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New Hereditary Ataxia-Disorders in Finland

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Contents

Abstract

During the last 15 years the causative mutations of many hereditary ataxias have been identified. However, adult-onset non-dominant ataxias are less well characterized. Despite comprehensive investigations, the majority of adult patients presenting with sporadic or apparently recessive ataxia have previously remained without a final genetic diagnosis. In this thesis, two distinct new types of non-dominant ataxias are clinically and genetically characterized. One of them, the mitochondrial recessive ataxia syndrome (MIRAS), proved to be the most common single hereditary ataxia disease in the Finnish population.

The scientific research of this thesis started with the unique clinical observations in patients showing recessive progressive adult-onset ataxia and bilateral thalamic lesions detected by MRI. This ataxia was found to be caused by recessive mutations in the *POLG1* gene, encoding mitochondrial polymerase gamma. Further explorations revealed other clinical features of these patients, that together with long term follow-up, completed the phenotype of the MIRAS disease: adult- or juvenile onset spinocerebellar type ataxia combined with sensory neuropathy, late cognitive impairment, oculomotor defects, myoclonus, tremor, psychiatric symptoms, and seizures.

Because the carrier frequency for the W748S in the *POLG1* gene proved to be very high in the Finnish population, and some family members reported neurological symptoms, we also investigated possible disease manifestations in heterozygote carriers belonging to the large original MIRAS family. Sensory neuropathy was a frequent subclinical finding, but definite clinical signs were not determined.

In our clinical cohort of non-dominant adult-onset patients we also identified a heteroplasmic 8993T>C mitochondrial DNA mutation underlying a second new distinct ataxia entity presented in this thesis. This is another adult-onset slowly progressive spinocerebellar type ataxia with axonal polyneuropathy, and without mitochondrial histochemical abnormalities in skeletal muscle.

Appropriate genetic diagnosis is important for the individual patients and their families and helps addressing the prognosis, genetic counselling and possible therapeutic implications of the disease. This research effort has improved the diagnostic strategy and accuracy for adult ataxia patients in Finland. However, there are still unsolved genetic backgrounds that will need further research in order to find additional causes for non-dominant adult-onset ataxia.

Aikuisiässä alkavan ataksian uusia perinnöllisiä syitä

Perinnölliset ataksiat ovat keskushermoston sairauksia, jotka johtuvat pikkuaivojen, aivorungon ja/tai selkäytimen asentotuntoa välittävien ratayhteyksien etenevästä vauriosta ja joiden merkittävin oire on tasapainohäiriö. Ataktisen kävelyn ja liikuntakyvyn heikkenemisen lisäksi sairastuneilla voi esiintyä dysartrista puhevaikeutta, silmien liikehäiriöitä kuten nystagmusta, raajojen koordinaatiovaikeuksia ja vaihtelevasti myös muita hermostoperäisiä oireita ja löydöksiä, kuten polyneuropatiaa. Aikuisalkuiset resessiivisesti periytyvät tai sporadisena ilmenevät ataksiat muodostavat puutteellisesti kuvatun sairausryhmän niin suomalaisväestössä kuin maailmanlaajuisestikin. Ataksian spesifinen diagnoosi jää usein puuttumaan ja samalla myös täsmällinen tieto sairauden laadusta, ennusteesta ja sen periytyvyydestä jälkipolville. Sairauksien geenivirheen löytyminen on tärkeä lähtökohta degeneratiivisten aivosairauksien patogeneettisten mekanismien ymmärtämiseksi ja hoidon kehittämiseksi.

Kliinisessä neurologisessa työssä havaitsimme useita tapauksia ja perheitä, joiden ataksian kuva ei sopinut tiedossa oleviin tauteihin, ja diagnoosi jäi avoimeksi huolimatta erittäin laajoista tutkimuksista. Väitöskirjatutkimuksessa kuvattiin kliinisesti kaksi uutta perinnöllisen ataksian tautimuotoa ja selvitettiin niiden molekyyligeneettinen syy. Toinen niistä – MIRAS-ataksia (Mitochondrial recessive ataxia syndrome) – on osoittautunut yleisimmäksi perinnölliseksi ataksiaksi suomalaisväestössä ja yleiseksi Pohjois-Euroopassa. Tutkimustulosten avulla voitiin huomattavasti parantaa aikuisiässä alkavan perinnöllisen ataksian taudinmäärittelyn mahdollisuuksia Suomessa ja maailmalla.

MIRAS-ataksian löytymiseen johtaneet tutkimukset käynnistyvät eteläpohjalaisesta perheestä vuonna 1997. Kolme sisarusta viidestä oli 30 vuoden iässä peräjälkeen sairastunut etenevään ataksiasairauteen. Kuvasimme uuden sairauden kliinisen ilmiasun sisältäen neurologisten oireiden ohella myös taudin neuroradiologiset ja neuropatologiset morfologiset löydökset, neuropsykologiset ja oftalmologiset piirteet sekä systemaattisen laboratoriotutkimusten ja geneettisten analyysien kulun. Molekyyligeneettisen tutkimuksen osalta pääsimme läpimurtoon vuonna 2003; sairauden syyksi löytyi tuman *POLG1*-geenistä uusi mutaatio W748S. Kyseisen geenivirheen seurauksena mitokondrioiden DNA:ta monistavaan polymeraasi gamma -entsyymiin (POLG) tulee häiriö, jonka seurauksena mitokondrioiden oma DNA-aines alkaa vioittua. Suomalaisten ja muiden eurooppalaisten MIRAS-potilaiden jatkotutkimukset täydensivät kuvaamme sairauden kliinisistä piirteistä: MIRAS on yleensä aikuis- tai nuoruusalkuinen spinoserebellaariataksia, johon liittyy tuntohermo-

jen neuropatia ja mahdollisina lisäpiirteinä silmien liikehäiriöt, myoklonus, vapina, epileptiset kohtaukset, psykiatriset oireet ja myöhäisvaiheessa kognitiivisen tason heikkeneminen.

MIRAS-ataksian geenivirhe periytyy kuvatuissa perheissä resessiivisesti, joten heterotsygootit mutaation kantajasukulaiset ovat terveitä. Kuitenkin osa ataksiapotilaiden sukulaisista oli huolissaan havaitsemistaan puutumis- ja kömpelyystuntemuksistaan ja pienellä osalla sukulaisista oli diagnosoitu neurologisia sairauksia. Kantajatutkimuksessa osalla mutaation kantajilla voitiinkin osoittaa subkliininen tuntohermojen neuropatia. Lisäksi osalla tutkituista havaittiin aivojen PET-kuvauksissa hypometabolia-alueita, minkä vuoksi laajemmat jatkotutkimukset ovat tarpeen löydöksen merkityksen selvittämiseksi. Kliinisiä tautilöydöksiä mutaation kantajilta ei kuitenkaan ollut todettavissa, mikä on tärkeä tieto perinnöllisyysneuvonnan kannalta. Kantajasukulaisten mahdollinen lieväkin neurologinen sairastumisalttius on merkittävä jatkokysymys geenivirheen yleisyyden vuoksi – suomalaisväestössä on noin 40 000 MIRAS-mutaation kantajaa.

Toinen tutkimuksessa kuvattu perinnöllinen ataksia – Neuropatia-ataksia 8993T>C – löytyi nelilapsisesta pohjalaisperheestä. Perheen yhdellä 1960-luvulla menehtyneellä lapsella oli diagnosoitu Friedreichin ataksia obduktiotutkimuksella. Perheen kolme muuta lasta olivat täysin terveitä, kunnes heille aikuisiässä kehittyi etenevän ataksian ja/tai polyneuropatian oireisto. Friedreichin ataksian geenitesti jäi negatiiviseksi. Erikoisen periytymistavan vuoksi päädyttiin koko mitokondriaalisen DNA:n sekvensointiin, jolloin löytyi harvinainen mutaatio 8993T>C, ensimmäistä kertaa aikuisalkuisen ataksian ja polyneuropatian syynä. Havainnon julkaisemisen jälkeen kyseistä ataksiaa on löydetty myös muualta.

Abbreviations

AOA   ataxia with oculomotor apraxia

ARSACS   autosomal recessive spastic ataxia of Charlevoix-Saguenay

AT   ataxia telangiectasia

ATP   adenosine triphosphate

AVED   ataxia with vitamin E deficiency

BAEP   brainstem auditory-evoked potential

CK   creatine kinase

CNS   central nervous system

COX   cytochrome *c* oxidase

CSF   cerebrospinal fluid

CT   computed tomography

DNA   deoxyribonucleic acid

DRPLA   dentatorubral-pallidolluysian atrophy

EEG   electroencephalogram

EMG   electromyography

ENG   electronystagmography

FDG   fluorodeoxyglucose

FRDA   Friedreich's ataxia

FRDA1   the gene encoding frataxin, a mitochondrial protein

FXTAS   fragile X-associated tremor/ataxia syndrome

IOSCA   infantile onset spinocerebellar ataxia

KSS   Kearns-Sayre syndrome

LHON   Lebers's hereditary optic neuropathy

MELAS   mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes

MERRF   myoclonus epilepsy with ragged red fibers

MIRAS   mitochondrial recessive ataxia syndrome

MNGIE   mitochondrial neurogastrointestinal encephalomyopathy

MRI   magnetic resonance imaging

mtDNA   mitochondrial DNA

NARP   neuropathy, ataxia and retinitis pigmentosa

NCV   nerve conduction velocity

OXPHOS   oxidative phosphorylation

PCR   polymerase chain reaction

PEO   progressive external ophthalmoplegia

PET   positron emission tomography

POLG   polymerase gamma, the mitochondrial DNA polymerase

POLG1 the gene encoding for the catalytic subunit of POLG

polyQ   polyglutamine

RNA   ribonucleic acid

RRF   ragged red fibers

SANDO   sensory ataxic neuropathy, dysarthria and ophthalmoparesis

SCA   dominant spinocerebellar ataxia

SCAN1   spinocerebellar ataxia with axonal neuropathy

SDH   succinate dehydrogenase

SEP   somatosensory-evoked potential

Twinkle mitochondrial DNA helicase

VEP   visual evoked potential

List of the original publications

This thesis is based on the following original publications, which are refereed to in the text by their Roman numerals.

I   Rantamäki M, Krahe R, Paetau A, Cormand B, Mononen I and Udd B (2001): Adult-onset autosomal recessive ataxia with thalamic lesions in a Finnish family. Neurology 57:1043-1049.

II   Van Goethem G, Luoma P*, Rantamäki M*, Al Memar A, Kaakkola S, Hackman P, Krahe R, Löfgren A, Martin JJ, De Jonghe P, Suomalainen A, Udd B and Van Broeckhoven C (2004): *POLG* mutations in neurodegenerative disorders with ataxia but no muscle involvement. Neurology 63:1251-1257.

III   Rantamäki M, Luoma P, Virta JJ, Rinne JO, Paetau A, Suomalainen A and Udd B (2007): Do carriers of POLG mutation W748S have disease manifestations? Clin Genet 72:532-537.

IV   Rantamäki MT, Soini HK, Finnilä SM, Majamaa K and Udd B (2005): Adult-onset ataxia and polyneuropathy caused by mitochondrial 8993T→C mutation. Ann Neurol 58:337-340.

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Data included also from the related publication:

Hakonen AH, Heiskanen S, Juvonen V, Lappalainen I, Luoma PT, Rantamäki M, Van Goethem G, Löfgren A, Hackman P, Paetau A, Kaakkola S, Majamaa K, Varilo T, Udd B, Kääriäinen H, Bindoff LA and Suomalainen A (2005): Mitochondrial DNA polymerase W748S mutation: a common cause of autosomal recessive ataxia with ancient European origin. Am J Hum Genet 77:430-441.

1 Introduction

The hereditary ataxias comprise a heterogenous group of neurological disorders, which all taken together result in a considerable health burden. The widely variable symptoms and signs, often overlapping with extracerebellar neurological involvement, complicate clinical assessment and cause diagnostic challenges for the neurologist. During the past 15 years a number of new genetic causes have emerged that increase the diagnostic spectrum and accuracy of ataxias. At the same time important advances have been made to uncover the molecular pathology of these disorders, and this ongoing process will open the window to understand and eventually to correct the abnormalities. Among the autosomal recessive ataxias a number of subgroups can be distinguished. Although most of them are early-onset forms, the differential diagnostic alternatives in the adult patient are still numerous. If the initial examinations fail to reveal the exact cause, the further investigation of these patients by a systematic approach is very demanding. Thus, providing guidelines for genetic testing based on clinical findings and frequencies in different ethnic groups is a major task. Appropriate genetic diagnosis is of utmost importance for the individual patients and their families and helps addressing the prognosis, genetic counselling and possible therapeutic implications of the disease.

The most common hereditary ataxia worldwide, Friedreich's ataxia, is an extremely rare disorder in Finland. Despite comprehensive investigations, the majority of our adult patients presenting with sporadic or apparently recessive ataxia have previously remained without a final genetic diagnosis. The aim of this study was to clinically and genetically characterize new types of adult-onset ataxias and develop a strategy for the diagnostic investigations in adult ataxia patients in Finland, with emphasis on the non-dominant genetic disorders.

2 Review of the literature

Ataxia, a medical term originated from the Greek language meaning "without order", refers to disturbances in the control of body posture and motor coordination (Berciano et al. 2000). Loss of balance and coordination can be debilitating for patients and a diagnostic dilemma for clinicians. The phenotype of progressive spinocerebellar ataxia can result from both acquired and hereditary disorders. In some patients, a symptomatic cause can be identified such as excessive alcohol consumption, drug or toxin exposure, stroke or multiple sclerosis. When potential acquired causes have been excluded, the differential diagnosis relies on the different genetic causes. The expanding number of genes causing spinocerebellar ataxia has created novel challenges for the clinician to define the most efficient diagnostic approach. Certain clinical characteristic features and ethnic predilection of some of the ataxias may help specific gene testing priorities (Pulst 2003). On the whole, a specific aetiology can be determined in more than half the patients presenting with familial ataxia and, approximately, in a third of those with sporadic progressive ataxia (Perlman 2003). For the individual patients and their families, it is very important to have a definite diagnosis. This provides insight into disease prognosis, enables adequate genetic counselling and may have implications for therapy or preventive screening in family members.

Friedreich's ataxia (FRDA) is the commonest form among the autosomal recessive ataxias in most Caucasian populations (Harding 1983, Cossée et al. 1997, Epplen et al. 1997, Pandolfo 2003, Fogel and Perlman 2007). However, due to the specific historic population background, FRDA ataxia is very rare in Finland, with only 5–6 families diagnosed despite extensive genetic diagnostic evaluations (Juvonen et al. 2002). This basic fact and the occurrence of families with distinct new phenotypes of ataxia in our clinics formed the incentives to search for new genes in Finnish non-dominant ataxia patients.

2.1 Clinical approach to ataxic patients

2.1.1 Defining the clinical phenotype

The key feature is ataxia, involving anatomically the cerebellum, brainstem, and/or spinocerebellar long tracts and/or peripheral sensory afferences. The patients usually exhibit a slowly progressive syndrome with various combinations of oculomotor disorders, nystagmus, dysarthria, dysmetria/kinetic tremor, and ataxia of gait or posture control. Peripheral neuropathy may further contribute to imbalance and incoordination of movements. A number of additional signs may occur in syndromic ataxias, such as pigmentary retinopathy, extrapyramidal movement disorders (parkinsonism, dyskinesias, dystonia, chorea), pyramidal signs, cortical symptoms (seizures, cognitive impairment/behavioural symptoms) and in some cases, extraneural multisystem signs such as cardiac or muscle pathology (Perlman 2003). In many degenerative ataxia syndromes features of cerebellar and proprioceptive defects occur in variable combinations. Sensory ataxia is distinguished from cerebellar ataxia by the presence of near-normal coordination when the movement can be visually observed by the patient, but marked worsening of incoordination when the eyes are closed or in darkness. Sensory ataxia also lacks associated features of cerebellar ataxia such as scanning dysarthria, nystagmus, broken pursuit eye movements and saccadic dysmetria (Mariotti et al. 2005).

The known genetic causes can be subdivided regarding age of onset, those starting before the age of two years (infantile), and those with early (< 25 years) or late (> 25 years) onset. The majority of the defined autosomal recessive disorders start in childhood, adolescence, or early adulthood. In contrast, most sporadic and acquired ataxias have a late onset. Many sporadic ataxia disorders with early onset are in fact manifestations of an autosomal recessive disorder (Klockhether 2000).

2.1.2 Family history

An accurate pedigree is extremely important. In patients with positive family history a detailed pedigree will often reveal the mode of inheritance. However, even if there are no family members with the same phenotype, a detailed pedigree is important because, as for some mitochondrial disorders, the underlying gene defect may cause considerable variability of clinical manifestations. The absence of a family history does not exclude a genetic diagnosis. In different studies, a genetic explanation has been clarified in 2–22% of patients with "sporadic ataxia", most frequently representing FRDA (Futamura et al. 1998, Schöls et al. 2000, Abele et al. 2002, Kerber et al. 2005). Autosomal recessive disorders very often present as sporadic diseases from non-consanguinous marriages. Even autosomal dominant spinocerebellar ataxias (SCAs) may present

as an apparently sporadic or recessive disease: 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, or the mutation can occur *de novo* (Schöls et al. 2004). On the other hand, recessive disorders may look like dominant diseases in case of pseudo-dominance in populations with high degree of remote consanguinity, such as the rural Finnish population (Norio 2003). Specific phenotype features of the patients and the family may suggest mitochondrial ataxia, often associated with other manifestations of mitochondrial disease (Gropman 2004, Di Donato 2009). Mitochondrial diseases have all kinds of different modes of inheritance because mutations may originate in the nuclear or the mitochondrial DNA (mtDNA). Furthermore, maternally inherited mtDNA mutations may mimic autosomal dominant, recessive or X-linked inheritance due to phenomena such us heteroplasmy and threshold effects for biochemical activity in relation to expression levels of the mutant protein. Moreover, large single deletions and insertions in mtDNA are often sporadic *de novo* mutations (Brusse et al. 2007).

2.1.3 Neuroimaging

Brain computed tomography (CT) or magnetic resonance imaging (MRI) identify structural lesions that may be the cause of symptomatic ataxias. In degenerative ataxic disorders, T1-weighted MRI images accurately reveal the degree of atrophy, and T2-weighted images are used to evaluate signal changes (Wüllner et al. 1993, Ormerod et al. 1994). Decreases in perfusion or glucose metabolism in the cerebellum, brainstem, cerebral cortex, and other brain regions may precede structurally visible atrophy. Positron emission tomography (PET) scanning can be used to identify abnormalities even at the stage of a still normal MRI scan (Soong and Liu 1998).

Significant cervical spinal cord atrophy instead of cerebellar atrophy is a characteristic finding in FRDA. However, in advanced stages atrophy of vermis and brainstem, as well as generalized cerebral atrophy, have been reported (Bhidayasiri et al. 2005). Cerebellar atrophy is a common finding in many recessive disorders such as in ataxia telangiectasia (AT) and in ataxia with oculomotor apraxia type 1 and 2 (AOA 1 and 2) (Fogel and Perlman 2007). In the autosomal dominant cerebellar ataxias three patterns of atrophy can be identified: pure cerebellar atrophy, olivopontocerebellar atrophy and global brain atrophy (Schöls et al. 2004). Abnormal signal intensity of transverse pontine fibres has been reported in patients with SCA2 and other spinocerebellar ataxias (Wüllner et al. 1993, Murata et al. 1998, Giuffrida et al. 1999). In some patients with dentatorubral-pallidolluysian atrophy (DRPLA), high-intensity signals on T2-weighted images have also been detected in the brainstem and in the thalamus, in addition to diffuse high-intensity signal areas in cerebral white matter (Koide et al. 1997). Typical brain MRI findings in mitochondrial disorders consist of supra- and infratentorial atrophy, focal or widespread hyperintense signal abnormalities in white matter, in deep gray nuclei, and in the brainstem (Kendall 1992, Bianchi et al. 2007). Increased signal intensities of the

middle cerebellar peduncles are a distinctive and common feature of the fragile X-associated tremor/ataxia syndrome FXTAS (Brunberg et al. 2002). All the leukodystrophies also show MRI white matter lesions in the brain and/or in the medulla/spinal cord (Schiffmann and van der Knaap 2004).

2.1.4 Neurophysiology

Hereditary ataxias are frequently associated with peripheral axonal neuropathy, most notably with loss of proprioception and vibration sense, as seen in the prototypical disorder, Friedreich's ataxia (Geoffroy et al. 1976, Harding 1981, Santoro et al. 1999). Conditions notable for the absence of a prominent neuropathy include SCA6 (in most cases), SCA7 and DRPLA (Schöls et al. 2004). Chronic denervation on electromyography (EMG) similar to motor neuron disease is the frequent finding in juvenile or adult-onset hexosaminidase A deficiency (Neudorfer et al. 2005). Patients with mitochondrial disorders may have myopathic EMG findings and mainly sensory axonal neuropathy (Nardin and Johns 2001). Those with paraneoplastic cerebellar degeneration and anti-Hu antibodies may also have prominent sensory neuronopathy even as the first clinical sign (Graus et al. 2004). External sphincter EMG and autonomic function tests may be useful in the diagnosis of the multiple system atrophy (Wenning et al. 2004), and findings on electroencephalogram (EEG) are indicative in the diagnosis of both sporadic and familial prion disease (Eggenberger 2007).

2.1.5 Establishing the diagnosis of hereditary ataxia

The differential diagnosis of hereditary ataxias includes acquired, non-genetic causes of ataxia, attributed to various toxic, vascular, inflammatory, paraneoplastic, endocrinal, or malabsorption conditions. Ruling out the possibility of an acquired cause needs to be considered in each individual with sporadic ataxia because specific treatment may be available (Brusse et al. 2007). Neuroimaging and electrophysiological studies can provide additional features to the observed phenotype and thus indicate some etiological possibilities (Perlman 2003). The genetic diagnosis is confirmed by finding a positive family history, a clinical phenotype characteristic of a genetic form of ataxia, and finally by a positive mutation analysis when the causative gene is identified.

2.2 Classification of inherited ataxias

2.2.1 Historical aspects of ataxia research

Friedreich's ataxia was described by the German pathologist Nicolaus Friedreich in 1863. He was the first to describe patients with a hereditary form of ataxia showing that this classic representative of autosomal recessive ataxia was a distinctive clinical syndrome. "Degenerative atrophy of the posterior columns of the spinal cord" occurred in several members of the same kindred and caused progressive ataxia, sensory loss, muscle weakness often associated with scoliosis, foot deformity, and heart disease. Friedreich's proposition, that the disorder he described was a distinct entity, initially met with considerable opposition. Charcot considered that Friedreich's patients suffered from multiple sclerosis, but two years after the death of Nicolaus Friedreich, he eventually conceded the existence of FRDA (Harding 1984, Pearce 2004).

For a number of decades FRDA and other forms of inherited ataxias have been subjected to debate about their clinical definition and classification. The problem of a rational nosologic classification of the degenerative ataxias has haunted clinicians for about 150 years. Traditionally, classification of ataxias was based on neuropathologic criteria. Thus, Holmes distinguished between olivipontocerebellar atrophy (OPCA), spinocerebellar degeneration, and primary parenchymatous degeneration of the cerebellum. Under each category, Holmes included cases with different modes of inheritance and varying clinical phenotypes. Holmes' neuropathologic classification was further developed by Greenfield, who divided the various types of degeneration into spinal, spinocerebellar and cerebellar forms (Berciano et al. 2000).

2.2.2 Harding's classification

In 1983 Harding introduced a new clinical classification of hereditary ataxias, based on known pathogenesis and on the age of onset, which takes credits for defining phenotypes that have been essential for the molecular genetic linkage studies. She divided the hereditary ataxias in four categories: congenital, metabolic, associated with defective DNA repair, and degenerative, the last group being the largest. The degenerative ataxias were further clinically classified, according to the onset below or after 25 years. In this classification the disorders with early onset were mainly autosomal recessive and this category comprised FRDA and the early-onset cerebellar ataxias with specific features, in particular those with retained reflexes. The proposed classification of late-onset autosomal dominant forms distinguished three types of spinocerebellar ataxias (ADCA): ADCA type I is ataxia plus impairment of other neuronal systems, ADCA type II is ataxia with retinal degeneration and ADCA type III was described as "pure" cerebellar ataxia (Harding 1983). Ashizawa

and Pulst have suggested adding a fourth type, which would include ataxia combined with epilepsy (Pulst 2003). Many presently known SCAs with repeat expansion mutations may be assigned to one of these categories if the neurologist can observe the full-blown disease phenotype.

