf14. **Chapter 3.2** focuses on the clinical phenotype in the 14 affected members of this family. Postural tremor and sometimes head titubation appeared to be the first disease symptom, with a mean estimated disease onset of 11 years (range 7-20 years). Ataxia typically started in the third or fourth decade (mean onset 34 years) and displayed a very slow progression. Orofacial dyskinesia was present in eight patients. MRI of the brain was normal in most patients and showed moderate cerebellar atrophy in the two eldest individuals. The affected relatives showed a remarkably lower education level and frequent behavioral problems, compared to the unaffected relatives. Neuropsychological evaluation in four patients revealed that intelligence, verbal and nonverbal memory tests were below

average in the three eldest patients; executive functioning was diminished in all four patients. We suggest that the presence of postural tremor and dyskinesia is suggestive of involvement of the basal ganglia circuitry in the *FGF14* related SCA phenotype that has been classified as SCA27. This correlates with animal studies, showing high levels of expression of FGF14 in the cerebellum but also in the hippocampus, cortex and striato-pallidal and striatronigral pathways.

Chapter 3.3 discusses the genotype-phenotype correlation in a family with autosomal recessive cerebellar ataxia. **Chapter 3.3** starts with a review of the autosomal recessive ataxias, including the childhood onset and congenital ataxias, summarizing their loci, genes, proteins and distinguishing clinical features. Then, we introduce a non-consanguineous Dutch family with five affected sibs displaying a spinocerebellar syndrome, combining cerebellar ataxia, pyramidal signs and posterior column involvement; two patients also displayed a postural tremor. All patients showed a childhood onset but progression of disease was remarkably variable: in three patients, progression was slow and symptoms began interfering with daily life from the age of 40 years, while two patients were completely wheelchair dependent in the fifth decade. MRI of the brain showed cerebellar atrophy. Using a genomewide scan we mapped the responsible gene for autosomal recessive ataxia in this family to a 5.9 cM interval on chromosome 11p15. No obvious candidate gene could yet be assigned.

In **chapter 3.4**, we describe a family with cerebellar ataxia related to a mitochondrial mutation. **Chapter 3.4** is focusing on new phenotypic characteristics related to a known mitochondrial G8363A tRNA(Lys) mutation in 10 relatives of a two-generation Dutch family. Two distinct onset phenotypes were seen in this family, both emerging in the second or third decade. One phenotype presented with myopathy and axonal neuropathy, followed by cerebellar ataxia. The other phenotype presented with cerebellar ataxia, often also developing myopathy. Cerebellar onset was related to a higher mutation load in urine and blood. Furthermore, disease onset correlated to mutational load in urine. Severity of ataxia (indicated as SARA score) correlated with disease duration, predicting a more severe clinical outcome in patients with earlier onset, related to higher mutational load. We confirm that the use of urine to define mutational load is preferred to the use of blood in mitochondrial genotype-phenotype studies.

The clinical implications of the studies described in this thesis, and indications for further research are discussed in **chapter 4.** We conclude that the diagnostic algorithm for subacute cerebellar ataxia has facilitated and standardized the diagnostic phase in our clinic, showing a satisfying diagnostic output: during the first four years of its use, a genetic diagnosis was found in almost 35% of 89 cases and a classifying describing diagnosis in almost all patients. We are currently involved in a multidisciplinary Dutch

committee to come to an updated, evidence-based nationwide directive on diagnosis and treatment of cerebellar ataxia. This directive should also address treatable causes of fatigue, as a disease symptom, at least in SCA patients. We discuss the central governor model as a general concept of fatigue and suggest to include the cerebellum in this model, to explain the role of specific clinical factors that we have found to predict fatigue in our SCA population. The study on fatigue should be expanded to other subtypes of cerebellar ataxia and treatment strategies should be evaluated.

SCA27 has appeared to be an infrequent cause of SCA. The FGF14 protein is involved in proper functioning of voltage-gated Na channels, classifying SCA27 as a Na-channelopathy. Expression studies on voltage-gated Na channels in post-mortem brain tissue of a SCA27 patients are planned.

No other cases of autosomal recessive cerebellar ataxia have yet been mapped to the locus that we described on chromosome 11p15, now termed SCAR7, and still no mutation has been detected, the results of further sequencing studies are expected. Since diagnosis and genetic counselling in mitochondrial disorders is challenging, we discuss the importance of proper genotype-phenotype correlations, as described in the family with the mitochondrial tRNA(Lys) mutation. Subsequently, we discuss the current concept of pathophysiology in cerebellar ataxia. We end with the recommendation to evaluate patients with cerebellar ataxia in a multidisciplinary neurogenetic setting .