2.2.3 Pathogenetic classification

Efforts to classify the hereditary ataxias by their clinical and neuropathological phenotypes are troubled by excessive heterogeneity, and neither the clinical features nor the findings at autopsy provide a satisfactory basis for selection of distinct categories and classification. Recently, the group of Filla (Table 1, modified after De Michele et al. 2004) suggested a pathogenetic approach to classify hereditary ataxias, and divided the disorders in mitochondrial ataxias (including FRDA), metabolic ataxias, ataxias associated with defective DNA repair, ataxias with abnormal protein folding and degeneration, ataxias caused by channelopathies, and a miscellaneous group with unknown pathogenetic mechanism.

Table 1. Classification of hereditary ataxias based on the pathogenetic mechanis according to the group of Filla (De Michele et al. 2004).

Classification of hereditary ataxias according to pathogenetic mechanism

Mitochondrial
- Nuclear DNA (Friedreich's ataxia, IOSCA, MIRAS)
- Mitochondrial DNA

Metabolic
- Urea cycle disorders
- Amino acid disorders
- Pyruvate disorders
- Vitamin E disorder
- Lipid disorders
- Storage disorders
- Peroxisomal disorders

Defective DNA repair
- Ataxia telangiectasia
- Ataxia with oculomotor apraxia 1
- Ataxia with oculomotor apraxia 2
- Spinocerebellar ataxia with axonal neuropathy
- Xeroderma pigmentosum
- Cockayne syndrome

Abnormal protein folding and degradation
- Autosomal recessive spastic ataxia Charlevoix-Saquenay type
- Polyglutamine disorders (SCA1, 2, 3, 6, 7, 17, DRPLA)
- Marinesco-Sjögren syndrome
- Prion protein disorders

Channelopathies
- Episodic ataxia type 1
- Episodic ataxia type 2

Others
- Congenital cerebellar ataxias
- Early onset cerebellar ataxia with retained reflexes (EOCA)
- Progressive myoclonus ataxias
- Dominant ataxias caused by untranslated expansions, point mutations or other mutation types (SCA 5, 8, 10, 11, 12, 13, 14, 15)
- Other dominant ataxias with identified loci
- Fragile X-associated tremor/ataxia syndrome (FXTAS)

2.2.4 Molecular genetic classification

Molecular genetic progress over the last decade has introduced a new classification of the autosomal dominant ataxias, consisting of at least 28 different types (Table 2, modified after Schöls et al. 2004, Soong and Paulson 2007, Schmitz-Hübsch and Klocgether 2008, Carlson et al. 2009). In the last few years autosomal recessive ataxias have increasingly been investigated and proven to be genetically heterogenous (Table 3, modified after Fogel and Perlman 2007). The genes involved have functions in metabolic or other fundamental cellular processes such as DNA repair, protein folding, or RNA processing. Altered biological pathways mediated by mutant proteins include protein aggregation and clearance, ubiquitin-proteosome system, alterations of calcium homeostasis and activation of pro-apoptotic routes, all of which may be key events in the pathogenesis of neuronal damage (van de Warrenburg et al. 2005, Dueñas et al. 2006, Soong and Paulson 2007, Carlson et al. 2009).

Table 2. Summary of autosomal dominant spinocerebellar ataxias.

Disease	Gene	Locus	Protein	Mean age at onset (range)	Characteristic signs	Geographic distribution
SCA1	*ATXN1*	6p23*	Ataxin-1	37 (4–74)	Pyramidal and extrapyramidal findings, ophtalmoplegia, peripheral neuropathy, executive dysfunction	Relatively common worldwide
SCA2	*ATXN2*	12q24*	Ataxin-2	32 (1-65)	Slow saccades, peripheral neuropathy, hyporeflexia, titubation, dementia, less frequent extrapyramidal findings	Relatively common worldwide, rare in Finland
SCA3 Machacho-Joseph disease	*ATXN3*	14q32*	Ataxin-3; Machado Joseph disease (MJD1) protein 1	36 (5–70)	Pyramidal, extrapyramidal and amyotrophic signs, peripheral neuropathy, lid retraction, diplopia	Most common type worldwide, rare in Finland
SCA4	*PLEKHG4*	16q22	Puratrophin-1	? (19–72)	Sensory axonal neuropathy, pyramidal signs	Families in the US, Japan and Germany
SCA5	*SPTBN2*	11p13	Spectrin beta chain, brain 2	30 (10–68)	Cerebellar syndrome, bulbar signs, slow course	Families in the US and Germany
SCA6	*CACNA1A*	19p13*	Voltage-dependent P/Q-type calcium channel alpha-1A subunit	52 (30–71)	Cerebellar syndrome, pyramidal signs, occasional sensory loss, normal life expectancy, lack of family history, late onset > 50	Relatively common, rare in Finland
SCA7	*ATXN7*	3p14*	Ataxin 7	35 (0–70)	Retinal degeneration, ophthalmoparesis, slow saccades, variable pyramidal signs	Relatively common worldwide
SCA8	*KLHL1AS*	13q21**	KLHL (Kelch-like) 1AS	40 (1–73)	Late spasticity, tremor, mild sensory neuropathy; caution in genetic testing	Relatively common worldwide, the most frequent dominant type in Finland
SCA9	–	Category not assigned	–	–	–	–
SCA10	*ATXN10*	22q13***	Ataxin-10	36 (26–45)	Frequent seizures, neuropathy	Mexican and Brazilian families
SCA11	*TTBK2*	15q15.2	Tau tubulin kinase-2	25 (15–43)	Pure cerebellar syndrome; rarely hyperreflexia, benign course	2 British families

Disease	Gene	Locus	Protein	Mean age at onset (range)	Characteristic signs	Geographic distribution
SCA12	*PPP2R2B*	5q32*	Brain-specific regulatory subunit of protein phosphatase 2A	35 (8–55)	Early tremor, late dementia, occasional dystonia, hyperreflexia	German family, common in India
SCA13	*KCNC3*	19q13	Kv3.3	Childhood (< 1–45)	Occasional mental retardation and delayed motor development, hyperreflexia, slow progression	French and Philipino families
SCA14	*PRKCG*	19q13.4	Protein kinase C, gamma subtype	27 (12–42)	Myoclonus or tremor with early onset, facial myokymia, dystonia, vibratory loss, late onset can be pure ataxia, slowly progressive	Families: Japanese, English, Dutch, French
SCA15	*ITPR1*	3p26	IP3-gated calcium channel	26 (10–50)	Cerebellar syndrome, slowly progressive	British, Australian and Japanese families
SCA16	allelic to SCA15			40 (20–66)	Cerebellar syndrome, head tremor, mental dysfunction, slowly progressive	Japanese families
SCA17	*TBP*	6q27*	TATA-box binding-protein	33 (6-48)	Dementia, extrapyramidal signs, slow saccades, epilepsy, dystonia, chorea, psychosis, widespread cerebral and cerebellar atrophy	French, Japanese, Italian and German families
SCA18	–	7q22–q32	–	15 (12–25)	Sensorimotor neuropathy	One American family of Irish ancestry
SCA19	–	1p21–q21	–	34 (11–45)	Cognitive impairment, hyporeflexia, occasional tremor and myoclonus	Dutch family
SCA20	–	11p13–q11	–	46 (19–64)	Early dysarthria, dystonia, dentate calcification on CT	Anglo-Celtic family in South-Eastern Australia
SCA21	–	7p21.3–p15.1	–	18 (7–30)	Cognitive defects, hyporeflexia, extrapyramidal features	French family
SCA22	–	1p21–q21	–	? (10–46)	Pure cerebellar syndrome, slow progression, hyporeflexia	Chinese Han family
SCA23	–	20p30–12.3	–	? (40–60)	Vibration loss, slow progression	Dutch family
SCA24	reserved	–	–	–	–	–

Disease	Gene	Locus	Protein	Mean age at onset (range)	Characteristic signs	Geographic distribution
SCA25	–	2p21–p13	–	? (1–39)	Severe sensory neuropathy, gastrointestinal symptoms	Southern French family
SCA26	–	19p13.3	–	–	Pure cerebellar syndrome	Norwegian (North Dakota) family
SCA27	*FGF14*	13q34	Fibroblast growth factor 14	11 (7–20)	Tremor, orofacial dyskinesias, psychiatric symptoms, cognitive deficits	Dutch family
SCA28	–	18p11.22–q11.2	–	–	Ophthalmoparesis, hyperreflexia	Italian family
SCA29	–	3p26	–	–	Nonprogressive ataxia, cognitive disability, vermian hypoplasia	–
SCA 30	–	4q34.3–q35.1	–	52 (45–76)	Pure cerebellar syndrome	Australian family of Anglo-Celtic entnicity
DRPLA	*DRPLA*	12p13.31*	Atrophin-1-related protein	30 (0–62)	Onset < 20 years with myoclonus, epilepsy; onset > 20 years with chorea, dementia, psychosis, often confused with Huntington disease	Common in Japan

mutation types:

* CAG repeat expansion

** CTG repeat expansion

*** ATTCT repeat expansion

Table 3. Autosomal recessive ataxias with identified gene defects.

Disease	Gene	Locus	Protein (Protein function)	Typical age at onset (range**)**	Distinctive clinical features	Geographic distribution
Friedreich's ataxia and similar disorders						
Friedreich's ataxia	*FRDA1*	9q13 (GAA repeat expansion)	Frataxin (mitochondrial iron metabolism)	8–15 (0–60)	Scoliosis, pes cavus, hyporeflexia, positive Babinski sign, deep sensory loss, cardiomyopathy, impaired glucose tolerance	Worldwide except North-East Asia and Sub-Saharan black population; rare in Finland: overall carrier frequency 1:500
Ataxia with vitamin E deficiency (AVED)	*TTPA*	8q13.1–13.3	α-tocopherol transfer protein (vitamin E homeostasis)	usually < 20 (3–52)	Similar to FRDA, characteristic head titubation, rarely cardiomyopathy; low vitamin E (< 10% of normal)	Worldwide, especially North-Africa, Mediterranean
Abetalipoproteinemia	*MTP*	4q22–24	Microsomal trygliceride transfer protein (lipoprotein metabolism)	2–17	Steatorrhea, areflexia, retinal degeneration; acanthocytosis, reduced serum LDL, low vitamin A, E and K	Worldwide, very rare
Refsum's disease (Heredopathia atactica polyneuritiformis)	*PHYH*	10qter–11.2	Phytanoyl-CoA hydroxy-lase (fatty-acid oxidation)	late childhood	Retinitis pigmentosa, deafness, polyneuropathy, cardiomyopathy; increased phytanic acid	Northern and Western Europe, especially Scandinavia
	PEX7	6q21-22.2	Peroxisomal biogenesis factor-7 (peroxisomal protein importation)			
Friedreich's ataxia-like disorders with cerebellar atrophy						
MIRAS (Mitochondrial recessive ataxia syndrome)	*POLG1*	15q22–26	DNA polymerase γ (mitochondrial DNA repair and replication)	juvenile or adult	Sensory axonal neuropathy, cognitive decline, seizures, myoclonus, migraine, late-onset ophtalmoplegia, minimal cerebellar atrophy at early stages	Europe, USA, Australia, and New Zeeland; prevalent in Finland and Norway with carrier frequencies 1:125 and 1:100
Spinocerebellar ataxia with axonal neuropathy (SCAN1)	*TPD1*	14q31–32	Tyrosyl-DNA phosphodi-esterase 1 (DNA repair)	adolescence	Axonal sensorimotor neuropathy, hypoalbuminaemia and high cholesterol levels	Saudi Arabian family
Cerebrotendinous xanthomatosis	*CYP27*	2q33-ter	Sterol 27-hydroxylase (bile acid synthesis)	After puberty (infancy – adulthood)	Axonal polyneuropathy, spasticity, dementia, tendon xanthomas, cataracts; increased serum/urine cholestanol and urine bile alcohols	A few hundred reported cases worldwide, clusters in Japan, in the Netherlands and in Israel

Disease	Gene	Locus	Protein (Protein function)	Typical age at onset (range)	Distinctive clinical features	Geographic distribution
Early-onset ataxia with cerebellar atrophy						
Ataxia telangiectasia (AT)	*ATM*	11q22–23	Phosphatidylinositol 3-kinase-type enzyme (DNA damage response)	1–6 (0–20)	Telangiectasias, immune deficiency, cancer, chromosomal instability; increased serum α-fetoprotein	Worldwide, rare in Finland (< 10 diagnosed cases)
Ataxia with oculomotor apraxia type 1 (AOA 1)	*APTX*	9q13	Aprataxin (DNA repair, possible RNA processing)	2–6 (2–18)	Oculomotor apraxia, choreoathetosis, cognitive impairment and learning difficulties, areflexia, flexor plantars, axonal sensorimotor neuropathy; hypoalbuminaemia and high cholesterol levels	Worldwide, especially Japan and Portugal
Ataxia with oculomotor apraxia type 2 (AOA 2)	*SEXT*	9q34	Senataxin (possible DNA repair, DNA transcription, or RNA processing)	11–22 (2–22)	Oculomotor apraxia, mild choreoathetosis and dystonia, absent lower limb reflexes, sensory neuropathy, extensor plantars; high serum α-fetoprotein	Possibly the second most common recessive ataxia after FRDA in European population, frequency in Finland unknown
Autosomal recessive spastic ataxia of Charlevoix-Saquenay (ARSACS)	*SACS*	13q11	Sacsin (possible protein folding)	1–20	Spasticity and hyperreflexia, peripheral neuropathy	French-Canadians (Quebec), also described in Europe, Eurasia, North Africa, and Japan
IOSCA (Infantile onset spinocerebellar ataxia)	*C10orf2*	10q24	Mitochondrial proteins Twinkle and Twinky (DNA replication)	9–18 months	Sensory axonal neuropathy, areflexia, hypotonia, optic atrophy, ophthalmopegia, hearing loss, involuntary movements, seizures	Finland: overall carrier frequency 1:200 (in Tampere region 1:50)
Marinesco-Sjögren syndrome	*SIL1*	5q31	SIL1 protein (possible protein folding)	infancy	Mental retardation, cataracts, short stature associated with hypogonadotropic hypogonadism, skeletal deformities, myopathy with rimmed vacuoles, muscle weakness and atrophy, peripheral neuropathy, epilepsy	Worldwide, Finnish patients: homozygous tetranucleotide duplication 506_509dupAAGA in exon 6, carrier frequency 1:96
Cayman ataxia	*ATCAY*	19p13.3	Caytaxin (possibly neurotransmitter metabolism)	infantile	Non-progressive cerebellar dysfunction, hypotonia, psychomotor retardation	Grand Cayman Island

2.3 Differential diagnosis of inherited ataxias

2.3.1 Introduction

Evaluation of adult-onset ataxia patients with non-dominant mode of inheritance is a challenge because of the broad spectrum of differential diagnostic alternatives, and a large spectrum of rare neurological disorders should be considered, in addition to the known and established specific genetic entities. Gene defects have been identified in several hereditary forms, but DNA analysis should not be restricted to ataxia patients with positive family history.

2.3.2 Autosomal recessive cerebellar ataxias

2.3.2.1 General features

Although age at onset can be quite diverse, most autosomal recessive ataxias with identified gene defects are early-onset forms. Autosomal recessive ataxias are frequently associated with peripheral sensorimotor neuropathy and some show systemic involvement outside the nervous system. Due to the heterogeneity of these disorders, further differentiation requires detailed assessment of the phenotype in order to establish the clinical diagnosis. For the different recessively inherited ataxias caused by inborn errors of metabolism, biochemical markers in blood, urine, or cerebrospinal fluid (CSF) are available (Worth 2004) (Table 4, modified after Worth 2004). Additional diagnostic studies, particularly neuroimaging, may provide clues for directing the DNA-genetic testing. For diagnostic purposes, categorisation of autosomal recessive ataxia (Table 3) either as the Friedreich's ataxia-like type, or as the early-onset type with cerebellar atrophy, may be useful (Fogel and Perlman 2007). The new ataxia syndrome MIRAS (Mitochondrial recessive ataxia syndrome), first reported in a Finnish family as part of this PhD research project, is described detailed in the Results and Discussion. Other autosomal recessive ataxias listed in the Table 3 are described in the Review of Literature.

Table 4. Non-genetic laboratory studies for the differential diagnosis of ataxia disorders.

Investigation	Disease
Thyroid function	Hypothyroidism
Vitamin B_{12}	Vitamin B_{12} deficiency
Glucose tolerance test	Friedreich's ataxia Mitochondrial diseases
Anti-gliadin and anti-endomysial antibodies	Coeliac ataxia
Anti-neuronal and anti voltage-gated calcium channel antibodies	Paraneoplastic cerebellar syndrome
Vitamin E	Ataxia with vitamin E deficiency (AVED) Vitamin E deficiency Abetalipoproteinaemia
Blood smear for acanthocytosis	Abetalipoproteinaemia Chorea-acanthocytosis
Cholesterol	Abetalipoproteinaemia Ataxia with oculomotor apraxia type 1 (AOA1)
Low density lipoprotein (LDL), very low density lipoprotein (VLDL)	Abetalipoproteinaemia
Immunoglobulins	Ataxia telangiectasia
α-fetoprotein	Ataxia telangiectasia, Ataxia with oculomotor apraxia type 2 (AOA2)
Albumin	AOA1
Cholestanol	Cerebrotendinous xanthomatosis
Very long chain fatty acids	X-linked adrenoleukodystrophy
Copper, caeruloplasmin	Wilson's disease
Phytanic acid	Refsum's disease
Serum/cerebrospinal fluid lactate, pyruvate	Mitochondrial diseases
Screening for lysosomal disorders urine oligosaccharides; leukocyte/fibroblast β-hexosaminidase, β-galactosidase, arylsulfatase-A, β-galactocerebrosidase, glucocerebrodidase, neuraminidase	GM2 gangliosidosis, GM1 gangliosidosis, metachromatic leukodystrophy, Krabbe, Gaucher, sialidosis
Skin biopsy	Niemann-Pick type C
Urine amino acids and organic acids	Aminoacidurias and organic acidemias
Urine glycosaminoglycans	Mucopolysaccharidoses

2.3.2.2 Epidemiology of autosomal recessive ataxias

Friedreich ataxia is worldwide the most common hereditary ataxia with a prevalence of approximately 1:30 000–1:50 000 in most populations. The carrier frequency is approximately 1:85 in Caucasian populations (Cossée et al. 1997). The Finnish population is an exception with a very low carrier frequency of FRDA mutation, only 1:500, and correspondingly positive DNA genetic tests are very rare (Juvonen et al. 2002). The prevalence of ataxia telangiectasia is variable but is estimated to be as high as 1:40 000 in the USA (Chun and Gatti 2004). Also ataxia telangiectasia was found to be exceptionally rare in the Finnish population with only 7 reported patients (Juvonen et al. 2002). The prevalence of ataxia with oculomotor apraxia type 2 (AOA2) is not known, but in the European population it might even be the second most frequent cause of autosomal recessive ataxia after Friedreich's ataxia (Le Ber et al. 2004). Some other forms of autosomal recessive ataxia have very specific geographical distributions: ataxia with vitamin E deficiency (AVED) in Mediterranean populations (Cavalier et al. 1998, Benomar et al. 2002), autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) in Canadian families of French origin (Engert et al. 2000), infantile-onset spinocebellar ataxia (IOSCA) in Finland (Nikali et al. 2005), whereas ataxia with oculomotor ataxia type 1 (AOA1) is relatively frequent in Portugal (Moreira et al. 2001), and Refsum's disease (Gibberd and Wierzbicki 2000) and MIRAS (Hakonen et al. 2005, Tzoulis et al. 2006, Hakonen et al. 2007) are more common in the Northern European populations.

2.3.2.3 Friedreich's ataxia (FRDA)

Although FRDA ataxia has been well recognized as a distinct disease entity, the use of the diagnosis was often less rigorous leading to frequent misclassification of cases. Geoffroy and colleagues and Harding brought clarity to the field by critically reviewing the literature and examining large series of patients personally (Geoffroy et al. 1976, Harding 1984). According to Harding, the classic clinical features are: autosomal recessive inheritance, onset before 25 years of age, progressive limb and gait ataxia, absent tendon reflexes in the legs, electrophysiologic evidence of axonal neuropathy, areflexia at all four limbs, distal loss of position and vibration sense, extensor plantar responses, pyramidal weakness of legs and dysarthria. Cardiomyopathy, scoliosis, pes cavus, and diabetes are common systemic findings (Harding 1981). Neuropathology shows loss of dorsal root ganglion cells with subsequent degeneration of the dorsal columns, degeneration of spinocerebellar and corticospinal tracts, and loss of cells in the cerebellar dentate nucleus (Koenig and Dürr 2000).

Most patients are homozygous for the expansion mutation of a GAA triplet repeat in the first intron of the *FRDA1* gene (Campuzano et al. 1996, Dürr et al. 1996, Filla et al. 1996), whereas about 4% of patients are compound heterozygous for the GAA expansion and a point mutation within the coding region of the gene (Cossée et al. 1999). *FRDA1* encodes a mitochondrial frataxin protein expressed in different tissues, albeit with highest expression levels in the spinal cord and dorsal root ganglia (Jiralerspong et al. 1997). Current evidence suggests that loss of frataxin impairs mitochondrial iron handling, antioxidant regulation and iron

sulphur regulation, resulting in mitochondrial respiratory chain dysfunction, increased oxidative stress and cellular damage (Campuzano et al. 1997, Lodi et al. 1999, Cavadini et al. 2002, Pandolfo and Pastore 2009).

After the introduction of direct molecular genetic DNA-testing (Campuzano et al. 1996), the phenotype has expanded so much that up to 25% of patients do not meet the original clinical diagnostic criteria of FRDA (Schöls et al. 1997a). In particular, two separate variants have been identified: a late FRDA form with onset after the age of 25 years and even as late as in the sixth decade (LOFA), and FRDA with retained reflexes (FARR). Late onset and retained reflexes are frequently concurrent/co-existing in the same patients and are suggestive of better prognosis. Both features are associated with significantly smaller GAA expansion on the shorter allele (Bhildayasiri et al. 2005). Variants presenting with spastic paraparesis, or chorea or pure sensory ataxia have also been reported (Berciano et al. 1997, Ragno et al. 1997, Hanna et al. 1998).

2.3.2.4 Ataxia with Vitamin E Deficiency (AVED)

Vitamin E deficiency may present with a phenotype that closely resembles FRDA. Like Friedreich ataxia, the age of onset is before 20 years, although decreased visual acuity or retinitis pigmentosa may be an early finding. Patients may have head titubation and a slower disease course. Cardiomyopathy and neuropathy seem to be less common than in Friedreich ataxia (Cavalier et al. 1998, Benomar et al. 2002). Mutations in the *TTP1* gene on chromosome 8q13, encoding the α-tocopherol transfer protein, are responsible for the disease (Ouahchi et al. 1995). Patients with a previous diagnosis of FRDA and negative test for FRDA should be tested for serum vitamin E levels, which are very low (< 5μg/ml) in AVED. Early replacement therapy may prevent progression of the disease, and may improve cerebellar ataxia to some extent (Gabsi et al. 2001).

2.3.2.5 Abetalipoproteinemia

Several diseases are known to cause secondary vitamin E deficiency. Abetaliporoteinemia is one of them with a neurological phenotype consisting of spinocerebellar degeneration, peripheral neuropathy, and retinitis pigmentosa presenting in young adults. Peripheral blood smear shows acanthocytosis ("thorny" red blood cells). Symptoms of abetaliporoteinemia begin in childhood, with stearrhoea and lipid malabsorption. Mutations in the microsomal triglyceride transfer protein (*MTP*) gene on 4q22-24 account for a part of the abetaliporoteinemia disorders (Narcisi et al. 1995). Vitamin E replacement therapy prevents further progression of the neurological symptoms (Berriot-Varoqueaux et al. 2000). Hypolipoproteinemia is a genetically distinct disorder with a clinical presentation similar to abetalipoproteinemia (Ohashi et al. 1998).

2.3.2.6 Ataxia telangiectasia

Ataxia telangiectasia (AT) is characterized by an early age at onset, progressive cerebellar ataxia, oculomor apraxia, telangiectasias, frequent infections, irradiation sensitivity, and increased risk for malignancies such as leukaemia and lymphoma. Diagnostic laboratory features are elevated serum alpha-fetoprotein and carcinoembryonic antigen concentrations, dysgammaglobulinemia and impairment of cellular immunity (Taylor and Byrd 2005). The *ATM* gene codes for a protein involved in DNA double strand break repair (Savitsky et al. 1995a, Savitsky et al. 1995b). The clinical variation with milder AT has been associated with relative preservation of ATM protein expression, leading to phenotypes with later onset, slower progression and no sensitivity to ionizing radiation (Sutton et al. 2004, Taylor and Byrd 2005).

2.3.2.7 Ataxia with oculomotor apraxia

Another ataxic disorder, very similar to ataxia telangiectasia, has recently been determined and found to be two distinct disorders. Ataxia with oculomotor apraxia type 1 (AOA1), identified first in members of Portuguese and Japanese families (Moreira et al. 2001), presents later than ataxia telangiectasia at about seven years of age and sometimes even much later (Le Ber et al. 2003, Criscuolo et al. 2004). AOA1 is characterized by the combination of cerebellar ataxia, severe peripheral neuropathy, oculomotor apraxia, extrapyramidal signs, and mild cognitive impairment. Laboratory studies show hypoalbuminaemia, hypercholesterolaemia, and normal serum α-fetoprotein (Le Ber et al. 2003). The disease is caused by mutations of the aprataxin gene, *APTX*, on chromosome 9p13 (Date et al. 2001). The protein is involved in DNA repair particularly of single-strand DNA breaks, although other additional roles, such as in RNA processing, have been suggested (Kijas et al. 2006).

Ataxia with oculomotor apraxia type 2 (AOA2) shares phenotypical features with type 1, but age at onset is later in the early teens and oculomotor apraxia, extrapyramidal signs, or cognitive impairment findings may be milder. In contrast to type 1, laboratory studies show normal albumin and high serum α-fetoprotein concentrations (Le Ber et al. 2004, Criscuolo et al. 2006). AOA2 is caused by mutations in the senataxin gene, *SETX*, on chromosome 9q34, and encodes a novel member of the superfamily 1 helicase proteins. Senataxin is expressed in the cytoplasm and the nucleolus, possible in a cell-cycle-dependent manner in cultured cells (Ursic et al. 2004, Mosesso et al. 2005). The functional role of human senataxin is unknown. Its orthologue in yeast, the protein encoded by *Sen1p,* is implicated in DNA transcription, DNA repair, and processing of non-coding RNAs (Moreira et al. 2004, Chen et al. 2006).

2.3.2.8 Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)

ARSACS is characterised by progressive cerebellar dysfunction, pyramidal signs such as spasticity with hyperreflexia, and sensorimotor neuropathy with muscular atrophy. Onset is typically between 1–5 years, but has been reported in the second decade in some families (Bouchard et al. 1998, El Euch-Fayache et al. 2003).

ARSACS was first described in Quebec region in Canada (Bouchard et al. 1978), and more recently identified in Europe, Eurasia, North Africa and Japan (Gomez 2004). The gene implicated in this disorder, *SACS*, encodes a protein known as sacsin predicted to be a chaperone involved in protein folding (Engert et al. 2000).

2.3.2.9 Cayman Ataxia

Cayman ataxia is so far restricted to one known population of Grand Cayman Island and characterized by hypotonia at birth, variable psychomotor retardation and cerebellar dysfunction (Nystuen et al. 1996). The pathogenetic mutations are located in the gene *ATCAY*, which encodes a protein called caytaxin expressed in neurons. Caytaxin contains a binding domain similar to that of α-tocopherol transfer protein, but likely binds a different and unknown ligand (Bomar et al. 2003).

2.3.2.10 Marinesco-Sjögren syndrome

Marinesco-Sjögren syndrome (MSS) is a rare infantile- or childhood-onset multisystem disorder characterised by cerebellar hypoplasia/atrophy and ataxia, congenital cataracts, mild to moderate mental retardation, severe rimmed vacuolar myopathy, short stature, hypogonatrotropic hypogonadism and skeletal deformities. Peripheral neuropathy and epilepsy may also occur (Anttonen et al. 2005, Senderek et al. 2005, Slavotinek et al. 2005). The severe progressive myopathy may remain frequently overlooked because of the CNS involvement (Mahjneh et al. 2006). The main causative gene for the MSS phenotype, *SIL1*, is located on chromosome 5q31 and encodes an endoplasmic reticulum resident cochaperone. Because SIL1 is ubiquitously expressed, it still remains unknown why mutational defects cause the specific syndromic features seen in the MSS phenotype (Anttonen et al. 2005, Senderek et al. 2005, Anttonen et al. 2008).

2.3.2.11 Spinocerebellar ataxia with axonal neuropathy

Spinocerebellar ataxia with axonal neuropathy (SCAN1) is a childhood-onset disorder, first identified in nine members of a Saudi Arabian family. This disease shows cerebellar ataxia and atrophy, distal muscle atrophy, pes cavus and peripheral axonal sensorimotor neuropathy that resembles Charcot-Marie-Tooth disease. The responsible gene for SCAN1 is *TDP1* on chromosome 14q31-32, which encodes tyrosyl-DNA phosphodiesterase 1 (TDP1) (Takashima et al. 2002). The protein is likely involved in repair of DNA-topoisomerase 1 complexes during transcription and replication in dividing cells, and topoisomerase 1-related single-stand break repair in postmitotic neurons. Oxidative stress and transcription may lead to single-strand breaks in the nervous system DNA, which become persistent in patients with TDP1 dysfunction, resulting in the neurodegenerative phenotype (El-Khamisy et al. 2005, El-Khamisy and Caldecott 2006).

2.3.2.12 Refsum's disease

The Refsum's disease is characterized by cerebellar ataxia, peripheral polyneuropathy, sensorineural deafness, retinis pigmentosa, and anosmia, cardiomyopathy, skeletal abnormalities, renal failure and ichthyosis as additional features. In patients, onset of first symptoms varies from early childhood to 50 years of age. Refsum's disease is genetically heterogenous; two genes, *PHYH* and *PEX*, have been identified to cause an identical phenotype (Jansen et al. 2004). The more common causes are mutations in the gene for the peroxisomal enzyme phytanoyl-CoA hydhydroxylase, *PHYH*, on chromosome 10pter-11.2 (Jansen et al. 1997, Mihalik et al. 1997). Less frequent are mutations in *PEX7* on chorosome 6q21-22.2, which encodes the peroxin 7 receptor. The protein is needed for peroxisomal import of proteins containing a type 2 peroxisomal targeting signal (van den Brink et al. 2003). Because of impaired branched-chain fatty acid α-oxidation, phytanic acid accumulates to high levels in body fat. Dietary restriction halts disease progression, which makes early identification essential (Jansen et al. 2004).

2.3.2.13 Cerebrotendinous xanthomatosis

Cerebrotendinous xanthomatosis (CTX) is a rare neurometabolic disease involving lipid metabolism and is caused by mutation of *CYP27* on chromosome 2, which encodes the mitochondrial enzyme sterol 27-hydroxylase (Cali et al. 1991). The classical phenotype is characterized by neurological dysfuction starting around age 20 years including ataxia with pyramidal and extrapyramidal signs, sensorimotor peripheral neuropathy, seizures, psychiatric proplems and cognitive decline. Associated features include juvenile cataracts, tendom xanthomas, early atherosclerosis, osteoporosis, and chronic diarrhoea. Early diagnosis is crurial because treatment with chenodeoxycholic acid may stop the deterioration and improve the neurological functions (Lorincz et al. 2005).

2.3.2.14 Infantile-onset spinocerebellar ataxia (IOSCA)

IOSCA, first described in Finland, is a severe ataxia syndrome with onset before the age of two years (Koskinen et al. 1994). Infants present with progressive cerebellar ataxia, muscle hypotonia, sensory neuropathy with areflexia, optic atrophy, ophthalmoplegia, hearing loss and athetosis. Female hypogonadism and epilepsy are late features. MRI shows atrophy of the cerebellum, brainstem and spinal cord with corresponding atrophic changes on neuropathology (Lönnqvist 1995, Lönnqvist et al. 1998). IOSCA is the second most common autosomal recessive ataxia in Finland with 22 identified patients (Nikali et al. 2005).

The gene, *C10orf2,* on chromosome 10q24 mutated in this disorder encodes Twinkle protein, a mitochondrial helicase involved in mtDNA replication, and Twinky protein with unknown functions. Severe MIRAS may resemble IOSCA in its clinical and morphological findings, including lack of mitochondrial myopathy, suggesting that similar pathogenetic mechanisms may cause both infantile-onset spinocerebellar ataxia and the *POLG1* related ataxic disorders (Nikali et al. 2005, Hakonen 2008). Both Twinkle and POLG

are essential mtDNA maintenance proteins. More recently, Twinkle-IOSCA was associated with brain-spesific mtDNA depletion (Hakonen et al. 2008).

2.3.3 Autosomal dominant cerebellar ataxias (SCAs)

2.3.3.1 Epidemiologic features

Dominantly inherited spinocerebellar ataxias (SCAs) are characterized by progressive cerebellar ataxia and variably associated with ophtalmoplegia, retinopathy, optic atrophy, extrapyramidal features, pyramidal signs, peripheral neuropathy, dementia or epilepsy (Schöls et al. 2004). They usually present between 30 and 50 years of age, although, early onset in childhood and onset in later decades after 60 years have been reported. Epidemiological data with prevalence of 5–7:100 000 indicate that SCAs might be more common than the previously estimated (Dueñas et al. 2006). SCAs are highly heterogenous and the prevalence of specific subtypes varies between 0.2–3.0:100 000 with large variations in different ethnic populations, and the most recent data suggest that SCA3 is the commonest subtype worldwide (Schöls et al. 2004). DRPLA has been reported to occur predominantly in Japanese individual, but has also been identified in European families (Koide et al. 1994, Warner et al. 1994, Norremolle et al. 1995). Detailed clinical assessment of dominant SCA families can reveal a specific diagnosis in about half of the cases and in about 4–10% if no inheritance pattern is recognized (Moseley et al. 1998, Schöls et al. 2000, Abele et al. 2002, Brusco et al. 2004, Juvonen et al. 2005).

Founder mutation effects contribute to the variable prevalence of specific SCA subtypes. At least SCA subtypes 1, 2, 6, 7, 8, and 17 have been identified among the Finnish patients. Mutation analyses for nine SCA subtypes showed that approximately 60% of the Finnish SCA patients with dominant family history still remain without specific diagnosis. Lack of SCA3 and relatively more SCA8 and SCA7 mutations have been found in Finnish SCA patients (Jonasson et al. 2000, Juvonen et al. 2000, Juvonen et al. 2005).

2.3.3.2 Molecular genetics

Since the identification of the first gene involved in dominant ataxia, SCA1 in 1993, at least 28 distinct loci and 9 genes have been described (Soong and Paulson 2007). SCAs 1, 2, 3, 6, 7, 17 and DRPLA share expanded repeats of coding CAG sequences as the basic mutation defect (Table 2). These repeats are translated into expanded polyglutamine (polyQ) stretches in the cognate proteins. These are all termed ataxins even if they are totally unrelated proteins. In these polyglutamine diseases the age of onset and severity of disease symptoms inversely correlate with the length of the CAG repeat expansion. The expanded repeats are unstable and tend to expand further in successive generations. This anticipation leads to earlier age at onset and a more severe phenotype in successive generations. SCA8, 10, and 12 are caused by noncoding CTG, ATTCT, and CAG repeat expansions. In SCA8 the upper and lower limits of the CTG repeat length are unknown and

there are several families in which the CTG expansion did not co-segregate with the disease. Because of this inconsistency, presymptomatic testing of SCA8 does not seem appropriate. A third category of SCAs are caused by conventional mutations in specific genes. At least four such SCAs are known: SCA5, 13, 14 and 27 (Schöls et al. 2004, Dueñas et al. 2006, Orr and Zoghbi 2007, Soong and Paulson 2007).

2.3.3.3 Spesific features in SCAs

If there is a strong clinical indicator present, such as retinopathy suggesting SCA7, or a known genotype in the family, single gene testing can be requested. Because of significant clinical overlap between the various SCAs and the phenotypic variability of single subtypes, it is difficult to clinically predict the SCA phenotype in individual patients (Schöls et al. 2004). Hungtington's disease and familiar prion disease are autosomal dominant diseases which in the early stages may show incoordination and may be considered in differential diagnosis (Wild and Tabrizi 2007).

Mutations in ataxia patients without family history are not restricted to recessive mutations. Due to anticipation, age at onset variation, or premature death of an at-risk parent, mutations in dominant SCA genes are seen in sporadic ataxia patients as well as in families showing a pattern suggestive of recessive inheritance. Small repeat expansions close to the normal repeat range, most commonly in the genes associated with SCA2 and SCA6, may have incomplete penetrance and may thus appear as a sporadic disease without any family history. In particular this is true for SCA6 in which the age of onset is relatively late and affected parents may have died before ataxia became apparent. Repeat expansions in the genes associated with SCA2 and SCA6 have been found up to 8% of the patients with sporadic disorder (Riess et al. 1997, Moseley et al. 1998, Schöls et al. 2000, Wardle et al. 2009). In addition, negative family history may be due to false paternity or to *de novo* mutations (Schöls et al. 1997b).

2.3.4 Fragile X-associated tremor/ataxia syndrome (FXTAS)

A novel neurodegenerative syndrome, named fragile X-associated tremor/ataxia syndrome (FXTAS), has been described in older males (> 50 years) carrying premutation alleles in the fragile-X mental retardation 1 (*FMR1*) gene with CGG repeat expansion size of 55–200 repeats (Hagerman and Hagerman 2002, Jacquemont et al. 2003). The full mutation in *FMR1* (> 200 repeats) causes fragile-X syndrome, a relatively frequent cause of mental retardation in boys (Verkerk et al. 1991, Oostra and Willemsen 2009). Core clinical features of FXTAS are progressive cerebellar gait ataxia, intention tremor, parkinsonism, polyneuropathy and cognitive decline (Jacquemont et al. 2007). Neuroradiological findings include global brain atrophy and prominent white-matter disease in the periventricular and subcortical regions, and in the middle cerebellar peduncles on T2-weighted MRI (Brunberg et al. 2002). The principal neuropathological characteristics of FXTAS are eosinophilic, ubiquitin-positive inclusions located in the nuclei of neurons and astrocytes widely

distributed throughout the brain and spinal column. These inclusions contain *FMR1* mRNA, which may exert a direct toxic gain-of-function effect leading to FXTAS (Hagerman and Hagerman 2004, Tassone et al. 2004, Greco et al. 2006).

The prevalence of *FMR1* premutation was reported to be 1:813 in males and 1:259 in females. The predicted lifetime cumulative risk of FXTAS among men in the general population is estimated to be 1:3000–1:8000 (Jacquemont et al. 2007). Female carriers are at risk for premature ovarial failure or early menopause (Wittenberger et al. 2007). *FMR1* gene screens in sporadic cerebellar ataxia in adult males above 50 years have revealed a prevalence of *FMR1* premutation in around 5% of the cases, suggesting that this is a frequent genetic cause of sporadic ataxia, and should thus be considered in differential diagnosis of spinocerebellar ataxia (Macpherson et al. 2003, Brussino et al. 2005, Van Esch et al. 2005). More recently, a few female mutation carriers with ataxia have also been described (Hagerman et al. 2004, Berry-Kravis et al. 2007, Coffey et al. 2008). In addition, identification of familial cases suggests that genetically undefined ataxia patients with positive family history should be tested for the *FMR1* premutation (Peters et al. 2006). FXTAS has also been identified in Finland (Rantamäki et al. 2007).

2.3.5 Idiopathic cerebellar degeneration

Adult-onset sporadic ataxias can be divided into three major categories. The first group comprises symptomatic ataxias that are due to identifiable exogenous causes such us chronic alcoholism, other toxic factors, malignant disease (paraneoplastic cerebellar degeneration), vitamin deficiency, other metabolic causes, inflammatory or immune-mediated cerebellar damage. The second group includes inherited ataxias manifesting as late-onset sporadic disease. In two recent studies (Schöls et al. 2000, Abele et al. 2002) the genetic basis was found in 15–19% of all sporadic ataxia patients. Thirdly, after all known symptomatic or genetic causes have been ruled out, a significant group of sporadic cerebellar ataxia patients still remains without further clarification, forming a category of idiopathic cerebellar degeneration.

Clinically, patients with idiopathic cerebellar ataxia (IDCA) may have a pure cerebellar syndrome or combined with additional extrapyramidal features. Recent work showed that 29% of sporadic adult-onset IDCA patients met the clinical criteria of possible or probable multiple system atrophy (MSA) (Abele et al. 2002). In the same work thorough investigations identified the genetic cause in 13%, and the disease remained unexplained in 58% (Abele et al. 2002). IDCA patients who initially do not meet diagnostic criteria of MSA may still develop MSA in a later phase of the disease (Klockgether et al. 1990). It is still not clarified whether idiopathic cerebellar ataxia and the cerebellar variant of MSA (MSA-C) represent distinct entities (Penney 1995, Gilman and Quinn 1996, Gilman et al. 2000). Patients with a MSA phenotype, on the other hand, do not have a high frequency of SCA mutations. Examination for the SCA1 and 3 genes in 80

MSA patients did not identify any repeat expansions (Bandmann et al. 1997). In another study, 20 MSA patients were examined for expansions in the SCA1, 2, 3, 6, 7, 8 and 12 genes and the GAA repeat expansion of the *FRDA1* gene without any mutations found (Schöls et al. 2000). However, rare patients with a clinical MSA-C ataxia phenotype and mutated SCA3 repeat expansion has been reported (Takiyama et al. 1997, Nirenberg et al. 2007, Wüllner et al. 2007).

2.3.6 Inborn errors of metabolism causing ataxia in adulthood

The number of diseases in humans associated with inherited defects in metabolism exceeds 500. The vast majority of metabolic dieseases is childhood-onset and recessively inherited conditions. Nevertheless, many adults with inborn errors may show mild or intermittent clinical symptoms at an early age, but these have often remained unrecognised. Neurological symptoms are the presenting and most prominent clinical features in many inherited metabolic disorders. The most common neurometabolic disorders are organic acidurias, neuronal lipofuscinoses, urea cycle disorders, congenital lactic acidosis, peroxisomal disorders, and less frequently: sphingolipodoses, mucopolysaccharidoses, glycoprotein degradation disorders and fatty acid oxidation disorders. A number of these diseases may have adult-onset forms (Swanson 1995, Clarke 2006). Recessive metabolic disorders, in which adult-onset ataxia may present as part of the systemic disease, are presented is Table 5 (modified after Brusse et al. 2007).

Table 5. Recessive metabolic disorders with ataxia as a part of systemic adult-onset syndrome.

Disease	Gene	Locus	Gene product	Age at onset	Disease characteristics	Biochemical tests
Metachromatic leukodystrophy	*ARSA*	22q13	Arylsulfase A	infancy to adulthood	Infantile: spasticity, optic atrophy, developmental delay, demyelinating polyneuropathy adult: cognitive, bahavioral and psychiatric problems	Leukocyte or fibroblast arylsulphatase A
Krabbe's disease (Globoid leukodystrophy)	*GALC*	14q31	Galactosylceramidase	infancy to fifth decade	Hemiparesis, spastic hemi or tetraparesis, visual failure	Leucocyte or fibrobast galactosylceremidase
Niemann-Pick C (Sphingomyelin storage disease)	*NPC1*	18q11–12	NPC1 protein	infancy to adulthood	Supranuclear ophtalmoplegia, dementia, dystonia, seizures, psychiatric illness, hepatosplenomegaly, bone marrow histology: foamy cells (lipid-laden macrophages)	Impaired cholesterol esterification and positive filipin staining in fibroblast
Late-onset Tay-Sachs disease (G_{M2}-gangliosidosis, hexosaminidase deficiency)	*HEXA*	15q23–24	β-hexosaminidase A	childhood to adulthood	Areflexia, proximal muscle weakness with muscle atrophy and fasciculations, psychiatric or behavioural problems	Leucocyte or fibroblast hexosaminidase A
GM1 gangliosidosis	*GLB1*	3p21.33	Beta-galactosidase	infancy and childhood (also adult/chronic cases)	Choreathetosis, dementia flattering of vertebral bodies, psychosis	Leucocyte or fibroblast ß-galactosidase, urine oligosaccharides
Wilson's disease	*ATP7B*	13q14–21	ATPase Cu transporting ß-polypeptide	3–50 years	Dysarthria, pseudopulbar palsy, parkinsonism, dementia, renal failure, liver disease, Kayser-Fleischer rings	Urine copper, plasma copper and caeruloplasmin
Aceruloplasminemia	*CP*	3q23–24	Ceruloplasmin	25–60 years	Retinal dystrophy, diabetes, anemia, movement disorders, cognitive dysfunction and dementia	Plasma and urine copper, plasma iron and ferritin
Chorea-acanthocytosis	*CHAC*	9q21	Chorein	first to seventh decades, mean age of onset 35 years	Chorea, parkinsonisn, dystonia, progressive cognitive and behavioural changes, seizures, myopathy	Acanthocytes, usually present in 5–50% of the red cell population
Sialidosis (mucolipidosis type 1)	*XK*	Xq	XK protein	adult	Chorea, neuropsychiatric symptoms, hepatopathy, myopathy, seizures	Acanthocytotosis, specific Kell subantigen marker
	Neu1	6p21.3	Neuraminidase	0–20 years	Visual defect with lens or corneal opacity, myoclonus, seizures	Leucocyte or fibroblast α-neuraminidase, urine oligosaccharides

2.4 Mitochondrial disorders and ataxia

2.4.1 Mitochondrial function

The main role of mitochondria, the powerhouses of our cells, is to generate energy as adenosine triphosphate (ATP) in the process called oxidative phosphorylation (OXPHOS), carried out by the mitochondrial respiratory chain. In addition to ATP production, mitochondria are involved in several other metabolic pathways, such us fatty acid metabolism, cholesterol synthesis, cellular iron homeostasis, heme biosynthesis, and intracellular calcium concentration regulation. Mitochondria have important functions in cell signalling, particularly in regulating apoptotic cell death (Chinnery and Schon 2003). Mitochondria interact with many specific proteins implicated in genetic forms of neurodegenerative diseases (Schapira 2006, DiMauro and Schon 2008). Cellular energy requirements control the number of mitochondria in each cell. The largest number of mitochondria is found in metabolically very active tissues, such us skeletal and cardiac muscle, liver and brain (Chinnery and Schon 2003). Because ATP is essential for all energy demanding cellular processes it is not surprising that a relative deficiency of ATP can lead to dysfunction of many different organs, and ultimately cause cell death, if the deficiency is severe and prolonged (Smeitink et al. 2006).

2.4.2 Mitochondrial genetics

Two distinct genetic systems encode mitochondrial proteins: mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). The genetics of mtDNA is unique in many respects. Each cell contains between a few hundred and many thousands copies of mtDNA, which are inherited exclusively down the maternal line (Zeviani and Di Donato 2004). Unlike nDNA, which replicates only once during each cell cycle, mtDNA is continuously recycled. Even in postmitotic cells, such us neurons or cardiac and skeletal muscle cells, and in cells with a very low mitotic index, e.g. hepatocytes, mtDNA undergoes continuous replication and will thus need lifelong maintenance (Chinnery and Samuels 1999, Van Goethem 2006, Krishnan et al. 2008). Mitochondrial DNA encodes 13 of the approximately 90 proteins in the respiratory chain system, besides two ribosomal and 22 transfer RNAs essential for their synthesis. The majority of respiratory chain proteins as well as all the other proteins involved in replication, repair, transcription and translation of mtDNA, are synthesised from genes in the cell nucleus (Mancuso et al. 2007, Spinazzola and Zeviani 2007).