Samenvatting

Hoofdstuk 1.1. opent met een algemene introductie, waarin de rol van het cerebellum in de regulatie van de motoriek kort wordt aangestipt en waarin een overzicht wordt gegeven van de terminologie die wordt gebruikt voor de classificatie van de heterogene groep van de cerebellaire ataxieën. Verder staan we stil bij de totstandkoming van het neurogenetisch spreekuur, dat de basis heeft gevormd voor dit proefschrift.

In **hoofdstuk 1.2** presenteren we een richtlijn voor een gestandaardiseerde diagnostische evaluatie van (jong) volwassen patiënten met een subacute cerebellaire ataxie. Hierbij worden de subacute cerebellaire ataxieën onderverdeeld in twee hoofdgroepen: hereditaire en sporadische cerebellaire ataxie. De classificatie van de hereditaire ataxieën is gebaseerd op het onderliggende genotype en breidt zich nog steeds verder uit. Gezien deze genetische classificatie is de richtlijn dan ook gebaseerd op de familieanamnese, waarbij in eerste instantie vier veronderstelde manieren van overerving worden onderscheiden: vermoedelijk autosomaal dominante, autosomaal recessieve, X-gebonden en sporadische cerebellaire ataxieën. Vervolgens wordt zo mogelijk gebruik gemaakt van karakteristieke klinische kenmerken om richting te geven aan de verdere diagnostiek. Bij de vermoedelijk dominante cerebellaire ataxieën wordt onderscheid gemaakt in episodische ataxieën (EA's) en niet-episodische spinocerebellaire ataxieën (de SCA's). Bij de vermoedelijk autosomaal recessieve cerebellaire ataxieën wordt allereerst Friedreichse ataxie (FA) uitgesloten, waarna het verdere diagnostische traject bepaald wordt door de beginleeftijd en het bestaan van specifieke symptomen als oculomotore apraxie, polyneuropathie, ophtalmologische kenmerken en afwijkingen van de witte stof op de MRI-scan van de hersenen. Bij een vermoedelijke X-gebonden overerving is ook de beginleeftijd bepalend voor de verdere diagnostiek, waarbij met name adrenoleukodystrofie en het Fragiele-X-geassocieerde tremor-ataxie syndroom (FXTAS) moeten worden overwogen. Bij vermoedelijk sporadische ataxieën moeten zowel aymptomatische en paraneoplastische cerebellaire syndromen worden overwogen, alsook multi-systeem atrofie (MSA). Daarnaast kan er bij een patiënt met een ogenschijnlijk sporadische ataxie toch sprake zijn van een erfelijke oorzaak, met name als de klachten ontstaan voor het 50e levensjaar: de richtlijn adviseert bij deze patiënten dan ook verder genetisch onderzoek in te zetten. De richtlijn voorziet niet in een categorie van vermoedelijk mitochondriële overerving, omdat mitochondriële aandoeningen verschillende overervingvormen kennen. Een mitochondriële oorzaak moet dan ook overwogen worden op basis van het klinisch beeld.

In dit hoofdstuk is ook een overzicht opgenomen van de verschillende hereditaire en sporadische, zowel symptomatische als idiopathische, cerebellaire ataxieën. Tenslotte bespreken we adviezen voor behandeling en begeleiding van patiënten en

benadrukken daarbij nog eens het belang van een goede diagnose en een adequaat erfelijkheidsadvies.

In **hoofdstuk 2** beschrijven we een studie naar vermoeidheid in een cohort van 123 patiënten met een autosomaal dominante cerebellaire ataxie (SCA), met of zonder genetische diagnose. Alle patiënten vulden zelf een serie onderzoeksenquêtes in en aansluitend werden 58 patiënten klinisch onderzocht. De ernst van de vermoeidheid werd gekwantificeerd met de Fatigue Severity Scale (FSS) en er werden gegevens verzameld over ziekteduur, ernst van de ataxie (Scale of assessment and rating of ataxia, SARA), mate van fysieke beperking, SCA subtype, medicatiegebruik, slaapstoornissen, depressie en kwaliteit van leven. We stelden vast dat vermoeidheid een belangrijk en beperkend ziekteverschijnsel is bij volwassen patiënten met SCA: 69% van onze populatie gaf een FSS score van 5 of hoger aan, hetgeen wijst op ernstige vermoeidheid, en bijna 70% van de patiënten rekende vermoeidheid tot de top drie van de meest beperkende ziekteverschijnselen. Het fysiek functioneren en depressie bleken de sterkst voorspellende factoren voor het bestaan van ernstige vermoeidheid te zijn. We vonden geen significante correlatie tussen de FSS score en de ernst van de ataxie (SARA). Dit impliceert dat het fysiek functioneren, dat is gerelateerd aan vermoeidheid, niet volledig bepaald wordt door de mate van cerebellaire ataxie. Inderdaad bleek bij de analyse van de subgroep die we klinisch onderzochten, dat visuele symptomen (die niet gemeten worden met de SARA), waren gerelateerd aan de FSS score. Vermoeidheid was niet gerelateerd aan geslacht, leeftijd, ziekteduur, SCA subtype, medicatiegebruik of co-morbiditeit. We vonden wel een significante relatie met slaapstoornissen. We concluderen dan ook dat vermoeidheid een vroeg symptoom kan zijn bij SCA patiënten en dat alle behandelbare factoren die effect kunnen hebben op vermoeidheid per patiënt tegen het licht moeten worden gehouden, met name depressie, visuele symptomen en slaapstoornissen.

Hoofdstuk 3 legt de focus op families met hereditaire cerebellaire ataxieën met onbekende genmutatie, die we identificeerden op het neurogenetisch spreekuur en de daaropvolgende genotype-fenotype correlatiestudies die we bij deze families uitvoerden.

Hoofdstuk 3.1 en **3.2** beschrijft een familie met een nieuw ADCA (autosomaal dominante cerebellaire ataxie) subtype. In **hoofdstuk 3.1**. beschrijven we de ontdekking van een mutatie in het *fibroblast growth factor 14* gen, gerelateerd aan ADCA, in een grote familie van drie generaties van Kaukasisch-Nederlandse oorsprong, waarin 14 aangedane familieleden een ziektebeeld vertonen met een tremor vanaf de kinderleeftijd, in de daaropvolgende decaden gevolgd door dyskinesieën en een langzaam progressieve cerebellaire ataxie. Een systematische genoomscreening resulteerde in positieve LOD scores voor naastgelegen markers op chromosome 13q34. De daaropvolgende haplotype analyse begrensde een kritische regio, waarbinnen de codering lag van meer dan 10

genen met een bekende functie, inclusief *fibroblast growth factor 14 (FGF14)*. Aangezien er een muismodel met ontbrekend Fgf14 eiwit was gepubliceerd, waarbij ataxie en paroxysmale dyskinesieën werden beschreven, analyseerden we *FGF14* als eerste kandidaat-gen in onze familie. We vonden een substitutie van serine in phenylalanine op positie 145 (F145S) in het *FGF14* gen bij deze familie, die segregeerde met het klinische fenotype. We voorspelden dat de F145S mutatie het Fgf14 eiwit destabiliseerde, met functieverlies van Fgf14 tot gevolg. **Hoofdstuk 3.2**. beschrijft in meer detail het klinische fenotype van de 14 aangedane familieleden. Het eerste ziekteverschijnsel is een posturele tremor, soms gecombineerd met titubatie van het hoofd; de gemiddelde geschatte beginleeftijd was 11 jaar (spreiding 7-20 jaar). De ataxie openbaarde zich meestal in de derde of vierde decade (gemiddeld op de leeftijd van 34 jaar) en was zeer langzaam progressief. Bij acht patiënten werden orofaciale dyskinesieën gezien. De MRI-scan van de hersenen was bij de meeste patiënten normaal; bij de twee oudste patiënten werd een milde cerebellaire atrofie gezien. Bij de aangedane familieleden was sprake van een opmerkelijk laag opleidingsniveau en frequente gedragsproblemen, in vergelijking met de gezonde familieleden. Vier patiënten ondergingen neuropsychologisch onderzoek, de drie oudste patiënten presteerden lager dan gemiddeld op het gebied van intelligentie, verbale en nonverbale geheugentaken en het executief functioneren was afwijkend bij alle vier patiënten. We denken dat de posturele tremor en dyskinesieën bij dit FGF14-gerelateerde SCA fenotype, geclassificeerd als SCA27, wijzen op betrokkenheid van de basale kernen. Dit komt overeen met bevindingen in dierstudies, waarbij naast het cerebellum ook een hoge expressiegraad van FGF14 werd gezien in de hippocampus, de cortex en in de striato-pallidale en in de striato-niagrale banen.