Mutations can affect all mtDNA copies in the individual (homoplasmy), or only some copies (heteroplasmy). Most mtDNA mutation associated diseases are characterized by the coexistence of wild type and a mutant mtDNA in various proportions (heteroplasmy). Typically the cells are able to tolerate high percentage

levels of mutated mtDNA (70–90%) before they develop a biochemical respiratory chain defect. In the case of heteroplasmy the proportion of mutant mtDNA may vary among individuals within the same family, and also among organs and tissues within the same individual. This is one explanation for the widely variable clinical phenotypes seen in individuals with pathogenic mtDNA disorders (Chinnery et al. 1997, Chinnery and Schon 2003). For example, in individuals harbouring the 8993T>G mutation, higher percentage levels of mutated mtDNA are seen in individuals presenting with Leigh syndrome than in those presenting with neurogenic weakness with ataxia and retinitis pigmentosa (NARP) (Holt et al. 1990, Tatuch et al. 1992, White et al. 1999a, White et al. 1999b). The proportion of mutated mtDNA in individual tissues may also undergo changes during development and throughout adult life, potentially influencing the phenotype over time in a certain individual. In addition, multiple independent factors can influence the biochemical expression of a mutation: mtDNA sequence changes may interact with each other, with nuclear genes, or with environmental factors to cause a disease (DiMauro 2004).

2.4.3 Genetic categories of mitochondrial diseases

Since mitochondrial proteins are encoded by both the nDNA and mtDNA, mitochondrial diseases can result from primary mutations in either of the genomes. Defect oxidative phosphorylation may be due to overall dysfunction of the respiratory chain or can be associated with single or multiple defects in the five different complexes (I to V) forming the total respiratory chain (DiMauro and Hirano 2005). Recent advances in the molecular genetic basis of the mitochondrial diseases have helped in the classification of these diseases. A number of nuclear genes that govern maintenance and replication of mtDNA have recently been shown to be important causes of mitochondrial disease. This disease group is characterized by secondary alterations in mtDNA, either multiple deletions and/or a reduced copy number of mtDNA (mtDNA depletion). Mutations in these nuclear genes affect either the enzymatic process itself, as with *POLG1* or *TWINKLE,* or the nucleotide pool available for mtDNA replication. Nuclear DNA genes also code for essential factors needed for intramitochondrial transcription and translation (Copeland 2008). One new and exciting pathogenic category of mitochondrial disorders is due to abnormal mitochondrial motility, fission and fusion (Berman et al. 2008). Genetic classification of mitochondrial diseases caused by disorders of the mitochondrial genome and nuclear genome is presented in Table 6 (modified after DiMauro and Hirano 2005, Filosto and Mancuso 2007, Copeland 2008).

Table 6a. Genetic classification of mitochondrial disorders (mtDNA defects).

Mitochondrial diseases caused by disorders of the mitochondrial genome

Sporadic rearrangements

    Kearns-Sayre syndrome (KSS)

    Pearson syndrome

    Sporadic chronic progressive external ophtalmoplegia (CPEO)

    Diabetes and deafness

Sporadic point mutations

    CPEO

    Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS)

    Exercise intolerance

    Isolated myopathy

Maternally inherited rearrangements

    CPEO

    Multisystemic syndromes

Maternally inherited mtDNA point mutations

    Point mutation in genes encoding structural proteins

        Leber hereditary optic neuropathy (LHON)

        Neuropathy, ataxia, retinitis pigmentosa syndrome (NARP)

        Leigh syndrome

    Point mutation in genes encoding tRNAs

        MELAS

        Myoclonic epilepsy with ragged red fibres (MERRF)

        Cardiomyopathy and myopathy (MiMyCa)

        CPEO

        Isolated myopathy

        Diabetes and deafness

        Sensorineural deafness

        Hypertrophic cardiomyopathy

        Tubulopathy

    Point mutations in genes encoding rRNAs

        Aminoglycoside-induced non-syndromic deafness

        Hypertrophic cardiomyopathy

Table 6b. Genetic classification of mitochondrial disorders (nDNA defects).

Mitochondrial diseases caused by disorders of the nuclear genome

Defects in genes encoding for structural proteins of the complexes of the respiratory chain

    Leigh syndrome

    Cardiomyopathy

    Paraganglioma

    Multisystemic syndromes

Defects in genes encoding factors involved in the assembling complexes of the respiratory chain (assembly genes)

    Leigh syndrome

    Multisystemic syndromes

Defects in genes altering mtDNA stability and integrity (defects in intergenomic communication)

mtDNA defect	Involved gene
mtDNA depletion	
Hepato-cerebral syndrome	*dGK*
Hepato-cerebral syndrome	*MPV17*
Encephalomyopathic syndrome	*SUCLA2*
Myopathic syndrome	*TK2*
Alpers-Huttenlocher syndrome	*POLG1*
Encephalomyopathic syndrome	*RRM2B*
IOSCA	*TWINKLE*
mtDNA multiple deletions	
ad/arPEO	*ANT-1*
adPEO	*TWINKLE*
ad/arPEO, ad/ar multisystemic syndromes, parkinsonism, SANDO, MIRAS	*POLG1*
mtDNA depletion and multiple deletions	
MNGIE	*TP*

Coenzyme Q10 deficiency

    Encephalomyopathy

    Encephalomyopathy with renal dysfunction

    Ataxic form

Defects in the lipid milieu

    Barth syndrome

Syndrome caused by defects in mitochondrial ribonuclear acid modifications

    Mitochondrial myopathy with lactic acidosis and sideroblastic anaemia (MLASA)

Defects in mitochondrial translation

    MRPS16 mutation syndrome

Defects in mitochondrial fission and fusion

    Autosomal dominant optic atrophy

In addition, many hereditary neurodegenerative disorders have been associated with other mitochondrial proteins, which are not obviously linked directly to the respiratory chain and energy production. This group includes frataxin, putatively involved in iron handling and iron-sulfur protein regulation (Zeviani and Di Donato 2004) (Table 7, modified after Zeviani and Di Donato 2004).

Table 7. Disorders due to nuclear DNA mutations indirectly involved in OXPHOS.

Nuclear DNA mutation and protein fuction	Disease	Phenotype
Autosomal recessive mutation in the *frataxin* gene (iron handler iron-sulfur cluster assembly)	Friedreich's ataxia	Ataxia, loss of deep tendon reflexes, sensory neuropathy, Babinski sign, cardiomyopathy, diabetes
Autosomal recessive mutation in the *ABC7* iron transporter	X-linked ataxia and sideroplastic anaemia	Ataxia, sideroplastic anaemia
Autosomal recessive mutation in the *SPG7* gene encoding a met-alloprotease	Hereditary spastic paraplegia	Spastic paraplegia. complex form: with mental retardation, ataxia, optic atrophy, deafness, cataracts, axonal neuropathy, amyotrophy, skin pigmentary abnormalities
X-linked recessive mutation in the *DDP1* gene encoding protein mitochondrial transporter	X-linked deafness-dystonia syndrome	Deafness and dystonia
Autosomal dominant mutations in the *OPA1* gene encoding a dynamin-related protein involved in mitochondrial fusion and cristae organisation	Autosomal dominant optic atrophy (*OPA1* gene)	Optic atrophy and visual failure
OPA1 mutations involving mtDNA instability		Complex form: also ataxia, axonal neuropathy, CPEO, deafness, mitochondrial myopathy

2.4.4 Diagnosis of mitochondrial diseases

Mitochondrial diseases are characterized by large heterogeneity and frequent multisystemic involvement. The peripheral and central nervous system (CNS), striated muscles, endocrine glands, auditory system, gastrointestinal tract, liver, kidney, bone marrow, and dermis are organs frequently clinically or subclinically affected in mitochondrial disorders. The range of central nervous system manifestations is broad, including fluctuating encephalopathy, seizures, stroke-like episodes, migraine, movement disorders, extrapyramidal abnormalities, spasticity, psychiatric abnormalities or neuropsychological impairment (Finsterer 2006, Di-Mauro and Schon 2008). In paediatric patients the most frequent clinical features are severe psychomotor delay, generalized hypotonia, lactic acidosis and signs of cardiorespiratory failure. The most common and well characterized early onset mitochondrial encephalopathy is Leigh syndrome or subacute necrotizing encephalomyelopathy. Adult patients frequently show signs of neuropathy or myopathy associated with variable involvement of the CNS (Zeviani and Di Donato 2004).

If mitochondrial disease is suspected, but the blood mtDNA tests for the common point mutations associated with MERRF, MELAS and NARP syndromes remains negative, the patient should have a tissue biopsy from an affected tissue, usually from skeletal muscle (McFarland et al. 2002). Characteristic findings on muscle histopathology include subsarcolemmal aggregations of mitochondria (ragged red fibres, RRF) and fibres negative for cytochrome *c* oxidase (COX, complex IV). Reduced COX reaction within some of the fibers (as a mosaic defect) suggests a mtDNA defect, whereas reduction affecting all the fibers within the entire biopsy suggests a nuclear genetic defect. Typical mitochondrial findings are usually present in the case of alteration of functional proteins (i.e. tRNAs), but more rarely when structural genes are involved (such us in Leber's hereditary optic neuropathy, LHON, and NARP) (Zeviani and Di Donato 2004). Biochemical studies of the activity of the individual respiratory chain complexes may also provide a clue to the underlying genetic defect. Further molecular testing on muscle is indicated if there is abnormal muscle histochemistry or respiratory chain complex assays, or a very strong clinical suspicion (Chinnery 2006).

Southern blot analysis is the traditional method used for detection of large (> about 500 bp) mtDNA rearrangements. Such single deletions are typically found in sporadic Kearns-Sayre syndrome (KSS) or PEO patients with mitochondrial myopathy (DiMauro 2004). Importantly, a normal muscle histochemistry and/or normal Southern blot analysis does not exclude the possibility of a mitochondrial defect. Multiple mtDNA deletions not observed by Southern blot may be possible to detect with sensitive PCR-based methods. Some nuclear genetic defects are associated with secondary mtDNA abnormalities causing multiple mtDNA deletions and/or depletion, mainly in postmitotic tissues. When multiple mtDNA deletions are present at very low levels, the technique called long-range PCR should be applied, which preferentially detects small fragments of mtDNA. Abnormalities can be further studied with real-time PCR or a Southern blot allowing for quantification of the rearrangements or the detection of mtDNA depletion (Chinnery 2006, Horvath et al. 2006, Chinnery and Zeviani 2008). If there are no deletions and no depletion, the next approach may be targeted sequencing of specific mtDNA genes, and ultimately, complete mtDNA genome sequencing if necessary. It is important to sequence muscle DNA because low levels of heteroplasmic mutations may not be detected in blood leukocyte DNA by automatic fluorescent sequencing especially in elderly patients (Chinnery 2006). If a mutation is heteroplasmic, high percentage levels are usually found in the pathologically abnormal muscle cells by single muscle fibre analysis. This strongly suggests that the mutation is causative (Chinnery and Schon 2003).

During the recent years mutations in the *POLG1* gene have proven to a common cause of neurodegenerative diseases. Detecting *POLG1* mutations in adult ataxia patients poses a particular challenge because these patients do rarely have abnormal muscle histopathology findings. Some laboratories have direct analytics for the two most common pathogenic mutations A467T and W748S, which may provide the exact diagnosis. Long-range PCR applied on muscle biopsy is a useful screening test in patients with neurological disease due

to *POLG1* mutations to detect multiple mtDNA deletions. The interpretation of the results is complicated by the fact that low amounts of multiple deletions are normally present in older individuals (Krishnan et al. 2008) or in patients with sporadic inclusion body myositis (IBM) disease (Jansson et al. 2000). Moreover, there are common polymorphisms in the *POLG1* gene that may complicate interpretation. Anyway, the presence of mtDNA deletions or depletion combined with clinical suspicion in a patient should lead to *POLG1* gene molecular genetic evaluations (Chinnery 2006, Horvath et al. 2006, Chinnery and Zeviani 2008).

2.4.5 Mitochondrial dysfuction: an important cause of spinocerebellar ataxia

Mitochondrial dysfunction is increasingly acknowledged as an important mechanism in the pathophysiology of spinocerebellar degeneration. Ataxia is a frequent associated feature in several classical mitochondrial syndromes, many of them associated with a specific type of primary mtDNA defect (Table 8, according to McFarland et al. 2002). In some of them ataxia may be a major clinical presentation, as in the myoclonic epilepsy with ragged red fibres (MERRF) caused by the 8344A>G mtDNA mutation; in the NARP syndrome caused by the 8993T>G mtDNA mutations, or in syndromes caused by a single large mtDNA deletion (Kearns-Sayre syndrome, KSS) (Zeviani and Di Donato 2004). The clinical features in adult-onset familiar progressive external ophthalmopegia (PEO) associated with multiple mtDNA deletions may include, beside PEO, ataxia and peripheral neuropathy and various additional symptoms such as deafness, cataracts, optic atrophy, psychiatric disorders, dysphagia, dysphonia, facial diplegia, hypogonadism, profound muscle weakness and extrapyramidal syndromes (Yuzaki et al. 1989, Zeviani et al. 1989, Servidei et al. 1991, Suomalainen et al. 1997, Van Goethem et al. 2003a, Van Goethem et al. 2003c, Luoma et al. 2004). Recently, coenzyme Q10 (CoQ_{10}) deficiency has been included among the respiratory chain disorders because of its central role in the electron transport from complex I and II to complex III. Deficiency of this enzyme has been associated with a spectrum of autosomal recessive conditions, predominantly affecting children, but adult patients may present with cerebellar ataxia or myopathy (Quinzii et al. 2007).

The newly recognized defects of proteins involved in mtDNA maintenance seem to be very important causes for autosomal recessive ataxias. Infantile onset spinocerebellar ataxia (IOSCA), one of the most severe forms of inherited ataxias included in the Finnish disease heritage and resembling severe FRDA, is associated with dysfunction of mitochondrial proteins Twinkle and Twinky (Nikali et al. 2005). Also the juvenile or adult-onset ataxia of spinocerebellar-type MIRAS reported in this thesis is associated with defective mitochondrial DNA polymerase gamma (POLG). Because Friedreich's ataxia may also be considered a mitochondrial disorder, it seems that mitochondrial dysfunction has a central role in the pathogenesis of many inherited ataxias.

Table 8. Clinical presentations of mtDNA diseases.

Clinical syndrome	Organs affected											
	Muscle	Brain	Peripheral nerve	Heart	Endocrine pancreas	Eye	Bone marrow	Ear	Kidney	Liver	Gut	Exocrine pancreas
CPEO	+			+		+++						
CPEO+	++	++	++	++	++	+++		++				
KS	++	++		++		+++						
PS	+	+					+++					+++
DS	+++	+++							+++	+++		
MNGIE	++	+++	++								+++	
MELAS	++	+++		++	+++			+++			+	
MERRF	+++	+++		+++								
NARP		+++	++			+++						
LHON		+		+		+++						
LS	+	+++	+			+++						

CPEO = chronic progressive external ophtalmoplegia; KS = Kearns-Sayre syndrome; PS =Pearson's syndrome; DS = depletion syndrome; MNGIE = mitochondrial neurogastrointestinal encephalopathy syndrome; MELAS = mitochondrial encephalopathy, lactid acidosis and stroke-like episodes; MERRF = myoclonus epilepsy with ragged red fibers; NARP = neuropathy, ataxia and retinitis pigmentosa; LHON = Leber's hereditary optic neuropathy; LS = Leigh's syndrome.

2.4.5.1 DNA polymerase gamma (POLG)

DNA polymerase gamma (POLG) is the only polymerase known to be involved in the replication of the mitochondrial genome (Kaguni 2004). The catalytic subunit of POLG protein contains DNA polymerase (i.e. replicative) and exonuclease (i.e. proofreading) activities, whereas the accessory subunit is a DNA binding factor responsible for processive DNA synthesis and tight binding of the POLG complex to DNA (Kaguni 2004, Graziewicz et al. 2006). The catalytic subunit of POLG is translated from the 22 exons of the nuclear *POLG1* gene on the chromosome 15q25. The polymerase and exonuclease domains in the catalytic subunit are separated by a linker region. Mutations in the linker region are thought to compromise efficiency and catalytic interaction with the accessory subunit. The two amino acid substitutions identified in MIRAS patients, W748S and A467T, affect this intervening linker region between DNA polymerase and exonuclease regions of POLG (Chan et al. 2005, Luo and Kaguni 2005, Luoma et al. 2005, Chan et al. 2006). There is a tendency for dominant late-onset-disease to be associated with mutations affecting the polymerase domain of POLG, whereas recessive mutations appear to be scattered throughout the gene (Hudson and Chinnery 2006, Chinnery and Zeviani 2008, Copeland 2008).

Primary mutations in the *POLG1* gene can lead to secondary defects in the maintenance of the mitochondrial genome, with DNA depletion, multiple deletions or multiple point mutations. These mtDNA abnormalities lead to defective or reduced synthesis of mtDNA-encoded components of the respiratory chain in clinically affected tissues, and are thought to accumulate with time, contributing to the progressive phenotype of these disorders (Spelbrink et al. 2000, Krishnan et al. 2008). Mutations in *POLG1* have been linked to a wide spectrum of paediatric and adult diseases, with three main disease phenotypes: Alpers syndrome and related

depletion disorders, progressive external ophthalmoplegia (PEO) and ataxia-neuropathy syndromes (Chinnery and Zeviani 2008, Wong et. al. 2008, Blok et al. 2009, Steward et al. 2009). Interestingly, knock-out mice lacking the proofreading activity of POLG accumulate high amounts of mtDNA mutations and develop symptoms associated with advanced aging, supporting the role of mtDNA in normal aging (Trifunovic et al. 2004). Furthermore, defect function of POLG has been associated with male subfertility (Rovio et al. 2001). Specific syndromes associated with mutations in *POLG1* including MIRAS, linked to homozygous or compound hereozygous W748S and A467T mutations, are described in more detail in the Discussion.

2.5 Treatment in hereditary ataxia

2.5.1 Symtomatic therapy

Despite an increasing insight into molecular genetic etiology underlying hereditary ataxias, the understanding of molecular pathogenetic mechanisms leading to neuronal cell death are still poor or unknown. Consequently, therapeutic options to curing or even modifying neurodegeneration are not available (Chinnery 2006). In the case of some ataxia syndromes caused by inborn error of metabolism therapies are useful, such us supplemention with vitamin E seems in AVED, dietary treatment in Refsum's disease, and treatment of cerebrotendinousus xanthomathosis with chenodeoxycholic acid (Brusse et al. 2007).

Although medical treatment of these neurodegenerative diseases has remained symptomatic, this does not diminish the importance of an adequate genetic diagnosis for the patient. Establishing the correct genetic diagnosis excludes a potentially treatable disorder, it allows for comprehensive genetic counselling and provides some resolution of the prognosis permitting preventive measures to avoid complications and adequate planning of rehabilitative measures. As with other chronic complex debilitating disorders, the appropriate management requires an integrated multidisciplinary team approach, which includes specialist nurses and physical, speech and occupational therapists, as well as medical professionals in other specialities such us ophthalmology, cardiology or psychiatry (Stevenson and Playford 2007). Palliative therapy is dictated by good medical practice and may include control of endocrine dysfunction, surgical procedures and anticonvulsant treatment (DiMauro and Mancuso 2007). Importantly, sodium valproate should be avoided in all patients with proven or suspected *POLG1* disease because of the increased risk of severe liver failure (II, Horvath et al. 2006, Tzoulis et al. 2006, Engelsen et al. 2008, McFarland et al. 2008, Uusimaa et al. 2008). Symptomatic therapy may be used to treat spasticity, and in selected patients botulin toxin may be considered. Dystonia, tremor or bradykinesia may be indications for dopaminergic and anticholinergic therapy. Intention tremor has been treated with betablockers, benzodiazepines or even thalamic stimulation. Muscle

cramps can be relieved with benzodiazepines (clonazepam) and seizures are treated with antiepileptic drugs (Perlman 2004).

2.5.2 Future aspects in hereditary ataxia

Functional molecular biology studies of hereditary ataxias are beginning to reveal pathways that are potential therapeutic targets for disease-modifying therapy. Cell and animal disease models in progress will increase our understanding of how the known mutations in different genes execute their subcellular pathogenetic mechanisms. The results of these studies will be useful for developing treatments (Schmitz-Hübsch and Klockgether 2008, Carlson et al. 2009, Tyynismaa and Suomalainen 2009). Potential therapeutic strategies already underway for the polyglutamine repeat disorders include silencing of gene expression, increasing protein clearance, reducing toxicity of the protein and influencing downstream pathways activated by the mutant protein (Shao and Diamond 2007). Possible other therapeutic approaches include removal of noxious metabolites, administration of artificial electron acceptors, metabolites or free radical scavengers, and gene therapy (van de Warrenburg et al. 2005, Dueñas et al. 2006, Orr and Zoghbi 2007, Soong and Paulson 2007).

There is increasing interest in the administration of reactive oxygen species scavengers both in primary mitochondrial diseases and in neurodegenerative diseases directly or indirectly related to mitochondrial dys-function (Pandolfo 2008). Given the current knowledge of the molecular pathogenetic mechanisms of Friedreich's ataxia, treatment options have been initiated directed at antioxidant protection. Treatment with the coenzyme Q10 (CoQ_{10}) analog idebenone has been applied in FRDA patients since it was demonstrated that it may improve cardiac and neurological functions (Rustin et al. 1999, Rustin et al. 2002, Di Prospero et al. 2007, Pineda et al. 2008). Ubiquinone replacement is shown to be of some value in CoQ_{10} deficiency (Artuch et al. 2006) and has been suggested also for other mitochondrial disorders (Mattiazzi et al. 2004, Chinnery et al. 2006), but seems not to be helpful in MIRAS patients (Udd, personal communication).

Aerobic exercise may prevent some of the deconditioning and improve exercise tolerance in patients with mitochondrial myopathy (Chinnery and Bindoff 2003). Removal of noxious metabolites has been focused on reducing lactic acidosis and was extended to other metabolites. Gene therapy is a challenge because of poly-plasmy and heteroplasmy in mitochondrial diseases, but interesting experimental approaches are being pur-sued and include decreasing the ratio of mutant to wild-type mitochondrial genomes (gene shifting), convert-ing mutated mtDNA to normal nuclear genes (allotropic expression), importing cognate genes from other species, or correcting mtDNA mutations with specific restriction endonucleases. Germline therapy raises ethical problems but is being considered for preventing maternal transmission of mtDNA mutations (Chin-nery and Schon 2003, DiMauro and Mancuso 2007, Gardner et al. 2007). Preventive therapy through genetic

counselling and prenatal diagnosis is becoming increasingly important for nuclear DNA-related disorders (DiMauro et al. 2006).

3 Aims of the study

During the last 15 years the causative mutations of many hereditary ataxias have been identified, and important scientific advance has unveiled the molecular pathology in some of them. However, with the exception of FRDA, adult-and late-onset recessive ataxias have rarely been described and are poorly understood. Correspondingly, final aetiology and diagnosis has remained uncertain in the majority of patients with adult onset non-dominant ataxia in Finland and elsewhere. After the available differential diagnostic tests had failed to reveal the cause of the ataxia in our clinical patients, we expanded the scientific studies:

1. to characterize the clinical and pathological features of previously undescribed adult-onset non-dominant ataxias in selected families in Finland
2. to clarify the molecular genetic aetiology of the studied new ataxia syndromes
3. to estimate the prevalence of these new ataxias in Finland, and to outline and improve the differential diagnostic process of adult onset non-dominant ataxia
4. to determine possible clinical effects in carriers of POLG mutation W748S in the MIRAS family.

4 Patients and Methods

This study was approved by the ethical review board of the Hospital District of Southern Ostrobotnia, and informed consent for sample collection and DNA analysis was obtained from the patients and their relatives. Clinical examinations and follow-up of patients on multiple occasions and of their family members were carried out by the author during ten years in 1997–2007.