Hoofdstuk 3.3 beschrijft de genotype-fenotype correlatiestudie in een familie met een autosomaal recessieve cerebellaire ataxie. **Hoofdstuk 3.3.** begint met een overzicht van de autosomaal recessieve cerebellaire ataxieën, inclusief de ataxieën van de kinderleeftijd en de congenitale ataxieën, waarbij de betrokken loci, genen, eiwitten en onderscheidende klinische symptomen worden samengevat. Daarna introduceren we een niet-consanguine Nederlandse familie met vijf broers en zussen met een spinocerebellair syndroom, met een combinatie van een cerebellaire ataxie, een piramidaalsyndroom en achterstrengstoornissen; twee patiënten hadden daarbij ook een posturele tremor. Bij alle patiënten begon de ziekte op de kinderleeftijd maar er was een opmerkelijke variatie in ziekteprogressie: bij drie patiënten was sprake van een langzame progressie en werden de symptomen pas merkbaar in het dagelijks leven vanaf het veertigste jaar, terwijl twee patiënten al volledig rolstoelgebonden waren in de vijfde decade. Op de MRI van de hersenen was cerebellaire atrofie te zien. Met een genoomscreening werd het verantwoordelijke gen voor autosomaal recessieve ataxie in deze familie gekoppeld aan een interval van 5.9cM op chromosoom 11p15. Tot nu toe kon nog geen kandidaat-gen worden aangewezen.

In **hoofdstuk 3.4** beschrijven we een familie met een cerebellaire ataxie gerelateerd aan een mitochondriële mutatie. **Hoofdstuk 3.4** belicht nieuwe fenotypische kenmerken gerelateerd aan een bekende mitochondriële G8363A tRNA(Lys) mutatie bij 10 Nederlandse familieleden uit twee generaties. In deze familie werden bij aanvang van de ziekte twee verschillende fenotypes gezien, allebei beginnend in de tweede of derde decade. Het eerste fenotype presenteerde zich met een myopathie en een axonale neuropathie, gevolgd door cerebellaire ataxie. Het tweede fenotype presenteerde zich met een cerebellaire ataxie, waarbij later in het beloop vaak ook een myopathie ontstond. Een cerebellaire presentatie was gerelateerd aan een hoger percentage gemuteerd mt.DNA in urine en bloed. Daarnaast correleerde ook de beginleeftijd met het percentage gemuteerd mt.DNA in urine. De ernst van de ataxie (SARA) correleerde met de ziekteduur en voorspelde een ernstiger klinisch beeld bij patiënten met een vroegere beginleeftijd, die weer is gerelateerd aan een hoger percentage gemuteerd mt.DNA. We bevestigen dat urine de voorkeur heeft boven het gebruik van bloed bij de bepaling van het percentage gemuteerd mt.DNA voor mitochondriële genetype-fenotype correlatie-studies.

De klinische implicaties van de studies uit dit proefschrift en suggesties voor verder onderzoek worden besproken in **hoofdstuk 4**. We concluderen dat de diagnostische richtlijn de diagnostiek bij patiënten met cerebellaire ataxie in ons centrum heeft vereenvoudigd en gestandaardiseerd en voldoende diagnostische opbrengst geeft: in de eerste vier gebruiksjaren werd een genetische diagnose gevonden bij bijna 35% van 89 patiënten en bij vrijwel iedereen kon een omschrijvende diagnose worden vastgesteld. Momenteel zijn we betrokken bij een multidisciplinaire Nederlandse commissie die een landelijke, actuele, evidence-based richtlijn ontwikkelt voor de diagnose en behandeling van cerebellaire ataxie. Deze richtlijn zou ook in moeten gaan op behandelbare oorzaken van vermoeidheid, als een symptoom bij SCA patiënten. We bespreken het 'central governor model' als een algemeen concept van vermoeidheid en doen de suggestie om het cerebellum aan dit model toe te voegen als verklaring voor de rol van de specifieke klinische symptomen die voorspellers van vermoeidheid bleken te zijn bij onze SCA populatie. Het onderzoek naar vermoeidheid zou moeten worden uitgebreid naar andere subtypes van cerebellaire ataxie en behandelstrategieën moeten worden geëvalueerd.