Detailed information of the patients and methods are given in the original articles I-IV. The individuals are refereed to in the text by their numbering presented in pedigrees (Figures 1–3). The new autosomal recessive ataxia described in Studies I–III was named MIRAS (MItochondrial Recessive Ataxia Syndrome) in collaboration with others (Hakonen et al. 2005), denoting the important mitochondrial aspect of this recessive ataxia syndrome.
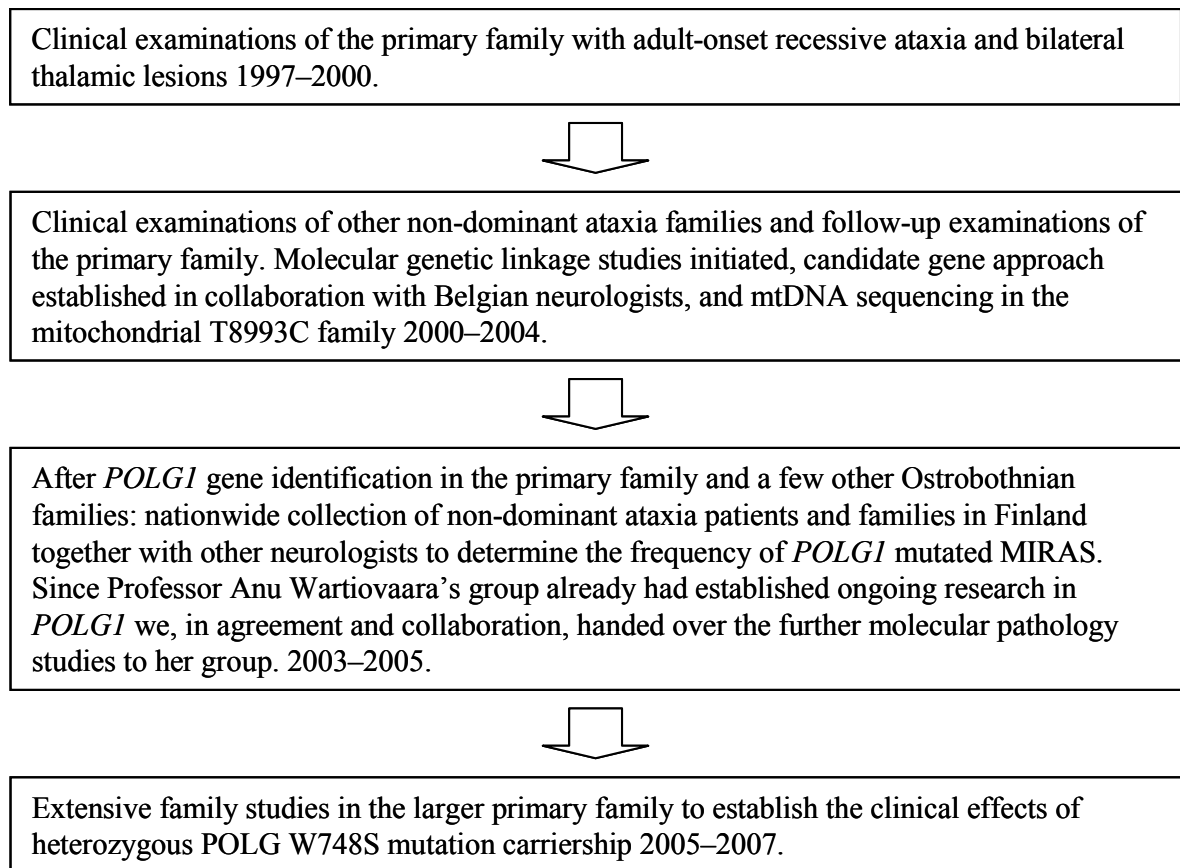
4.1 Patients

Study I. Clinical neurological examinations at Seinäjoki and Vaasa Central Hospitals identified unusual kindred with recessive adult-onset ataxia and thalamic lesions detected by MRI. Three siblings out of five were affected, whereas no other family members, including the parents, were affected by any similar disease (Figure 1).

Study II. After the first clinical report (I) we started a molecular genetic linkage project as a homozygosity mapping approach to determine the chromosomal locus of the disorder. However, before this project was concluded we were contacted by our Belgian collaborators, who had observed our clinical report and suggested to check a candidate gene in our family. Our Belgian collaborators had identified *POLG1* mutations in some of their families with similar clinical phenotype and the subsequent sequencing of *POLG1* in our patients gave a direct positive result: the homozygous W748S mutation was the cause of the disease. The collaborative report in Study II included a total of eight ataxia patients from five European families with a recessive ataxia syndrome due to homozygous or compound heterozygous *POLG1* mutations. Two of the surviving patients (F1.III-4, F1.III-5) from study I were re-examined by the author. The other six patients were two sporadic cases, one British and one Finnish, and two Belgian sib pairs from two different families.

Study III. During our primary studies of MIRAS disease, many family members reported minor neuro-logical symptoms, and some family members had even been diagnosed with neurological disease. This prompted for a careful study of possible clinical signs in heterozygous carriers of the W748S mutation, both for genetic counselling needs in the families concerned and for general health care aspects in populations with high carrier frequencies. Forty-one individuals belonging to the original MIRAS family were included to the study III (Figure 2).

Study IV. The separate non-dominant ataxia-neuropathy family compromised four affected siblings and healthy parents (Figure 3). Two of the three living sibs presented with an adult- or late-onset slowly progres-sive gait ataxia and dysarthria and polyneuropathy, and all three had peripheral axonal neuropathy.

4.2 General study design and agenda

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┌─────────────────────────────────────────────────────────────────────────┐
│ Clinical examinations of the primary family with adult-onset recessive     │
│ ataxia and bilateral thalamic lesions 1997–2000.                           │
└─────────────────────────────────────────────────────────────────────────┘
                                    ⬇
┌─────────────────────────────────────────────────────────────────────────┐
│ Clinical examinations of other non-dominant ataxia families and follow-up  │
│ examinations of the primary family. Molecular genetic linkage studies      │
│ initiated, candidate gene approach established in collaboration with        │
│ Belgian neurologists, and mtDNA sequencing in the mitochondrial T8993C      │
│ family 2000–2004.                                                          │
└─────────────────────────────────────────────────────────────────────────┘
                                    ⬇
┌─────────────────────────────────────────────────────────────────────────┐
│ After POLG1 gene identification in the primary family and a few other       │
│ Ostrobothnian families: nationwide collection of non-dominant ataxia        │
│ patients and families in Finland together with other neurologists to        │
│ determine the frequency of POLG1 mutated MIRAS. Since Professor Anu          │
│ Wartiovaara's group already had established ongoing research in POLG1 we,    │
│ in agreement and collaboration, handed over the further molecular pathology  │
│ studies to her group. 2003–2005.                                           │
└─────────────────────────────────────────────────────────────────────────┘
                                    ⬇
┌─────────────────────────────────────────────────────────────────────────┐
│ Extensive family studies in the larger primary family to establish the      │
│ clinical effects of heterozygous POLG W748S mutation carriership 2005–2007. │
└─────────────────────────────────────────────────────────────────────────┘
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4.3 Epidemiological studies of MIRAS disease in Finland (Hakonen et al. 2005)

After the gene defect in the original MIRAS family was identified, four out of five of the next studied candidate patients proved also to carry the same homozygous *POLG1* mutation. This indicated MIRAS might be relatively frequent and prompted for a larger study including the ataxia samples gathered over the years at the Medical Genetics Department in Turku. The study was extended with a founder haplotype study comparing Finnish samples with other Northern European MIRAS samples and a mutation frequency study.

4.4 Examination of patients and relatives in MIRAS families (Studies I, II, III)

The patients in the original MIRAS family (Figure 1) and all available 36 family members were examined by the author (Figure 2), including physical examination and thorough neurological examination. Reports of five deceased relatives were extracted from hospital records. For the additional six ataxia patients included in study II, clinical data was collected from hospital records by co-investigators.

4.5 Clinical investigations of the MIRAS patients (Studies I, II)

Patients F1.III-7 and F1.III-5 underwent detailed clinical investigations: brain CT and MRI imaging, electrophysiologic investigation comprising motor and antidromically determined sensory nerve conduction velocity (NCV) measurements using surface electrodes, sensory threshold measurements, EMG, tibial and medial nerve somatosensory-evoked potentials (SEP), brainstem auditory-evoked potentials (BAEP), and reversal pattern visual-evoked potentials (VEP), together with flash VEP, electroretinography and blink reflexes, and EEG. Other examinations included neuropsychologic tests, ophthalmologic examinations, electrocardiography and a set of tests to examine functions of the autonomous nervous system. Muscle and sural nerve biopsies and bone marrow aspiration were performed on Patient F1.III-7. After her sudden death, autopsy and neuropathologic studies were conducted. One of the affected individuals (Patient F1.III-4) primarily refused hospital investigations, but agreed later to electrophysiological investigations during Study II.

Comprehensive laboratory tests from blood, urine, and cerebrospinal fluid (CSF) tests were performed in Patients F1.III-7 and F1.III-5 to rule out inherited metabolic disorders or other known conditions in the differential diagnostic approach for progressive ataxia. Laboratory tests included: complete blood counts, search for acanthocytosis in the peripheral blood smears, fasting glucose, glucose tolerance, electrolytes, creatine, calcium, vitamin E, vitamin B_{12} folic acid, creatine kinase, lactate dehydrogenase, serum immu-

noglobulins, α-fetoprotein, very long chain fatty acids, phytanic acid, cholestanol, liver and thyroid function tests, cholesterol and triglyceride levels, copper and ceruloplasmin values, lactate and puryvate levels, blood ammonia and urinary organic acids. Lysosomal storage diseases tests included urinary screening for muco-polysaccharides and oligosaccharides by thin layer chromatography, and determination of the enzyme activity of arylsulfatase A, galactocerebrosidase, β-galactosidase, glucocerebrosidase, hexosaminidase A and B, and α-neuraminidase in fibroblasts. CSF protein level, cell count, and glucose, lactate, and IgG indexes were investigated.

Summary of clinical investigations performed to eight genotyped patients (Studies I, II) is presented in Table 9 in the Results.
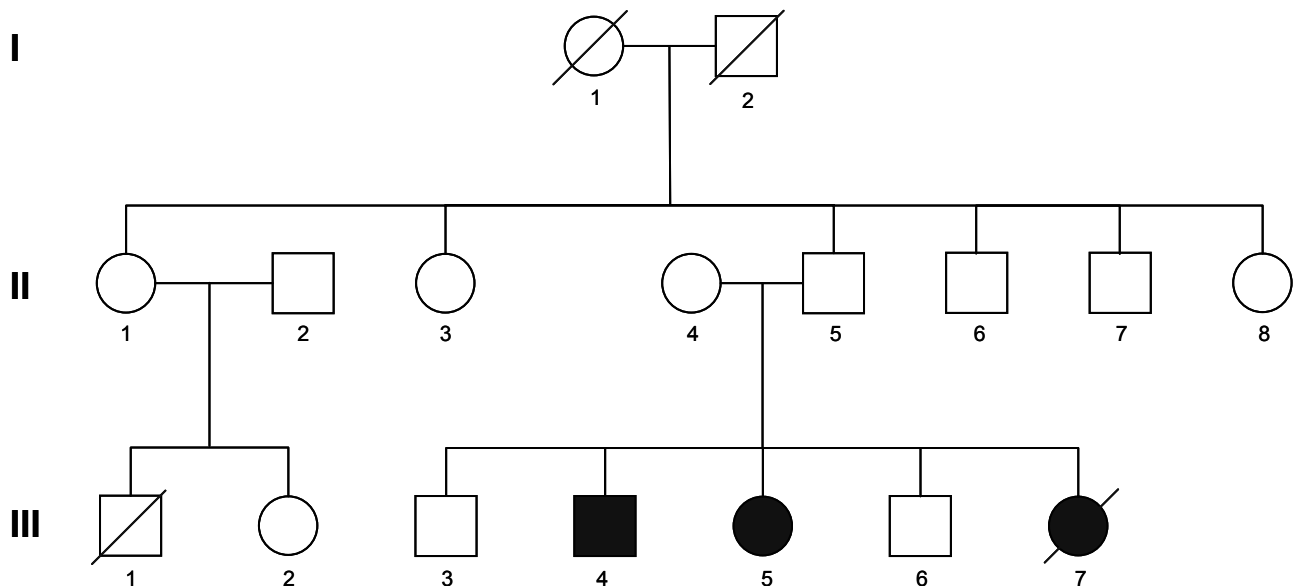
Figure 1. Pedigree from the original Finnish MIRAS family (F1). Filled symbols indicate affected family members.
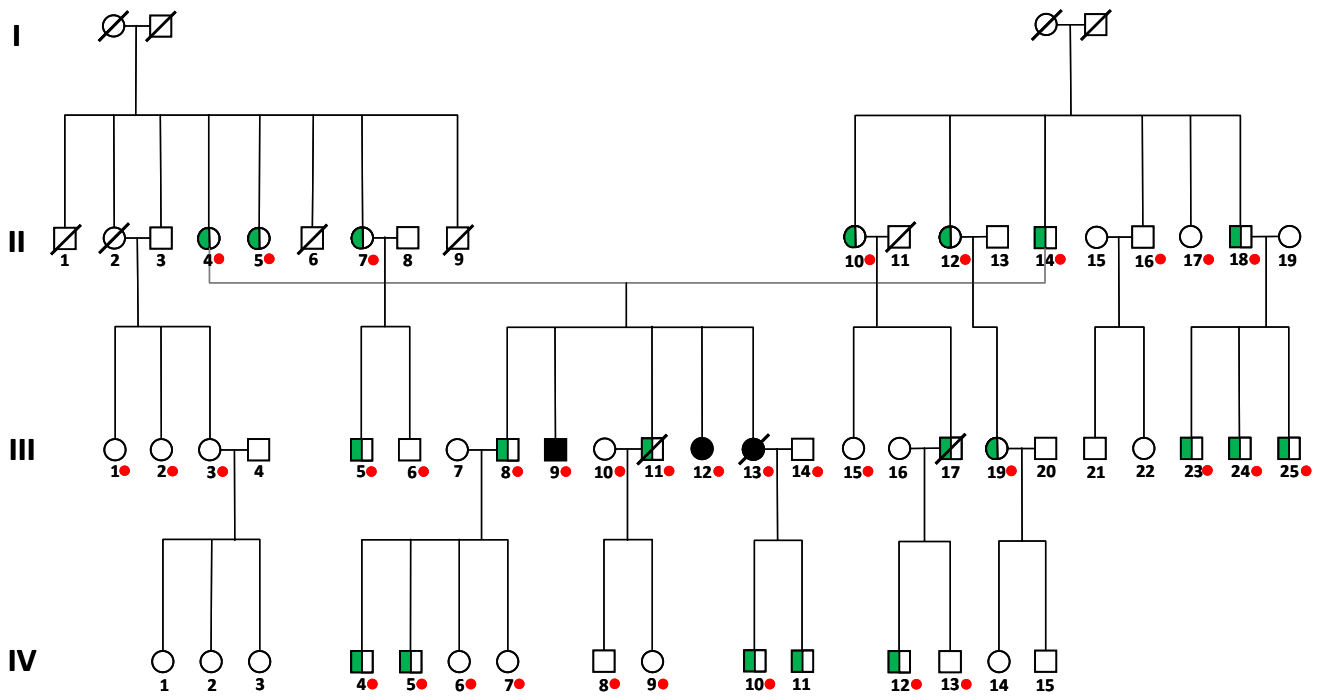
Figure 2. Pedigree of the extended family shows segregation of the POLG mutation W748S. Filled symbols indicate MIRAS patients harbouring homozygous W748S mutation and semifilled symbols indicate heterozygote carriers. Red circles beside numbers indicate available samples for DNA study.

4.6 Clinical investigations in the W748S carriers (Study III)

36 individuals were available to the clinical examination by the author. Motor and sensory NCV studies were performed in seven carriers (II-7, II-12, II-14, II-18, III-8, III-25, IV-12). During the study, one carrier (III-11) deceased, and autopsy and neuropathological examination were conducted. F-18 fluorodeoxyglucose positron emission tomography (FDG-PET) with MRI was completed in one MIRAS patient (III-12), in four carriers (III-8, III-23, IV-5, IV-12), and in six control individuals (two of the controls were non-carrier members of this family: IV-6, IV-13). Figure 2.

4.7 Examination of the non-dominant ataxia-neuropathy family (Study IV)

The detailed physical and neurological examination of the Study IV patients was performed by the author, and the medical history from other family members shown in the Figure 3 was extracted from hospital re-

cords. The results of clinical hospital investigations performed before the defined molecular cause was established are described in detailed case reports in the Results.
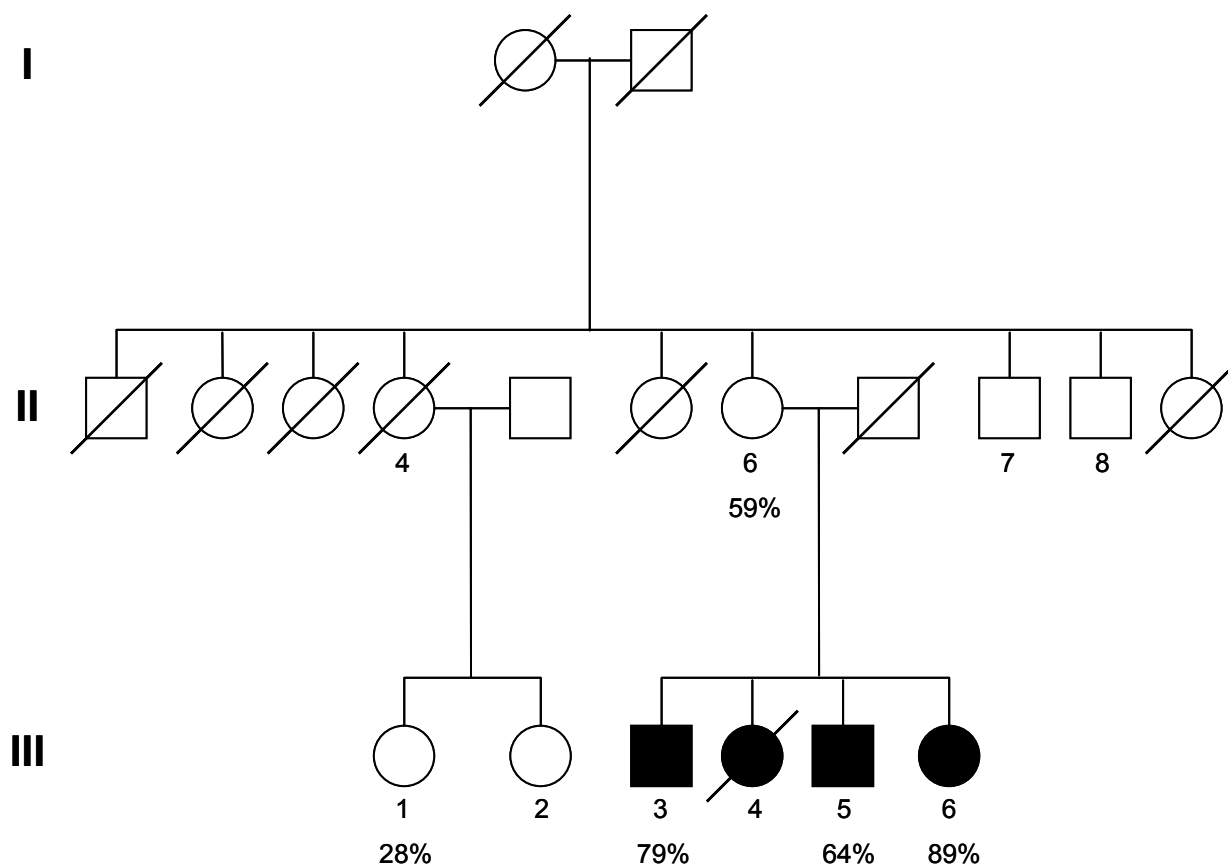
Figure 3. Pedigree of the non-dominant ataxia- neuropathy family with the 8993T>C mutation in mtDNA. Filled symbols indicate affected individuals.The proportion of the mutant genome in blood DNA is shown. None of individuals II-4, II-6, II-7 or II-8 had reported neurological symptoms. Individual III-1 has been diagnosed with multiple sclerosis with accordance with Poser's diagnostic criteria.

4.8 Molecular genetic studies

The molecular genetic methods are described in detail in the original articles and only general principles are summerized here. Genomic DNA was isolated and analyzed from peripheral blood and/or skeletal muscle samples using standard methods.

4.8.1 Linkage studies and mutation analyses (Study I)

Linkage to the *FRDA1* and *ARSACS* gene regions was evaluated by microsatellite marker analysis.

Detection of the $(GAA)_n$ expansion in intron 1 of the *FRDA1* gene was performed as previously described (Campuzano et al. 1996) with minor modifications, and gene sequencing of PCR-amplified fragments was performed to exclude other mutations of the *FRDA1* gene.

Repeat numbers in the autosomal dominant SCA types 1, 2, 3, 6, 7, 8, 10, and 12 and DRPLA were estimated from PCR-amplified fragments by gel electrophoresis.

MtDNA mutation analyses for the common point mutations at positions 8344, 3243, and 8993 associated with myoclonus epilepsy with ragged red fibers (MERRF), mitochondrial encephalomyopathy-lactid acidosis, stroke-like symptoms (MELAS), neuropathy, ataxia with retinitis pigmentosa (NARP), and mitochondrial deletion screening was carried out from muscle samples of patient F1.III-7.

4.8.2 Analysis of the *POLG1* gene (Study II)

The coding exons of *POLG1* gene were sequenced from PCR-amplified genomic DNA. In index patients carrying *POLG1* mutations, mutations in *ANT1* and *C10orf2* were subsequently excluded.

One hundred sixty-eight Belgian and 70 Finnish control individuals were examined for the presence of the c.2243G>C (W748S) and the c.3428A>G (E1143G) alterations by sequencing. Control subjects included 46 individuals from the genetic isolate of North Karelia in Finland, from which family F1 originated.

4.8.3 Mitochondrial DNA analysis (Study II)

Mitochondrial DNA deletions were evaluated both by Southern blotting and by long-range PCR methods, the latter preferentially detecting small fragments of mtDNA. Quantification of mtDNA was perfomed by Southern blotting analysis to detect possible mtDNA depletion.

Diagnostic muscle DNA samples from healthy siblings of patients with *POLG1* mutations or *POLG1* patients' leukocyte DNA were used as controls. A muscle sample of a patient with a dominant *POLG1* mutation (Y955C), known to harbour multiple mtDNA deletions, and a fibroblast DNA sample from a patient with Kearns–Sayre syndrome carrying a single mtDNA deletion in muscle were used as disease controls for PCR reactions.

4.8.4 Screening for carrier status of POLG mutation W748S (Study III)

Blood samples from 30 family members were analysed for the W748S mutation (i.e. the 2243G>C substitution) by sequencing.

4.8.5 MtDNA sequencing and measurement of mtDNA heteroplasmy (Study IV)

The entire coding region of mtDNA was analyzed from overlapping PCR-amplified fragments by conformation sensitive gel electrophoresis followed by sequencing of fragments with altered mobility. Heteroplasmy for the 8993T>C mutation was determined by restriction fragment analysis.

5 Results

5.1 Clinical features in the original MIRAS family (Studies I and II)

5.1.1 Family history and development of clinical symptoms

This family had been removed to Western Finland during population evacuations in Karelia during World War II. Genealogic studies revealed no relationship between the parents over the past 150 years. However, all four grandparents were born in the same rural area making the distant common ancestry probable. Three siblings of five were affected and both sexes were affected without major variation in the phenotype. The inheritance was most consistent with an autosomal recessive pattern. Because specific diagnosis in this family remained elusive for years even after comprehensive investigations, and because the findings were not compatible with any other established spinocerebellar ataxia, we started the scientific research in 1997 aiming to define the new entity by clinical and molecular genetic methods.

Early developmental and adolescence had been normal in all affected siblings. The presenting and major feature was progressive ataxia from age 30, which proved to be a combination of spinocerebellar and sensory deficits. Dysarthria started in their early 40's. Nystagmus and jerky pursuit eye movements suggestive of cerebellar and brainstem dysfunction were observed. Later in the fifth decade mild ptosis and moderate ophthalmoparesis of mainly central origin occurred. Mild cognitive decline was observed as an early feature of the disease in all three siblings. The index patient developed refractory epilepsy and she died at age 36, without signs of peripheral external ophthalmoplegia. Ataxia of gait and stance worsened over the years, and the patients became wheelchair dependent in their late 40's, about 20 years after first manifestations of the disease.

5.1.2 Case reports (later developments after the Study I)

Patient F1.III-4.