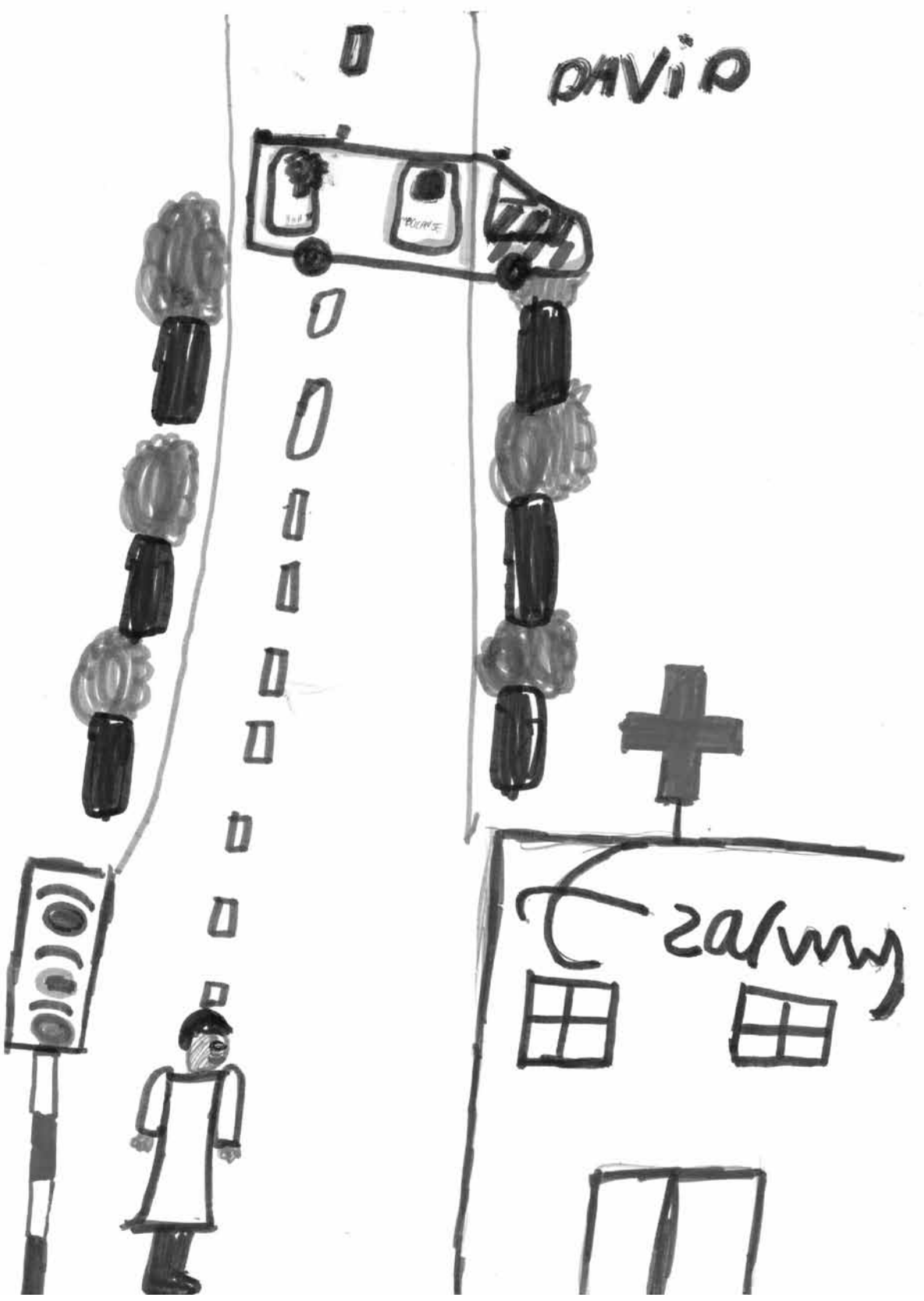
SCA27 blijkt een zeldzaam SCA subtype te zijn. Het FGF14 eiwit speelt een rol bij het functioneren van de voltage-gated natrium kanalen en SCA27 kan dus worden beschouwd als een natrium-kanalopathie. Onderzoek naar expressie van voltage-gated natrium kanalen in post-mortem hersenweefsel van een SCA27 patiënt zijn in voorbereiding.