He developed recurrent tonic-clonic seizures at age 49. His epilepsy became seizure-free with lamotrigin treatment. Repeated examination at age 50 (study II) revealed severe progression of gait ataxia. He was able to walk only few ten meters with help of another person and a walker, but mainly he moved around with a

wheelchair. Ophthalmoparesis was exacerbated and limitation of eye movements was observed in all directions except downwards. Eye movements were less limited on single eye testing suggesting a combination of both central and peripheral paresis. Fluctuating mild ptosis had developed within the last two years. There was diffuse mild muscle weakness and slight atrophic changes in distal lower limbs. On examination at age 53, his speech was severely dysarthric with only some comprehensive words. His cognitive impairment was moderate, he was restless and needed constant surveillance. Balance difficulties progressed and he was able to walk only few meters with the support of one or two persons and walker. In 2009, he died from acute myocardial infarction at age 55.

Patient F1.III-5.

On examination at age 46 (study II) the patient showed mild bilateral ptosis. In addition to restriction of upward gaze, ocular movements were now limited mildly in abduction and adduction. Memory difficulties were aggravated and nystagmus caused impaired vision. Gait ataxia had progressed and she needed a personal assistance and a walker. Only mild weakness was observed in the lower limbs. In subsequent years ataxia progressed, and a wheelchair was necessary. At age 50, cognitive decline was moderate, and dysarthria and dysphagia were deteriorated. She needed help for her all daily activities. More recently, at age 51, she developed tonic-clonic seizures, and antiepileptic medication was initiated. EEG recording showed generalized slowing with epileptic activity in temporo-parietal regions.

5.1.3 Genetic analyses and other laboratory examinations

Investigations in previously known genetic causes of ataxia including FRDA, ARSACS and dominant SCAs had proven negative. Mitochondrial mutation analysis for large scale deletions, and common point mutations associated with MERRF, MELAS, and NARP were all negative. Laboratory screening tests for progressive ataxia associated with biochemical abnormalities remained negative. Results of basic cardiologic examination, ECG and chest-X-rays were normal. Echocardiography showed minor mitral valve insufficiency but no signs of cardiomyopathy.

5.1.4 Electrophysiologic findings

Sensory and motor CNVs were slightly to moderate reduced, and sensory action potentials in were undetectable or had markedly decreased amplitudes, corresponding to axonal neuropathy predominantly involving sensory nerves. Threshold testing exhibited impaired vibration sense, and cold and heat thresholds were also abnormal suggesting thin-fiber neuropathy. SEP and BAEP were abnormal suggesting a central disorder in the somatosensory pathway. Reversal pattern VEP, flash VEP, blink reflex, and electroretinograms were

within normal limit. Autonomic function tests showed mild cardiovascular dysfunction. EEG recording revealed diffuse backround slowing.

5.1.5 Neuropsychological findings

Extensive examination of the patients F1.III-7 and F1.III-5 confirmed cognitive and behavioural changes. The overall cognitive processing was at low-medium level: the full scale IQ was 86–88, although the primary IQ level of both patients was supposed to be normal. Verbal and visual memory and visuomotor functions were impaired. Patient F1.III-7 also had mild anomia and flattering of the affect, and both patients had some loss of insight.

5.1.6 Brain imaging findings

The index patient F1.III-7 underwent three brain MRIs, at ages 32, 33, and 35. The main findings included increased signal intensity lesions in the T2-weighted series bilaterally in the thalamus and in the upper part of the medulla, primarily in the gray matter. In addition, bilateral high-intensity signal were seen in the cerebellar white matter, and minor infra-and supratentorial atrophy. Brain CT performed at age 32 was normal.

Patient F1.III-5 underwent a brain MRI at age 40. Thalamic and cerebellar high signal lesions and mild atrophy similar to those observed in her sister were detected in T2-weighted series. Cerebellar atrophy had progressed to a moderate stage on MRI at age 48 (study III). Symmetric low density areas in the thalamus and cerebellar white matter were also observed on brain CT performed at age 37.

5.1.7 Neuropathology findings

Light and electron microscopic examination of muscle biopsy specimens showed no significant atrophy, no findings suggesting storage or mitochondrial disease. Distal sural nerve biopsy showed marked decrease of large myelinated fibers, but no active demyelination; these findings were consistent with axonal neuropathy. On bone marrow examinations no findings indicating storage disease were detected.

On autopsy (Figure 4) degenerative pathology was observed in the cerebellum, brainstem, and spinal cord. In the cerebellum, the cortex was quite well preserved, except for a slight and patchy dropout of Purkinje cells, whereas severe changes were seen in the dentate. In the pulvinar and dorsomedial nuclei of the thalamus autopsy revealed neuronal degeneration with a peculiar vacuolar change, probably representing a transsynaptic degeneration in response to deafferentation. Prion immunoreactive was negative excluding

CJD disorders. Cerebral hemipheres, basal ganglia, hippocampi and white matter were well preserved. No signs of neuronal storage disease could be observed. Autopsy findings were not compatible with mitochondrial Leigh's disease. Similar findings have been detected in subsequent autopsies of a few Finnish patients with MIRAS ataxia (Anders Paetau, personal communication).
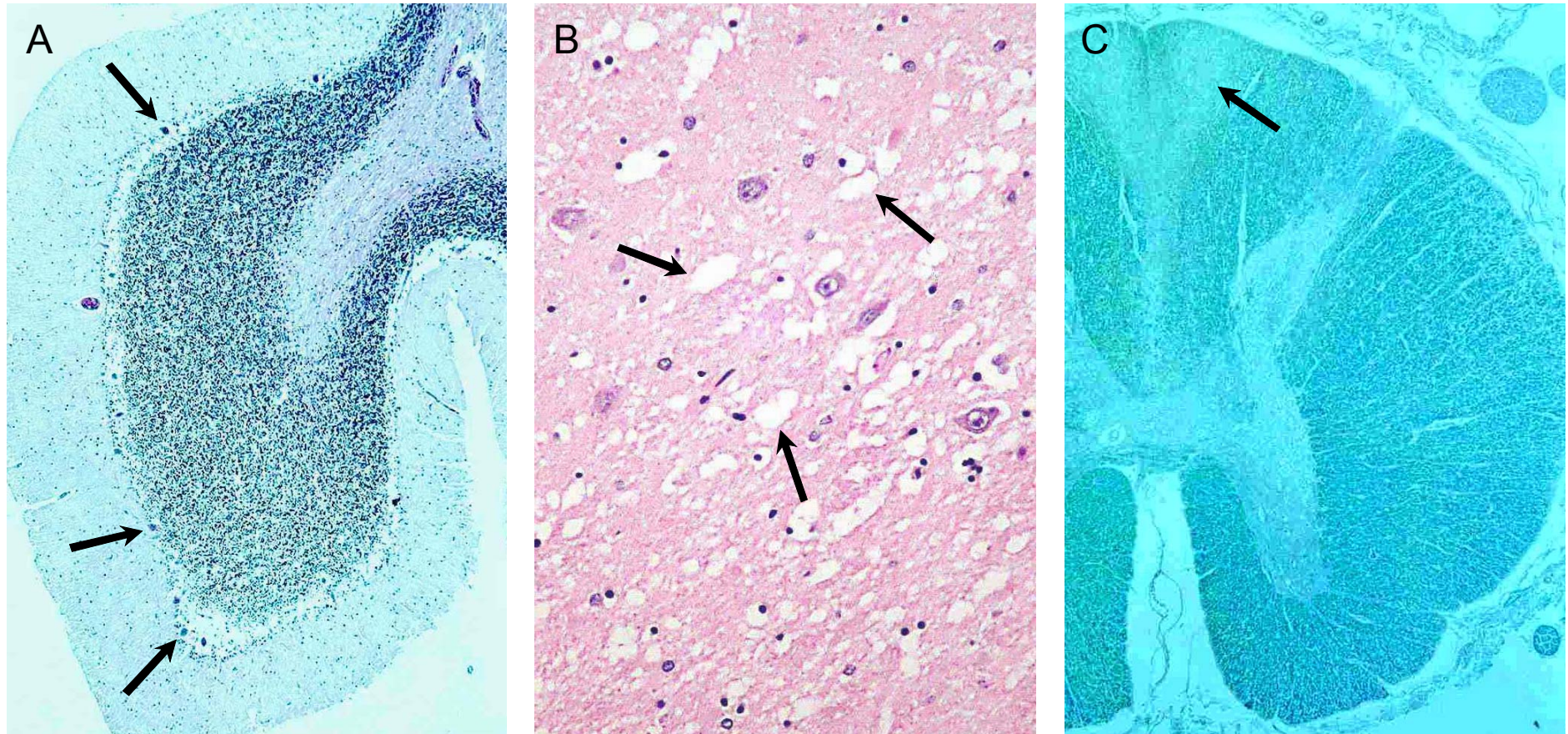
Figure 4. Neuropathology findings of MIRAS patient.

A) Cerebellar cortex with patchy drop-out of Purkinje cells. Arrows indicate few preserved Purkinje cells. Luxor fast blue (LFB) / cresyl -violet staining.

B) A vacuolar change (arrows) and some neuronal loss can be seen in this section of the dorsomedial thalamic nucleus. Immunohistochemistry for PrP was negative, so the change probably represents transsynaptic degeneration. C) Spinal cord at C7 level. Posterior columns, especially the gracile (arrow), shows pallor of myelin staining. LFB staining. Paraffin sections; original magnification x 40 (A), x 200 (B), x 20 (C).

5.2 Clinical features of one sporadic Finnish MIRAS patient (Study II)

Study II characterized clinical features of totally eight European ataxia patients harbouring *POLG1* mutations (Table 9), includig three siblings from the original MIRAS family and one additional Finnish male patient (F2.II-1). This man was diagnosed with metabolic syndrome. At age 30, he noted disturbed balance, and ataxia and severe axonal neuropathy were diagnosed. On examination at age 32, he had slowed ocular pursuit movements but full range of ocular motility. He had severe gait ataxia, clumsiness of hands, and dysarthric speech. Brain MRI showed bilateral symmetric intense signals in the cerebellar white matter.

Laboratory features of all eight MIRAS patients (Study I and II) are summarized in Table 9.

Table 9. Laboratory features of patients presenting with ataxia caused by recessive mutations in POLG1 gene.

Findings	F1.III-4	F1.III-5	F1.III-7	F2.II-1	B1.II-3	B2.IV-1	B2.IV-2	UK1.II-1
	Gender/ age, yrs at study							
	M/50	F/46	F/36	M/33	M/39	M/49	F/47	M/52
Lactic acidosis	NP	–	–	–	++	NP	NP	NP
Elevated ck	–	–	–	–	+	–	–	–
CSF protein	NP	normal	normal	normal	elevated	NP	NP	NP
CSF lactate	NP	normal	normal	NP	normal	NP	NP	NP
EEG background slowing	NP	–	++	NP	++	NP	–	NP
Axonal neuropathy								NP
Sensory	+++	+++	+++	+++	+++	+++	+++	
Motor	+	+	+	+	+	+	–	
Neurogenic EMG	+	+	–	++	–	++	–	NP
BAEP abnormalities	NP	NP	+	NP	NP	NP	+	NP
MRI lesions	NP	thalamus med. oblongata cerebellum	thalamus med. oblongata cerebellum	cerebellum	–	cerebellum	–	minor atrophy
Muscle biopsy / age, yrs	NP	NP	31	33	34	NP	NP	52
Ragged red fibers			–	–	–			–
EM mt abnormalities			–	NP	+			NP
Muscle mtDNA	NP	NP				NP	NP	NP
Southern blot			–	–	–			
Long PCR mtDNA			deletions	NP	deletions			
Nerve biopsy / age, yrs	NP	NP	31	NP	NP	NP	NP	52
Axonal neuropathy			+					+

+ Finding present; ++ prominent finding; +++ severe finding; – finding absent; NP not performed

5.3 Molecular genetic findings in MIRAS disease (Study II)

5.3.1 *POLG1* analysis

A new pathogenic mutation in *POLG1*, c.2243G>C, causing a W748S substitution, was identified in all four Finnish ataxia patients (F1.III-4, -5, and -7 and F2.II-1). This mutation occurred in the homozygous state in all patients and in the heterozygous state in unaffected siblings and parents of Family F1. The British ataxia patient was compound heterozygous for W748S and for the previously reported A467T mutation (Van Goethem et al. 2001). Three Belgian patients were homozygous for the previously known A467T mutation.

In addition, all four Finnish patients and the British patient also carried the c.3428A>G change, predicting an E1143G substitution, previously designed as a polymorphism (http://www.ncbi.nlm.nih.gov/SNP/). E1143G always occurred together with W748S.

In two Finnish families F1 and F2, haplotype analysis using five informative polymorphic DNA markers surrounding *POLG1,* revealed a common ancestral origin of the W748S/E1143G mutation.

5.3.2 Mitochondrial DNA analysis

Southern blot hybridization of total muscle DNA showed the 16.5-kb signal derived from normal mtDNA in control samples, in muscle of Patient F1.III-7, and in leucocytes of Patient F2.II-1. Absence of muscle mtDNA deletions on diagnostic Southern analysis correlated with the absence of clinical and morphologic abnormalities in muscle. However, long-range PCR, which selectively amplified short deleted molecules, did show low levels of multiple mtDNA deletions in muscle samples of Patients B1.II-3 and F1.III-7, suggesting that the proportion of deleted mtDNA in muscle might be < 5%. MtDNA depletion was not present in any of the patients.

5.4 Epidemiological features in Finnish MIRAS (Hakonen et al. 2005)

In the collaborative study (Hakonen et al. 2005) the homozygous W748S mutation in the *POLG1* gene, with carrier frequency 1:125, was found to be the most common hereditary ataxia in Finland. Genotyping in the Finnish patients showed three different haplotypes, suggesting the major introduction of the mutation came

with the Finnish founder population from the south-east, and minor imports came, probably with settlers, from the south-west and west.

5.5 Clinical phenotype in W748S carriers of the original MIRAS family (Study III)

20 carriers and 17 non-carriers were found, and in four deceased individuals the genetic status remained unknown (Fig. 2). Summary of medical history and neurological examinations is given in the original article. Neurological symptoms and signs were present in a number of family members. Clinical examination of family members showed no neurological findings of ataxic disorder or peripheral neuropathy. Some members were diagnosed with Alzheimer disease, epilepsy or migraine, or with different psychiatric conditions. However, clearly defined neurological diseases did not segregate consistently with the W748S mutation.

5.5.1 Electrophysiological findings

All seven examined carriers showed normal motor and sensory NCVs. Distal sensory and motor amplitude response latencies were within normal limits, but five out of seven carriers showed slightly low distal sensory amplitudes (SD values between –1.0 and –2.5). In lack of other known causes for incipient axonal sensory neuropathy, these slightly low or borderline low distal sensory amplitudes in a majority of examined carriers indicate a subclinical sensory neuropathy manifestation in carriers.

5.5.2 Neuropathology findings

III-11 (W748S carrier, sudden death at age 49): Distinct spinocerebellar pathology as we reported in a homozygous MIRAS patient was not seen in this individual. The only slightly pathologic finding was a minimal dropout of Purkinje cells in the cerebellar cortex.

5.5.3 PET and MRI findings

FDG-PET imaging in the MIRAS patient at age 48 showed reduced glucose metabolism in all examined brain regions. Greatest declines were seen in the thalamus (55% of the control value), cerebellum (59%) and occipital cortex (75%). T2-weighted MRI showed moderate cerebellar atrophy and minor supratentorial cortical atrophic changes. In addition, the previously reported increased signal lesions bilaterally in the cerebellar white matter, in the thalamus and upper part of medulla oblongata were present.

In two out of four carriers decreased glucose metabolism levels were detected. They had no deficits in their cognitive capacity. In the 56-years-old carrier (III-8) the cerebral glucose metabolism was reduced to 80–95%. Greatest declines were found in the temporal cortex (80%), cerebellum (81%) and hippocampus (83%) On MRI, only three small subcortical ischemic lesions in the parietal and frontal white matter were observed, but no atrophic changes were seen.

In the other W748S carrier (III-23), at age 41, also considerable decline (64–79%) in all brain regions was detected, most prominent in the medial frontal cortex (64%) and thalamus (65%). Brain MRI was normal. In two other younger carriers (IV-5, age 29 years; IV-12, age 32 years) no significant changes were detected in PET or in MRI.

5.6 The separate non-dominant neuropathy-ataxia family (Study IV)

5.6.1 Case reports including later developments

Pedigree of the family with the mtDNA 8993T>C mutation is shown in Figure 3. The father had died at age 90 and the mother was still alive at age 89 years, both without neuromuscular symptoms. One sibling had died at age 19 because of early-onset spinocerebellar ataxia, determined and diagnosed as Friedreich's ataxia at autopsy in the 1960's. Her younger sister was referred to neurological evaluation at age 36 because of slowly progressive ataxia and polyneuropathy starting eight years earlier. Other aetiologies than Friedriech's ataxia had not been considered in this family before the gene test for *FRDA1* expansion mutation was available and proved to be negative. In spite of comprehensive laboratory examinations the specific diagnosis remained unsolved.

After several years the two brothers of the proband also presented with neurological symptoms, one with ataxia and polyneuropathy and the other with polyneuropathy only. Because the neurological disease had ultimately segregated to all four children with highly variable severity, a mtDNA mutation was further suspected. Previous screening for the common mtDNA point mutations, for large scale deletions and rearrangements analyzed by Southern blot analysis, and a muscle biopsy, proved to be negative regarding mitochondrial abnormalities. Sequencing of the entire mtDNA was then initiated.

Patient III-6. At age 51, her gait was grossly ataxic and she used a walker. Romber's test was positive. She had mild limb ataxia and dysarthria. She showed gaze-evoked nystagmus, but eye movements were un-

restricted and fundoscopy was normal. There was mild muscle weakness and atrophy. Memory and cognitive functions were normal.

Brain MRI showed mild supratentorial and infratentorial atrophy. Electrophysiological studies were compatible with axonal sensorimotor polyneuropathy (NCV upper limb: sensory 51 m/sec, motor 56 m/sec; NCV lower limb: sensory 40 m/sec, motor 36 m/sec). Histological examination of muscle biopsy revealed mild chronic neurogenic changes, and no COX deficient or ragged red fibers were detected. CK was mildly elevated and lactate was normal.

Patient III-3. Slight balance difficulties and parestesias of legs manifested at age of 57 years. Clinical examination at age 65 showed wide-based walking and mild distal leg muscle weakness, but no overt ataxia nor dysarthria or retinitis pigmentosa. Brain MRI showed slight periventricular white matter abnormalities and mild atrophy in cerebellar vermis. Electrophysiological examination revealed severe axonal sensorimotor polyneuropathy (NCV upper limb: sensory 46 m/sec, motor 56 m/sec; NCV lower limb: sensory no potential, motor 39 m/sec). Serum lactate and CK levels were normal. Muscle histology was normal.

Patient III-5. Manifest muscle fatique and balance difficulties occurred at 45 years. On examination at age 58 his gait was ataxic, but he could walk without support. Romberg´s test was positive. His speech was dysarthric and pursuit eye movements were jerky, but fundoscopy was normal. Limb movements were slightly dysmetric. Brain computed tomography scan was normal. Muscle histology and electrophysiological studies confirmed axonal sensorimotor neuropathy.

5.6.2 Genetic analysis results

Sequencing of the entire mtDNA coding region revealed the 8993T>C mutation in the ATP synthase subunit 6 gene (*MTATP6)*, which has previously been described to be associated with infantile or childhood-onset phenotypes, ranging from Leigh syndrome to neurogenic weakness, ataxia and retinitis pigmentosa (NARP) syndrome. The mutation was heteroplasmic in blood and was found in several family members (Figure 3). The proportion of the mutant genome was 89% in the proband (III-6), 79% (III-3) and 64% (III-5) in her two affected older brothers, and 59% in their unaffected mother. Interestingly, the mutation was also found at a low heteroplasmy (28%) in a maternal cousin (III-1), who had been diagnosed with relapsing remitting multiple sclerosis at age 35. However, the very low degree of 8993T>C heteroplasmy primarily suggests an independent cause of her MS disease.

6 Discussion

6.1 Clinical features of MIRAS

The first ever description of MIRAS patients in Study I reported three Finnish adult siblings presenting after the age of 30 years with gait and limb ataxia, dysarthria, nystagmus, axonal mainly sensory neuropathy, mild cognitive impairment, and in one patient, epilepsy. The main differential diagnosis for this disorder was late-onset FRDA (Schöls et al. 1997a). MRI, however, showed bilateral symmetric thalamic lesions and white matter changes in the cerebellum constituting a characteristic finding that had not been reported in FRDA or any other recessive ataxia. Already on clinical grounds we classified this recessive ataxia with thalamic lesions as a probable distinct new clinical entity.

The logical next step was to search for the causative gene defect. Because of the family structure remote consanguinity in the parents of the patients was most likely and thus a genome wide scan as a homozygosity mapping project was initiated. Halfway through the genome we were contacted by the Belgian collaborators who suggested a candidate gene approach. Their candidate gene, *POLG1*, then proved to be the successful hit. This new entity of recessive ataxia reported in this thesis, MIRAS, was found to be associated with homozygous or compound heterozygous POLG mutations W748S and A467T (I, II). The novel W748S mutation was identified in all our Finnish patients and this mutation was always found to co-segregate *in cis* with E1143G (II, Hakonen et al. 2005). The latter was previously considered a polymorphism, but its functional significance and contribution to the phenotype remains unsettled (Hisama et al. 2005, Winterthun et al. 2005, Chan et al. 2006, Horvath et al. 2006, Bindoff 2007, Craig et al. 2007). Belgian patients were homozygous for the previously known A467T, and, in a British patient W748S occurred in compound heterozygosity with A467T.

The study II included totally eight genotyped patients with *POLG1* mutations, from five unrelated European families. In addition, the ninth case from Belgian MIRAS family had been examined for acute fatal encephalopathy at age 17, but DNA of this patient was not available for molecular genetic studies. The most common first symptom and major feature in the patients was progressive gait unsteadiness/ataxia. The range of the onset in disease symptoms varied from 12 to 30 years. The differences in phenotypic expression were large, and data on familial patients showed that clinical variability was larger between families than within families, suggesting a contribution from the genetic background in the phenotypic expression of recessive *POLG1* mutations. Three patients of eight were diagnosed with epilepsy, and in one, epileptic seizures pre-

ceded ataxia for five years. Valproate-induced hepatotoxicity was observed in one patient, indicating that hepatocytes with a low mitotic index may become affected with sodium valproate treatment. Thus, *POLG1* mutations should be considered in syndromes with epilepsy and adverse reaction on sodium valproate (Ferrari et al. 2005, Tzoulis et al. 2006). One patient had hand tremor as the first symptom at age 12, followed by tingling in both hands and legs in his late 20's, and then followed by ataxia in the early fourth decade. In some patients, ataxia occurred in combination with other CNS abnormalities, such as myoclonus, transient hemiparesis, cognitive decline, psychiatric symptoms and seizures. Two of eight patients died at ages 36 and 39 years without extraocular muscle involvement. In the other six patients, PEO was absent at clinical presentation, although minor signs of incipient PEO appeared as a late feature. Gastrointestinal dysmotility, weight loss or overweight, and cardiomyopathy were observed in some patients. Electrophysiological findings and/or absent or decreased reflexes in lower extremities indicating polyneuropathy was observed in all patients. Some patients may show sensory neuropathy only. Lactate levels in the cerebrospinal fluid were normal in the three patients tested. Notably, there was no apparent skeletal muscle involvement. Typical findings of mitochondrial disease, such as RRFs, COX-negative fibres and deletions on Southern blot, were absent. However, long-range PCR did show low levels of multiple mtDNA deletions in analysed muscle samples suggesting this method can be used for screening purposes. Recently, MIRAS was also associated with multiple mtDNA deletions and subtle mtDNA depletion in the brain (Hakonen et al. 2008). FDG-PET imaging showed declines in two different cerebral regions: in the thalamus and in the cerebellum, correlating well with atrophy and other degenerative changes observed on MRI. In addition, decreased glucose metabolism in temporal regions, striatum and hippocampus without evident MRI abnormality suggest that metabolic changes precede atrophic loss of tissue.

Our later unpublished experience with MIRAS syndrome largely follows the phenotypic range outlined above. However, the phenotype variation is definitely larger, including patients with very early-onset undetermined clumsiness, neuropsychiatric symptoms causing the major disability in adolescence and neuropathy occurring as a pure sensory neuropathy. The course of the disease varies, but often it seems to be quite rapidly progressive leading to a severe disability because of incapable of moving and cognitive decline, requiring permanent institutional care before the age of 50 years.

Further studies in patients from Finland, Norway and other European countries have completed the phenotype of MIRAS disease. The large collaborative study in Finland (Hakonen et al. 2005), including 19 patients with homozygous W748S+E1143G mutations, confirmed the phenotypic variation ranging from acute encephalopathy in adolescence to late-onset polyneuropathy. Epilepsy was seen in 50% of cases and could be the only symptom of the patient for years, sometimes developing to treatment resistant grand mal seizures, recurrent status epilepticus and death. Abnormal eye movements were not were not typical for external ophthalmoplegia, but mainly coordination problems of central origin. Histological and biochemical findings characteristic to mitochondrial disease were lacking. The MRI findings, described initially in our previous

report of the index patients (I, II), were established as being typical for the disease and seen in most cases: symmetrical lesions of high signal intensity in the white matter of the cerebellum, and minor atrophy of the cerebellum or the vermis, sometimes combined with symmetrical high signal lesions of the thalamic nuclei. These patients lack any evidence of hepatic involvement. However, recently the homozygous W748S mutation was detected in three Finnish patients with juvenile-onset Alpers syndrome, migraine, intractable seizures or status epilepticus and acute liver failure (Uusimaa et al. 2008).

Previous reports on patients with *POLG1* mutations described disorders associated with CNS features and neuropathy in some (Lamantea et al. 2002, Van Goethem et al. 2003a, Van Goethem et al. 2003b, Van Goethem et al. 2003c). However, in all these patients PEO was a consistent feature, except for one teenager with myoclonus (Van Goethem et al. 2003b). One sporadic patient was reported with the clinical triad of sensory ataxic neuropathy, dysarthria, and ophthalmoplegia (SANDO) (Van Goethem et al. 2003c). In SANDO syndrome sensory ataxia due to peripheral nerve involvement can precede PEO by several decades (Fadic et al. 1997, Van Goethem et al. 2003a). Our patients differed from those with SANDO phenotype because of the clear evidence of central lesions in cerebellum, brainstem and thalamus connected to the ataxia and late ophthalmoparesis in MIRAS. A key clinical point is that both in SANDO and MIRAS phenotypes muscle biopsy may lack findings of mitochondrial myopathy on light microscopy, on biochemical analysis of respiratory chain enzymes and on diagnostic Southern analysis of mtDNA rearrangements (I, II, Hakonen et al. 2005, Winterthun et al. 2005, Tzoulis et al. 2006). Recently, *POLG1* mutations have been detected in patients having axonal Charcot-Marie-Tooth disease and subsequently developing profound cerebellar ataxia and tremor but without ophthalmoplegia (Harrower et al. 2008).

6.2 Epidemiology of MIRAS

MIRAS has proven to be the most prevalent hereditary ataxia in Finland and common in Northern Europe (Hakonen et al. 2005, Tzoulis et al. 2006, Bindoff 2007, Craig et al. 2007, Hakonen et al. 2007, Criscuolo et al. 2008). In Finland more than 40 patients have been identified. The carrier frequency for W748S is very high in the general population in Finland 1:125 (Hakonen et al. 2005), for A467T in Sweden 1:200 (Kollberg et al. 2006) and for both mutations in Norway 1:100 (Tzoulis et al. 2006), indicating that MIRAS is common in Scandinavia.

In Norway, Winterthun and co-workers reported six patients with recessively inherited ataxic syndrome caused by the A467T and W748S mutations confirming our observation that mutations in *POLG1* are a new important cause of recessively inherited ataxia (Winterthun et al. 2005). Following the diagnosis of a large number of MIRAS cases in Scandinavia several subsequent reports have been published.

Tzoulis and co-workers reported 26 patients belonging to 20 families caused by the A467T and W748S mutations (Tzoulis et al. 2006). This study demonstrates the clinical spectrum of disorder that combines features of Alpers disease and a later onset mitochondrial cerebellar ataxia with epilepsy and headache. Irrespective of genotype, the patients exhibited a progressive neurological disorder usually starting in their teens (ranging from 2 to 32 years), characterized by epilepsy, headache, ataxia, neuropathy, myoclonus and late onset ophthalmoplegia, delayed cognitive and psychomotor development and subsequent cognitive decline. Epilepsy was found to be one of the most common manifestations; epilepsy was present in 76% of all patients and in the majority of these there was an occipital EEG focus. MIRAS is also termed SCA-E or MSCAE (Mitochondrial spinocerebellar ataxia with epilepsy) in the literature emphasizing epilepsy as a frequent clinical manifestation in patients with *POLG1*-linked ataxia.

In a later study including 19 patients with *POLG1* mutations and seizures (Engelsen et al. 2008), all patients developed status epilepticus and 11 deaths were all related to prolonged convulsive status epilepticus, including two patients with liver failure apparently precipitated by treatment with sodium valproate. The median survival time for all patients, regardless of mutation, was eight years following the onset of epilepsy, highlighting the need for close follow-up and lifelong anti-epileptic treatment in those who developed epilepsy.

6.3 Carriers of POLG mutation W748S show minor subclinical disease manifestations

Although segregation of the MIRAS phenotype in families has shown an autosomal recessive inheritance, some recent observations have raised the question about a possible dominant effect of the MIRAS mutations. Clinical and functional studies have indicated that the A467T can also behave as a dominant mutation causing a mild phenotype with late-onset ptosis (Luoma et al. 2005). In addition, a recent study reported a heterozygous W748S mutation carrier, who developed epilepsy, ataxia, mild parkinsonism and peripheral neuropathy in late adulthood, suggesting the possibility of a dominant effect also with the W748S mutation (Tzoulis et al. 2006, Bindoff 2007). Patients with compound heterozygosity of the W748S and A467T mutations had significantly more severe phenotypes and poorer survival compared to patients being homozygous for either the A467T or W748S, suggesting a possible co-dominant negative effect (Tzoulis et al. 2006).

The original large MIRAS family was re-visited to investigate whether the heterozygous W748S carriers manifest disease symptoms or not. Clinically, none of the carriers showed signs of ataxia or peripheral neuropathy, but the carriers had uniformly low/borderline sensory amplitudes on electrophysiological examination. Diabetes, heart disease, hypertension and depression occurred commonly as did dementia, but none of these could be specifically related to the presence of the *POLG1* mutation. Three individuals with Alz-

heimer's disease and the majority of individuals with psychiatric symptoms were clustered in the maternal branch of the family, whereas the paternal branch showed no increased prevalence of neurological problems. This asymmetric segregation of neurological symptoms in the family, as well as lack of complete co-segregation with the W748S status suggests that these disorders probably were mediated by other inherited genetic factors in the maternal branch of the pedigree.

Episodic symptoms such us migraine-like headache and epilepsy can present in MIRAS (Tzoulis et al. 2006), but are also fairly common symptoms in the general population (Hirtz et al. 2007). In this family, migraine was more frequent in the non-carrier group. Epilepsy occurred in three heterozygous individuals, although, in two this was related to excessive alcohol consumption. Autopsy findings in one carrier showed only slight loss of Purkinje cells in the cerebellar cortex. These subtle atrophic changes in the cerebellar cortex were considered to be caused by chronic alcohol abuse (Harper 2007). However, involvement of the heterozygous *POLG1* mutation cannot be excluded, because the patient's alcohol abuse, toxic for cerebellum, may have exacerbated effects of the W748S heterozygosity.

The conclusion of this study was that no definite clinically manifest phenotype could be associated with W748S heterozygosity. However, mild sensory neuropathy seems to occur as a frequent subclinical finding on electrophysiological studies. Moreover, in two out of four W748S carriers FDG-PET study showed decreased glucose metabolism levels. Because of the independent occurrence of Alzheimer disease in this family it is tempting to speculate that PET abnormalities, found especially in the temporal cortex and hippocampus, could be due to such another genetic background. However, FDG-PET in these individuals with normal cognitive capacity revealed reduced glucose metabolism also in the cerebellum without findings on MRI. Hypometabolism in this region has not been associated with early Alzheimer pathology (Mosconi 2005). Thus, subclinical CNS abnormalities as shown by PET results and susceptibility to develop neurological symptoms when combined with environmental toxic or genetic factors can not be excluded as consequences of W748S mutation carrier status. These PET abnormalities in two of the heterozygous carriers are of considerable interest and implicate further investigations, not the least because there are some 40 000 mutation carriers in the Finnish population.

6.4 Mutations in *POLG1*: a major cause of neurological diseases

POLG1 encodes the catalytic subunit of DNA polymerase gamma (POLG), essential for mitochondrial DNA replication and repair (Clayton 1982, Kaguni 2004). In 2001, Van Goethem and co-workers identified the first pathogenic mutations in *POLG1* associated with autosomal dominant PEO with multiple deletions and COX deficient muscle fibres (Van Goethem et al. 2001). Subsequently, mutations of *POLG1* were found to be the most important cause of both dominant and recessive PEO (AD- or AR-PEO) with mitochondrial

myopathy (Lamantea et al. 2002). Soon clinical heterogeneity with *POLG1* mutations became apparent as myopathy, ataxia, peripheral neuropathy, parkinsonism, psychiatric syndromes, myoclonus epilepsy mimicking MERRF and gastrointestinal symptoms mimicking mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) were found in various families (Van Goethem et al. 2003b, Van Goethem et al. 2003c, Luoma et al. 2004, II, Winterthun et al. 2005, Van Goethem 2006). Over the recent years, mutations in *POLG1* have been linked to a wide range of neurological diseases with heterogeneous clinical presentations in adults and children (Luoma 2007, Wong et al. 2008). Importantly, *POLG1* mutations have also been described in patients with AD or AR-PEO and parkinsonism, or with neuropathy and parkinsonism without PEO (Luoma et al. 2004, Mancuso et al. 2004, Davidzon et al. 2006, Remes et al. 2008). A continuous spectrum of *POLG1*-related disorders has been reported from severe progressive encephalopathies such us Alpers-Huttenlocher syndrome (Naviaux and Nguyen 2004, Davidzon et al. 2005, Ferrari et al. 2005, Kollberg et al. 2006, Nguyen et al. 2006, Wiltshire et al. 2008) to milder phenotypes such as sensory neuropathy or late-onset ptosis (Di Fonzo et al. 2003, Luoma et al. 2005, Horvath et al. 2006). Although some patients may develope classic features of mitochondrial disease in the early course, a large proportion of patients have common neurological symptoms, such as epilepsy or migraine, as the only manifestation of disease for many years (Tzoulis et al. 2006, Engelsen et al. 2008). Isolated mild manifestations such as fatigue, muscle weakness, and muscle pain have also been observed with *POLG1* mutations (Horvath et al. 2006). The A478T and W748S are the most common mutations in the ataxia-neuropathy spectrum of *POLG1*-disorders, but have also been associated with classic Alpers-Huttenlocher hepatocerebral syndrome and related childhood encephalopathies, and also with severe epilepsy and liver failure in adults (Copeland 2008, Uusimaa et al. 2008, Wong et al. 2008, Stewart et al. 2009).

The expanding spectrum of *POLG1* –related diseases is characterized by multiple mtDNA deletions or quantitative loss of this genome, mtDNA depletion, in affected tissues. Accumulation of these secondary mtDNA defects lead to biochemical dysfunction of the respiratory chain, which at least partly determine the disease phenotypes (Longley et al. 2005, Luo and Kaguni 2005, Luoma et al. 2005, Graziewicz et al. 2006, Horvath et al. 2006, Hudson and Chinnery 2006, Hakonen et al. 2008, Krishnan et al. 2008). Most *POLG1* mutations affect post-mitotic tissues such us neuronal parenchyme, cardiac and skeletal muscle, and tissues with low mitotic index such us liver (Van Goethem 2006). Since 2001, over 160 pathogenic mutations have been identified. An updated mutation database can be found at http://tools.niehs.nih.gov/polg/index.cfm. Over the last few years it has become clear that *POLG1* is a major human disease gene, possibly accounting for up to 25% of all adult presentations of mitochondrial disease (Chinnery and Zeviani 2008). Defining the molecular genetic diagnosis in *POLG1*-related disease may be challenging. Some *POLG1* mutations can behave as both dominant and recessive alleles (Luoma et al. 2005, Tzoulis et. al 2006, Bindoff 2007). Moreover, some patients have even three or four different *POLG1* mutations. Both the A467T and the W748S are recurrent in many recessively inherited conditions, which may help diagnostic workout in suspected cases (II, Hakonen et al. 2005, Winterthun et al. 2005, Horvath et al. 2006, Tzoulis et al. 2006). It is not clear why

the same mutations can cause severe depletion of mtDNA in Alpers-Huttenlocher syndrome, or a mild late-onset PEO phenotype with no depletion and hardly detectable multiple deletions. Polymorphic genetic variants (found in up to 4% of the population), seem to be able to modulate the phenotypic outcome, possibly explaining some of the observed clinical heterogeneity (Chan and Copeland 2008, Chinnery and Zeviani 2008).

6.5 Adult-onset ataxia and polyneuropathy caused by the 8993T>C mitochondrial DNA mutation

Mitochondrial dysfunction is involved in the pathogenesis of several recessively inherited ataxias, such as in Friedreich's ataxia, MIRAS and IOSCA. Mitochondrial diseases caused by primary mtDNA defects may also cause cerebellar ataxia, with or without corticospinal tract involvement, and have to be considered in the differential diagnosis of hereditary ataxias. Large single mtDNA rearrangements are usually sporadic and may cause ataxia as part of Kearns Sayre syndrome. Myopathy, hearing loss, seizures, polyneuropathy, pigmentary retinopathy or, more rarely, movement disorders may be diagnostic clues suggestive of mtDNA disease in ataxia patients (Finsterer 2006). Sometimes ataxia can be an important feature of the clinical phenotype, as in the MERRF caused by the 8344A>G mtDNA mutation, and in syndromes associated with mtDNA deletions (Chinnery and Schon 2003, Zeviani and Di Donato 2004). In adults, the most common mtDNA mutations are the 3243A>G causing MELAS, and the 11778G>A and 3460G>A causing Leber's hereditary optic neuropathy LHON (Majamaa et al. 1998, Man et al. 2003, Schaefer et al. 2008), but these mutations are rarely associated with adult-onset ataxia (Majamaa et al. 1998, Chinnery et al. 2002).

We identified a heteroplasmic 8993T>C mtDNA mutation in a Finnish kindred with adult-onset slowly progressive ataxia and polyneuropathy. The 8993T>C mutation in the ATP synthase *MTATP6* gene has previously been described in the literature to be associated with infantile or childhood-onset phenotypes, ranging from Leigh syndrome to neurogenic weakness, ataxia and retinitis pigmentosa (NARP) syndrome (Holt et al. 1990, de Vries et al. 1993, Fujii et al. 1998, Debray et al. 2007). However, adult-onset axonal polyneuropathy combined with slowly progressive ataxic gait and pyramidal signs has not been described in patients with 8993T>C. The 8993T>C mutation causes less impairment of the ATP synthase protein function compared with the more common 8993T>G mutation (Vazquez-Memije et al. 1998). This is consistent with a wider variation of the phenotype in 8993C>T patients, and possibly with a greater impact of other genetic factors, such as mtDNA haplotype or polymorphisms in nuclear genes encoding mitochondrial proteins (Debray et al. 2007). In our family one sibling had died from an early-onset disease well in line with previously reported clinical features associated with the 8993T>C mutation. Because this disease was diagnosed as Friedreich's ataxia on autopsy in the 1960's, the ataxia in the sister was also diagnosed as FRDA until DNA testing became available. Our observation of the adult-onset ataxia phenotype caused by the 8993T>C

mtDNA mutation was confirmed in the recent study by Craig and co-workers (2007). They studied 308 patients with unexplained ataxia and 98 patients with suspected Charcot-Marie-Tooth disease and found one three-generation family with heteroplasmic 8993T>C mutation. Two patients developed an adult-onset slowly progressive ataxia and axonal neuropathy, and one patient presented with episodic ataxia and transient hemiparesis, expanding the phenotype even further.

Mitochondrial abnormalities are rarely apparent in skeletal muscle in patients with *MTATP6* mutations (Filosto and Mancuso 2007). The correct diagnosis may easily be missed, when these clinical features occur in a sporadic patient without any other findings suggestive of a mitochondrial disease. In small pedigrees it may be impossible to distinguish between maternal or other modes of transmissions. Unlike most other heteroplasmic mutations, the mutation load of 8993 mtDNA mutations does not vary significantly between different tissues in the same subject, nor does it change over time (White et al. 1999a, White et al. 1999b). Unlike MELAS mutation 3243A>G, the 8993T>G mutations can reliable be detected in blood samples at any age (Chinnery 2006). Our and subsequent (Craig et al. 2007, DiMauro et al. 2009) findings suggest that the 8993T>C mtDNA mutation should be considered in the differential diagnosis of adult-onset ataxia and/or axonal neuropathy.

6.6 Differential diagnostic evaluation in non-dominant adult-onset ataxia

In the past few years, new genes have been identified increasing the diagnostic spectrum of ataxias to an extent that the clinician may need guidelines for the accurate differential diagnostic effort. Algorithms outlining the shortcuts can provide assistance in the diagnostic process. The focus of the algorithm in Figure 5 is on genetic causes of adult-onset non-dominant ataxias.

The homozygous W748S mutation in *POLG1* has been found to be the most frequent genetic cause of juvenile or adult-onset ataxia in Finland, and be should be considered in the first-line genetic investigation. In patients with features compatible with MIRAS, the straightforward strategy is to check for the W784S (and possible for another MIRAS mutation, the A467T) by DNA-analysis. If these *POLG1* defects are excluded, the next steps are screening of FRDA, and also in older male patients FXTAS. Incomplete penetrance of the specific dominant SCAs (SCA 8, 7, 1, 6, 2, 17), that have been found in a Finnish population (Juvonen et al. 2005), may be considered in apparently sporadic or recessive unexplained ataxia. Screening for common mtDNA point mutations in blood sample or more reliably in skeletal muscle is part of the diagnostic process of unexplained ataxia, especially if specific phenotypic features of the patient or the family are suggestive of mitochondrial ataxia. Importantly, because the 8993T>C mtDNA mutation can present with slowly progressive spinocerebellar-type ataxia, this mutation should be included in mtDNA point mutation analysis.
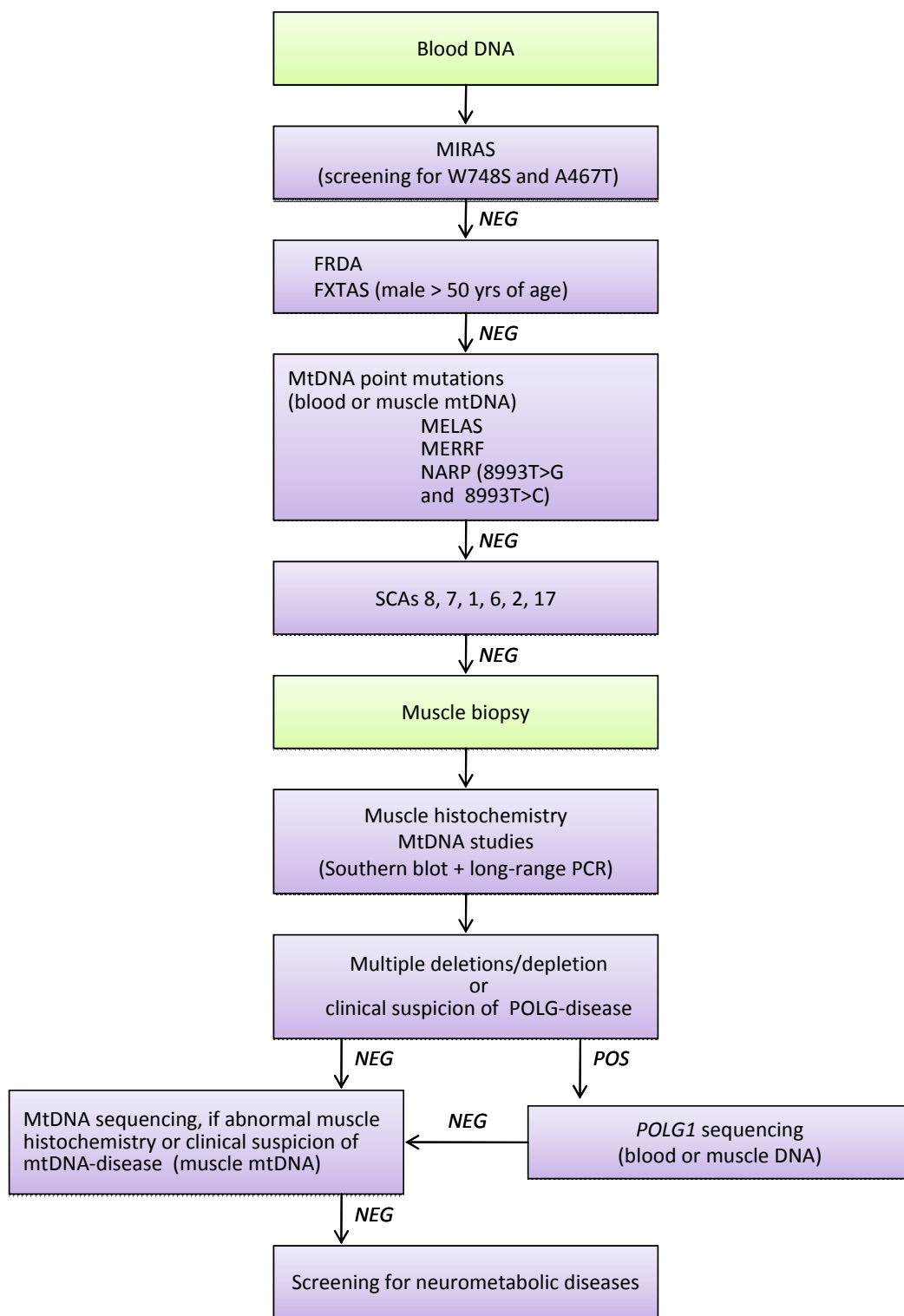
Figure 5. Diagnostic assessment of an adult patient with a suspected non-dominant ataxia.

If the blood DNA tests listed above remain negative in suspected *POLG1*-related or other mitochondrial disease, a tissue biopsy from a clinically affected organ (usually muscle or liver) should be performed to

detect biochemical evidence of mitochondrial dysfunction. Patients with mitochondrial defects usually have abnormal muscle pathology including COX deficient and ragged red fibres (Tzoulis et al. 2006, Chinnery and Zeviani 2008, Milone et al. 2008, Stewart et al. 2009). Particularly in children assaying the activity of the mitochondrial respiratory chain complexes is a preferred way to screen for mitochondrial dysfunction (Sarzi et al. 2007, Chinnery and Zeviani 2008, Rahman and Poulton 2009). Because mutations in *POLG1* gene have been identified in patients with normal histochemistry and respiratory chain enzyme analysis, further molecular genetic investigations should be done in every patient whom a clinical suspicion persists despite normal findings on muscle biopsy histopathology. The next step is assessment of multiple mtDNA deletions. Southern blot analysis of muscle mtDNA may remain normal, and it is important to use the long-PCR-based assay to detect smaller amount of deleted mtDNA fragments. The presence of multiple mtDNA deletions or mtDNA depletion in muscle biopsy tissue should directly guide to *POLG1* sequencing. Ultimately the decision to sequence *POLG1* is based on clinical suspicion in the light on supporting clinical and laboratory investigations.

Sequencing of entire mitochondrial genome is advised in patients with abnormal mitochondrial pathology in skeletal muscle when the above mentioned tests remain negative. Screening for rare inherited metabolic disorders may also be indicated in unexplained adult patients, as listed in Tables 4 and 5 in the Review of the Literature.