Tot nu toe zijn geen andere gevallen van autosomaal recessieve cerebellaire ataxie gekoppeld aan het door ons beschreven locus op chromosoom 11p15, tegenwoordig aangeduid als SCAR7 en er is nog geen mutatie gevonden: de resultaten van verder sequencing-onderzoek worden op korte termijn verwacht. Diagnostiek en erfelijkheids-onderzoek bij mitochondriële aandoeningen is gecompliceerd en daarom benadrukken we het belang van goed genotype-fenotype onderzoek, zoals bij de familie met de mitochondriële tRNA(Lys) mutatie. Aansluitend beschrijven we de huidige stand van zaken met betrekking tot de pathofysiologie van de cerebellaire ataxie. We besluiten met de aanbeveling om patiënten met een cerebellaire ataxie te analyseren in een multidisciplinaire neurogenetische setting.

List of abbreviations

aBL:	abetalipoproteinemia
ADCA:	autosomal dominant cerebellar ataxia
AOA:	ataxia oculomotor apraxia
AR:	autosomal recessive
ARCA:	autosomal recessive cerebellar ataxia
ARSACS:	autosomal recessive spastic ataxia of Charlevoix-Saguenay
AT:	ataxia teleangiectasia
AVED:	ataxia with vitamin E deficiency
BDI:	Beck Depression Inventory
CAF:	central activation failure
CMAP:	compound muscle action potential
(C)PEO:	(chronic) progressive external ophtalmoplegia
CNS:	central nervous system
COX:	cytochrome oxidase
CT:	computed tomography
CTX:	cerebrotendinous xantochromatosis
cM:	centimorgan
DRPLA:	dentatorubral-pallidoluysian atrophy
EA:	episodic ataxia
EMG:	electromyography
ER:	endoplasmic reticulum
ESS:	Epworth Sleepiness Scale
FA:	Friedreich ataxia
FGF:	fibroblast growth factor
FHM:	Familial hemiplegic migraine
FP-CIT:	[123I]N-fluoropropyl-2βcarbomethoxy-3β-(4-iodophenyl) nortropane
FSS:	Fatigue Severity Scale
FXTAS:	fragile-X-associated tremor ataxia syndrome
IBZM:	[123I]iodobenzamide
ILOCA:	idiopathic late onset cerebellar ataxia
IOSCA:	infantile-onset spinocerebellar ataxia
IQ:	intelligence quotient
LOD-score:	logarithm of odds-score
MELAS:	myopathy, encephalopathy, lactic acidosis and stroke-like episodes
MERFF:	myoclonic epilepsy and ragged red fibers
MIRAS:	mitochondrial recessive ataxic syndrome
MLD:	metachromatic leukodystrophy
MMSE:	Mini Mental State Examination

MRI:	magnetic resonance imaging
MRC:	Medical Research Council
MS:	multiple sclerosis
MSA:	multisystem atrophy
mt:	mitochondrial
NARP:	neuropathy, ataxia, retinitis pigmentosa
Na_v:	voltage-gated sodium channels
Nb-MCS:	norm-based mental component score
Nb-PCS:	norm-based physical component score
NCV:	nerve conduction velocity
PCR:	polymerase chain reaction
PD:	Parkinson's disease
PNS:	peripheral nervous system
PSQI:	Pittsburgh Sleep Quality Index
RD:	Refsum's disease
RHS:	Rotterdam Handicap Scale
SARA:	Scale for the Assessment and Rating of Ataxia
SCA:	spinocerebellar ataxia
SCAN:	SCA with axonal neuropathy
SF-36:	Short Form SF-36 health survey
SPECT:	single-photon-emission computed tomography
SSEP:	somatosensory evoked potentials
tRNA:	transfer-ribonucleic acid
VGCC:	voltage-gated calcium-channel
VGSC:	voltage-gated sodium channel
X-ALD:	X-linked adrenoleukodystrophy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Curriculum vitae

Esther Brusse was born on May 20th, 1970 in Aalten, The Netherlands. She attended the Christelijk Lyceum Almelo (Gymnasium β) and graduated in 1988. She started medical school at the Rijksuniversiteit Groningen and obtained her medical degree in June 1996. She started working as a resident at the department of Neurology at the Sint Elisabeth Hospital and Tweesteden Hospital in Tilburg, where she was subsequently trained as a neurologist under dr. C.C. Tijssen from 1998 to 2003. This included an 11- months residency in neurogenetics in 2001 at the Erasmus MC University Medical Center (prof. dr. F.G.A. van der Meché and prof. dr. P.J. Koudstaal), in co-operation with the department of Medical Genetics, and a three-months residency at the department of Neurosurgery of the Erasmus MC University Medical Center (prof. dr. C.J.J. Avezaat) in 2003.
Since 2004 she works as a neurologist at the Erasmus MC University Medical Center.
In 2006 she obtained a special qualification in neuromyology after a traineeship under supervision of prof. dr. P.A. van Doorn. Her expertise in neurology concerns neuromuscular disorders, neurogenetics and cerebellar ataxias. She is a member of the internal visitation committee of the Erasmus MC University Medical Center and of the website committee of the ISNO (Interuniveristair Steunpunt Neuromusculair Onderzoek).
She has recently started as the chairman of a quality-committee, initiated by the Dutch society for Neurology (Nederlandse Vereniging voor Neurologie), to develop a nationwide multidisciplinary guide on diagnosis and management of cerebellar ataxia.

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18. Nijssen PCG, **Brusse E**, Leyten ACM, Martin JJ, Teepen LJM, Roos, RAC. Autosomal dominant adult neuronal ceroid lipofuscinosis: parkinsonism due to both striatal and nigral dysfuction. *Movement Disorders 2002;17(3):482-487*.

PhD Portfolio Summary

Summary of PhD training and teaching activities

	Year	Workload (Hours/ECTS)
1. PhD training		
Research skills		
- EWP01 Introduction to Clinical research	2009	0.9
- EWP22 Biostatistics for Clinicians	2009	1.0
In-depth courses (e.g. Research school, Medical Training)		
- Biemond courses 2001, 2003-2009 (8×)		3.2
- Neuromuscular teaching course; WMS Bruges	2006	1.0
- Boerhaave Prinses Beatrix Fonds symposium neuromusculaire ziekten 2004-2010 (7×)		1.4
Presentations		
- Klinische kenmerken van een familie met spinocerebellaire ataxie, geassocieerd met een mutatie in het fibroblast growth factor 14 gen; 2e tweejaarlijks Nederlandstalig congres van de neurologie	2003	1.0
- A new locus for distal Hereditary Motor Neuronopathy maps to chromosome 16p. WMS Newcastle	2008	1.0
- Cerebellar ataxia, myopathy and sensorimotor neuropathy associated with a mitochondrial m.8363G>A tRNA(Lys) gene mutation: a clinical and biochemical study; WMS Geneva	2009	1.0
- Vermoeidheid, stemming en slapen bij ADCA; ADCA patiëntendag	2008, 2009	0.3
International conferences		
- International clinical symposium 'Epilepsy and Sleep Update'; Kempenhaeghe	2003	1.0
- International Symposium on Neuromuscular Diseases; Brussels	2005	1.0
- World Muscle Society (WMS); Gothenburg	2004	1.0
- World Muscle Society (WMS); Bruges	2006	1.0
- World Muscle Society (WMS); Giardini Naxos	2007	1.0
- World Muscle Society (WMS); Newcastle	2008	1.0
- World Muscle Society (WMS); Geneva	2009	1.0
Seminars and workshops		
- Belgisch-Nederlandse neuromusculaire studieclub 2004-2010		0.7
- ISNO retraite (2×)	2006, 2009	0.4
- Time-and stress management	2009	0.3

	Year	Workload (Hours/ECTS)
2. Teaching activities		
Lecturing		
- Het neurofysiologisch onderzoek bij neuromusculaire transmissiestoornissen; Boehaave Prinses Beatrix Fonds symposium neuromusculaire ziekten	2007	1.0
- Proximale spierzwakte; een richtlijn voor de praktijk; Boerhaave Prinses Beatrix Fonds symposium neuromusculaire ziekten	2010	1.0
- Annual training of skills in examination of hyperkinetic movement disorders for medical students; (5×)	2006-2010	1.0
- Annual training of skills in the examination of muscle weakness for medical students; (5×)	2006-2010	1.0
- Neuromuscular disorders for residents in Rehabilitation Medicine	2008	0.5
- Neuromuscular disorders for residents in Clinical Genetics	2009	0.5
Other		
- Reviewing papers for peer reviewed journals and reviewing research proposals applying for fund raising	2007-2010	2.0
- Chairman of the multidisciplinary Dutch committee developing a directive for diagnosis and management of adult onset cerebellar ataxia	2010	1.0
Total		27.2