7 Conclusions

In this thesis, two distinct new types of adult-onset non-dominant ataxias are clinically and genetically characterized. The scientific research of this thesis started with the unique clinical observations in patients showing recessive adult-onset ataxia and bilateral thalamic lesions detected by MRI. Further explorations revealed other clinical features of these patients that together with long term follow-up completed the phenotype of the MIRAS disease: adult- or juvenile onset spinocerebellar-type ataxia combined with sensory neuropathy, late cognitive impairment, oculomotor defects without typical PEO features, myoclonus, tremor, psychiatric symptoms, seizures in some patients even as a first symptom, and thalamic and cerebellar white matter changes on MRI. In muscle biopsy typical findings of mitochondrial disease were lacking even though long-range PCR did show low levels of multiple mtDNA deletions, suggesting that this method can be used for screening purposes. Autopsy findings showed a spinocerebellar degeneration with peculiar vacuolar change in thalamus, correlating with atrophy and other degenerative changes observed on MRI, and decreased glucose metabolism on FDG-PET. Because the specific genetic cause remained elusive for years even after comprehensive investigations, we continued with molecular genetics with the aim to define this new entity also by molecular genetic methods. The effort was successful and homozygous W748S mutation on POLG was found to be the cause of MIRAS in Finland.

In the collaborative epidemiological study together with Anna Hakonen et al. MIRAS proved to be the most common single genetic cause of hereditary ataxia in Finland and a relatively common cause in many northern European populations. The carrier frequency for the W748S proved to be very high in the Finnish population, 1:125, indicating that there are some 40 000 mutation carriers. Because some family members reported minor neurological symptoms or had even been diagnosed with neurological disease, we had to investigate possible disease manifestations in heterozygote carriers. However, definite clinical signs were not obtained. Sensory neuropathy was a frequent subclinical finding, and in two out of four W748S carriers FDG-PET imaging revealed decreased glucose metabolism levels.

In our clinical cohort of non-dominant adult-onset patient we also identified a heteroplasmic 8993T>C mitochondrial DNA mutation as a second new distinct ataxia entity presented in this thesis. This is also an adult-onset slowly progressive spinocerebellar-type ataxia and/or axonal polyneuropathy combined with pyramidal signs, and without mitochondrial histochemical abnormalities in skeletal muscle. The prevalence of this mutation in Finland remains to be determined in later studies. Our results expanded the phenotypic

spectrum associated with mtDNA 8993T>C mutation and suggest that this disorder should be considered in the differential diagnosis of unexplained adult-onset ataxia and axonal neuropathy.

The results of the scientific research presented in this thesis provide new basic insight and new possibilities for the patients and the clinicians:

1. The diagnostic accuracy for ataxia patients and their families has improved.
2. Living with a chronic progressive neurological disease is a heavy destiny. Not knowing the cause of the problem imposes an additional burden. Making the correct final genetic diagnosis is thus a relief of some of the total burden.
3. Achievement of the basic genetic cause is the first step towards therapeutic possibilities in hereditary diseases and corresponding hypothetical approaches are already investigated.
4. In the total cohort of patients there are still unsolved genetic backgrounds that will need further research in order to find additional causes for non-dominant adult-onset ataxia.

Acknowledgements

References

Abele M, Burk K, Schols L, Schwartz S, Besenthal I, Dichgans J, Zuhlke C, Riess O and Klockgether T (2002): The aetiology of sporadic adult-onset ataxia. Brain 125:961-968.

Anttonen AK, Mahjneh I, Hämäläinen RH, Lagier-Tourenne C, Kopra O, Waris L, Anttonen M, Joensuu T, Kalimo H, Paetau A, Tranebjaerg L, Chaigne D, Koenig M, Eeg-Olofsson O, Udd B, Somer M, Somer H and Lehesjoki AE (2005): The gene disrupted in Marinesco-Sjogren syndrome encodes SIL1, an HSPA5 cochaperone. Nat Genet 37:1309-1311.

Anttonen AK, Siintola E, Tranebjaerg L, Iwata NK, Bijlsma EK, Meguro H, Ichikawa Y, Goto J, Kopra O and Lehesjoki AE (2008): Novel SIL1 mutations and exclusion of functional candidate genes in Marinesco-Sjogren syndrome. Eur J Hum Genet 16:961-969.

Artuch R, Brea-Calvo G, Briones P, Aracil A, Galvan M, Espinos C, Corral J, Volpini V, Ribes A, Andreu AL, Palau F, Sanchez-Alcazar JA, Navas P and Pineda M (2006): Cerebellar ataxia with coenzyme Q10 deficiency: diagnosis and follow-up after coenzyme Q10 supplementation. J Neurol Sci 246:153-158.

Bandmann O, Sweeney MG, Daniel SE, Wenning GK, Quinn N, Marsden CD and Wood NW (1997): Multiple-system atrophy is genetically distinct from identified inherited causes of spinocerebellar degeneration. Neurology 49:1598-1604.

Benomar A, Yahyaoui M, Meggouh F, Bouhouche A, Boutchich M, Bouslam N, Zaim A, Schmitt M, Belaidi H, Ouazzani R, Chkili T and Koenig M (2002): Clinical comparison between AVED patients with 744 del A mutation and Friedreich ataxia with GAA expansion in 15 Moroccan families. J Neurol Sci 198:25-29.

Berciano J, Combarros O, De Castro M and Palau F (1997): Intronic GAA triplet repeat expansion in Friedreich's ataxia presenting with pure sensory ataxia. J Neurol 244:390-391.

Berciano J, Pascuel J and Polo JM (2000): History of ataxia research. In: Handbook of ataxia disorders, pp. 77-100. Ed. T Klockgether, Marcel Dekker, Inc., New York, Basel.

Berman SB, Pineda FJ and Hardwick JM (2008): Mitochondrial fission and fusion dynamics: the long and short of it. Cell Death Differ 15:1147-1152.

Berriot-Varoqueaux N, Aggerbeck LP, Samson-Bouma M and Wetterau JR (2000): The role of the microsomal triglyceride transfer protein in abetalipoproteinemia. Annu Rev Nutr 20:663-697.

Berry-Kravis E, Abrams L, Coffey SM, Hall DA, Greco C, Gane LW, Grigsby J, Bourgeois JA, Finucane B, Jacquemont S, Brunberg JA, Zhang L, Lin J, Tassone F, Hagerman PJ, Hagerman RJ and Leehey MA (2007): Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. Mov Disord 22:2018-2030.

Bhidayasiri R, Perlman SL, Pulst SM and Geschwind DH (2005): Late-onset Friedreich ataxia: phenotypic analysis, magnetic resonance imaging findings, and review of the literature. Arch Neurol 62:1865-1869.

Bianchi MC, Sgandurra G, Tosetti M, Battini R and Cioni G (2007): Brain magnetic resonance in the diagnostic evaluation of mitochondrial encephalopathies. Biosci Rep 27:69-85.

Bindoff L (2007): Reply. Brain 130:E70.

Blok MJ, Van den Bosch BJ, Jongen E, Hendrickx A, de Die-Smulders CE, Hoogendijk JE, Brusse E, de VM, Poll-The BT, Bierau J, de C, I and Smeets HJ (2009): The unfolding clinical spectrum of POLG mutations. J Med Genet e-pub.

Bomar JM, Benke PJ, Slattery EL, Puttagunta R, Taylor LP, Seong E, Nystuen A, Chen W, Albin RL, Patel PD, Kittles RA, Sheffield VC and Burmeister M (2003): Mutations in a novel gene encoding a CRAL-TRIO domain cause human Cayman ataxia and ataxia/dystonia in the jittery mouse. Nat Genet 35:264-269.

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

Bouchard JP, Richter A, Mathieu J, Brunet D, Hudson TJ, Morgan K and Melancon SB (1998): Autosomal recessive spastic ataxia of Charlevoix-Saguenay. Neuromuscul Disord 8:474-479.

Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis EM, Grigsby J, Leehey MA, Tassone F, Brown WT, Greco CM and Hagerman PJ (2002): Fragile X premutation carriers: characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. AJNR Am J Neuroradiol 23:1757-1766.

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

Brusse E, Maat-Kievit JA and van Swieten JC (2007): Diagnosis and management of early- and late-onset cerebellar ataxia. Clin Genet 71:12-24.

Brussino A, Gellera C, Saluto A, Mariotti C, Arduino C, Castellotti B, Camerlingo M, de A, V, Orsi L, Tosca P, Migone N, Taroni F and Brusco A (2005): *FMR1* gene premutation is a frequent genetic cause of late-onset sporadic cerebellar ataxia. Neurology 64:145-147.

Cali JJ, Hsieh CL, Francke U and Russell DW (1991): Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. J Biol Chem 266:7779-7783.

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

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

Carlson KM, Andersen JM and Orr HT (2009): Emerging pathogenic pathways in the spinocerebellar ataxias. Curr Opin Genet Dev In press: DOI:10.1016/j.gde.2009.02.009.

Cavadini P, O'Neill HA, Benada O and Isaya G (2002): Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. Hum Mol Genet 11:217-227.

Cavalier L, Ouahchi K, Kayden HJ, Di Donato S, Reutenauer L, Mandel JL and Koenig M (1998): Ataxia with isolated vitamin E deficiency: heterogeneity of mutations and phenotypic variability in a large number of families. Am J Hum Genet 62:301-310.

Chan SS, Longley MJ and Copeland WC (2005): The common A467T mutation in the human mitochondrial DNA polymerase (POLG) compromises catalytic efficiency and interaction with the accessory subunit. J Biol Chem 280:31341-31346.

Chan SS, Longley MJ and Copeland WC (2006): Modulation of the W748S mutation in DNA polymerase gamma by the E1143G polymorphismin mitochondrial disorders. Hum Mol Genet 15:3473-3483.

Chen YZ, Hashemi SH, Anderson SK, Huang Y, Moreira MC, Lynch DR, Glass IA, Chance PF and Bennett CL (2006): Senataxin, the yeast Sen1p orthologue: characterization of a unique protein in which recessive mutations cause ataxia and dominant mutations cause motor neuron disease. Neurobiol Dis 23:97-108.

Chinnery P, Majamaa K, Turnbull D and Thorburn D (2006): Treatment for mitochondrial disorders. Cochrane Database Syst Rev Issue 1. Art. No.: CD004426. DOI: 10.1002/14651858.CD004426.pub2.

Chinnery PF (2006): Could it be mitochondrial? When and how to investigate. Practical Neurology 6:90-101.

Chinnery PF and Bindoff LA (2003): 116th ENMC international workshop: the treatment of mitochondrial disorders, 14th-16th March 2003, Naarden, The Netherlands. Neuromuscul Disord 13:757-764.

Chinnery PF and Samuels DC (1999): Relaxed replication of mtDNA: A model with implications for the expression of disease. Am J Hum Genet 64:1158-1165.

Chinnery PF and Schon EA (2003): Mitochondria. J Neurol Neurosurg Psychiatry 74:1188-1199.

Chinnery PF and Zeviani M (2008): 155th ENMC workshop: Polymerase gamma and disorders of mitochondrial DNA synthesis, 21-23 September 2007, Naarden, The Netherlands. Neuromuscul Disord 18:259-267.

Chinnery PF, Howell N, Lightowlers RN and Turnbull DM (1997): Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes. Brain 120 (Pt 10):1713-1721.

Chinnery PF, Brown DT, Archibald K, Curtis A and Turnbull DM (2002): Spinocerebellar ataxia and the A3243G and A8344G mtDNA mutations. J Med Genet 39:E22.

Chun HH and Gatti RA (2004): Ataxia-telangiectasia, an evolving phenotype. DNA Repair (Amst) 3:1187-1196.

Clarke JTR (2006): A clinical guide to inherited metabolic diseases. Cambridge University Press, Cambridge.

Clayton DA (1982): Replication of animal mitochondrial DNA. Cell 28:693-705.

Coffey SM, Cook K, Tartaglia N, Tassone F, Nguyen DV, Pan R, Bronsky HE, Yuhas J, Borodyanskaya M, Grigsby J, Doerflinger M, Hagerman PJ and Hagerman RJ (2008): Expanded clinical phenotype of women with the FMR1 premutation. Am J Med Genet A 146:1009-1016.

Copeland WC (2008): Inherited mitochondrial diseases of DNA replication. Annu Rev Med 59:131-146.

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

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

Craig K, Ferrari G, Tiangyou W, Hudson G, Gellera C, Zeviani M and Chinnery PF (2007): The A467T and W748S POLG substitutions are a rare cause of adult-onset ataxia in Europe. Brain 130:E69.

Criscuolo C, Mancini P, Sacca F, De Michele G, Monticelli A, Santoro L, Scarano V, Banfi S and Filla A (2004): Ataxia with oculomotor apraxia type 1 in Southern Italy: late onset and variable phenotype. Neurology 63:2173-2175.

Criscuolo C, Chessa L, Di Giandomenico S, Mancini P, Sacca F, Grieco GS, Piane M, Barbieri F, De Michele G, Banfi S, Pierelli F, Rizzuto N, Santorelli FM, Gallosti L, Filla A and Casali C (2006): Ataxia with oculomotor apraxia type 2: a clinical, pathologic, and genetic study. Neurology 66:1207-1210.

Criscuolo C, Mancini P, Ammendola S, Cicala D, Banfi S, De Michele G and Filla A (2008): Screening for *POLG1* mutations in a Southern Italian ataxia population. J Neurol 255:454-455.

Date H, Onodera O, Tanaka H, Iwabuchi K, Uekawa K, Igarashi S, Koike R, Hiroi T, Yuasa T, Awaya Y, Sakai T, Takahashi T, Nagatomo H, Sekijima Y, Kawachi I, Takiyama Y, Nishizawa M, Fukuhara N, Saito K, Sugano S and Tsuji S (2001): Early-onset ataxia with ocular motor apraxia and hypoalbuminemia is caused by mutations in a new HIT superfamily gene. Nat Genet 29:184-188.

Davidzon G, Mancuso M, Ferraris S, Quinzii C, Hirano M, Peters HL, Kirby D, Thorburn DR and DiMauro S (2005): *POLG* mutations and Alpers syndrome. Ann Neurol 57:921-923.

Davidzon G, Greene P, Mancuso M, Klos KJ, Ahlskog JE, Hirano M and DiMauro S (2006): Early-onset familial parkinsonism due to *POLG* mutations. Ann Neurol 59:859-862.

De Michele G, Coppola G, Cocozza S and Filla A (2004): A pathogenetic classification of hereditary ataxias: is the time ripe? J Neurol 251:913-922.

de Vries DD, van Engelen BG, Gabreels FJ, Ruitenbeek W and van Oost BA (1993): A second missense mutation in the mitochondrial ATPase 6 gene in Leigh's syndrome. Ann Neurol 34:410-412.

Debray FG, Lambert M, Lortie A, Vanasse M and Mitchell GA (2007): Long-term outcome of Leigh syndrome caused by the NARP-T8993C mtDNA mutation. Am J Med Genet A 143A:2046-2051.

Di Donato S (2009): Multisystem manifestations of mitochondrial disorders. J Neurol.

Di Fonzo A, Bordoni A, Crimi M, Sara G, Del Bo R, Bresolin N and Comi GP (2003): POLG mutations in sporadic mitochondrial disorders with multiple mtDNA deletions. Hum Mutat 22:498-499.

Di Prospero NA, Baker A, Jeffries N and Fischbeck KH (2007): Neurological effects of high-dose idebenone in patients with Friedreich's ataxia: a randomised, placebo-controlled trial. Lancet Neurol 6:878-886.

DiMauro S (2004): Mitochondrial medicine. Biochim Biophys Acta 1659:107-114.

DiMauro S and Hirano M (2005): Mitochondrial encephalomyopathies: an update. Neuromuscul Disord 15:276-286.

DiMauro S and Mancuso M (2007): Mitochondrial diseases: therapeutic approaches. Biosci Rep 27:125-137.

DiMauro S and Schon EA (2008): Mitochondrial Disorders in the Nervous System. Annu Rev Neurosci 31:91-123.

DiMauro S, Hirano M and Schon EA (2006): Approaches to the treatment of mitochondrial diseases. Muscle Nerve 34:265-283.

DiMauro S, Milone M and Keegan BM (2009): A 41-year-old woman with progressive leg weakness and numbness, dizziness, and myalgia. Neurology 72:1262-1268.

Dueñas AM, Goold R and Giunti P (2006): Molecular pathogenesis of spinocerebellar ataxias. Brain 129:1357-1370.

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

Eggenberger E (2007): Prion disease. Neurol Clin 25:833-842.

El Euch-Fayache G, Lalani I, Amouri R, Turki I, Ouahchi K, Hung WY, Belal S, Siddique T and Hentati F (2003): Phenotypic features and genetic findings in sacsin-related autosomal recessive ataxia in Tunisia. Arch Neurol 60:982-988.

El-Khamisy SF and Caldecott KW (2006): TDP1-dependent DNA single-strand break repair and neurodegeneration. Mutagenesis 21:219-224.

El-Khamisy SF, Saifi GM, Weinfeld M, Johansson F, Helleday T, Lupski JR and Caldecott KW (2005): Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1. Nature 434:108-113.

Engelsen BA, Tzoulis C, Karlsen B, Lillebø A, Lægreid LM, Aasly J, Zeviani M and Bindoff LA (2008): *POLG1* mutations cause a syndromic epilepsy with occipital lobe predilection. Brain 131:818-828.

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

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

Fadic R, Russell JA, Vedanarayanan VV, Lehar M, Kuncl RW and Johns DR (1997): Sensory ataxic neuropathy as the presenting feature of a novel mitochondrial disease. Neurology 49:239-245.

Ferrari G, Lamantea E, Donati A, Filosto M, Briem E, Carrara F, Parini R, Simonati A, Santer R and Zeviani M (2005): Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA polymerase-gammaA. Brain 128:723-731.

Filla A, De Michele G, Cavalcanti F, Pianese L, Monticelli A, Campanella G and Cocozza S (1996): The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich ataxia. Am J Hum Genet 59:554-560.

Filosto M and Mancuso M (2007): Mitochondrial diseases: a nosological update. Acta Neurol Scand 115:211-221.

Finnilä S, Hassinen IE, Ala-Kokko L and Majamaa K (2000): Phylogenetic network of the mtDNA haplogroup U in Northern Finland based on sequence analysis of the complete coding region by conformation-sensitive gel electrophoresis. Am J Hum Genet 66:1017-1026.

Finsterer J (2006): Central nervous system manifestations of mitochondrial disorders. Acta Neurol Scand 114:217-238.

Fogel BL and Perlman S (2007): Clinical features and molecular genetics of autosomal recessive cerebellar ataxias. Lancet Neurol 6:245-257.

Fujii T, Hattori H, Higuchi Y, Tsuji M and Mitsuyoshi I (1998): Phenotypic differences between T-->C and T-->G mutations at nt 8993 of mitochondrial DNA in Leigh syndrome. Pediatr Neurol 18:275-277.

Futamura N, Matsumura R, Fujimoto Y, Horikawa H, Suzumura A and Takayanagi T (1998): CAG repeat expansions in patients with sporadic cerebellar ataxia. Acta Neurol Scand 98:55-59.

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

Gardner JL, Craven L, Turnbull DM and Taylor RW (2007): Experimental strategies towards treating mitochondrial DNA disorders. Biosci Rep 27:139-150.

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

Gibberd FB and Wierzbicki AS (2000): Heredopathia Atactica Polyneuritiformis. In: Handbook of ataxia disorders, pp. 235-256. Ed. T Klockgether, Marcel Dekker, Inc., New York, Basel.

Gilman S and Quinn NP (1996): The relationship of multiple system atrophy to sporadic olivopontocerebellar atrophy and other forms of idiopathic late-onset cerebellar atrophy. Neurology 46:1197-1199.

Gilman S, Little R, Johanns J, Heumann M, Kluin KJ, Junck L, Koeppe RA and An H (2000): Evolution of sporadic olivopontocerebellar atrophy into multiple system atrophy. Neurology 55:527-532.

Giuffrida S, Saponara R, Restivo DA, Trovato SA, Tomarchio L, Pugliares P, Fabbri G and Maccagnano C (1999): Supratentorial atrophy in spinocerebellar ataxia type 2: MRI study of 20 patients. J Neurol 246:383-388.

Gomez CM (2004): ARSACS goes global. Neurology 62:10-11.

Graus F, Delattre JY, Antoine JC, Dalmau J, Giometto B, Grisold W, Honnorat J, Smitt PS, Vedeler C, Verschuuren JJ, Vincent A and Voltz R (2004): Recommended diagnostic criteria for paraneoplastic neurological syndromes. J Neurol Neurosurg Psychiatry 75:1135-1140.

Graziewicz MA, Longley MJ and Copeland WC (2006): DNA polymerase gamma in mitochondrial DNA replication and repair. Chem Rev 106:383-405.

Greco CM, Berman RF, Martin RM, Tassone F, Schwartz PH, Chang A, Trapp BD, Iwahashi C, Brunberg J, Grigsby J, Hessl D, Becker EJ, Papazian J, Leehey MA, Hagerman RJ and Hagerman PJ (2006): Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). Brain 129:243-255.

Gropman AL (2004): The neurological presentations of childhood and adult mitochondrial disease: established syndromes and phenotypic variations. Mitochondrion 4:503-520.

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

Hagerman RJ and Hagerman PJ (2002): The fragile X premutation: into the phenotypic fold. Curr Opin Genet Dev 12:278-283.

Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, Tassone F, Hessl D, Harris SW, Zhang L, Jardini T, Gane LW, Ferranti J, Ruiz L, Leehey MA, Grigsby J and Hagerman PJ (2004): Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the *FMR1* premutation. Am J Hum Genet 74:1051-1056.

Hakonen AH (2008): Molecular characterization of mitochondrial ataxias – with emphasis on MIRAS and IOSCA. PhD-thesis, University of Helsinki. Helsinki University Print, Helsinki.

Hakonen AH, Heiskanen S, Juvonen V, Lappalainen I, Luoma PT, Rantamäki M, Goethem GV, Löfgren A, Hackman P, Paetau A, Kaakkola S, Majamaa K, Varilo T, Udd B, Kääriäinen H, Bindoff LA and Suomalainen A (2005): Mitochondrial DNA polymerase W748S mutation: a common cause of autosomal recessive ataxia with ancient European origin. Am J Hum Genet 77:430-441.

Hakonen AH, Davidzon G, Salemi R, Bindoff LA, Van Goethem G, DiMauro S, Thorburn DR and Suomalainen A (2007): Abundance of the POLG disease mutations in Europe, Australia, New Zealand, and the United States explained by single ancient European founders. Eur J Hum Genet 15:779-783.

Hakonen AH, Goffart S, Marjavaara S, Paetau A, Cooper H, Mattila K, Lampinen M, Sajantila A, Lonnqvist T, Spelbrink JN and Suomalainen A (2008): Infantile-onset spinocerebellar ataxia and mitochondrial recessive ataxia syndrome are associated with neuronal complex I defect and mtDNA depletion. Hum Mol Genet 17:3822-3835.

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

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

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

Harding AE (1984): The hereditary ataxias and related disorders. Churchill Livingstone, Edinburgh, London, Melbourne and New York.

Harper C (2007): The neurotoxicity of alcohol. Hum Exp Toxicol 26:251-257.

Harrower T, Stewart JD, Hudson G, Houlden H, Warner G, O'Donovan DG, Findlay LJ, Taylor RW, De Silva R and Chinnery PF (2008): *POLG1* mutations manifesting as autosomal recessive axonal Charcot-Marie-Tooth disease. Arch Neurol 65:133-136.

Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR and Zalutsky R (2007): How common are the "common" neurologic disorders? Neurology 68:326-337.

Hisama FM, Mancuso M, Filosto M and DiMauro S (2005): Progressive external ophthalmoplegia: a new family with tremor and peripheral neuropathy. Am J Med Genet A 135:217-219.

Holt IJ, Harding AE, Petty RK and Morgan-Hughes JA (1990): A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 46:428-433.

Horvath R, Hudson G, Ferrari G, Fütterer N, Ahola S, Lamantea E, Prokisch H, Lochmüller H, McFarland R, Ramesh V, Klopstock T, Freisinger P, Salvi F, Mayr JA, Santer R, Tesarova M, Zeman J, Udd B, Taylor RW, Turnbull D, Hanna M, Fialho D, Suomalainen A, Zeviani M and Chinnery PF (2006): Phenotypic spectrum associated with mutations of the mitochondrial polymerase gamma gene. Brain 129:1674-1684.

Hudson G and Chinnery PF (2006): Mitochondrial DNA polymerase-gamma and human disease. Hum Mol Genet 15 Spec No 2:R244-R252.

Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des P, V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F and Hagerman PJ (2003): Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 72:869-878.

Jacquemont S, Hagerman RJ, Hagerman PJ and Leehey MA (2007): Fragile-X syndrome and fragile X-associated tremor/ataxia syndrome: two faces of *FMR1*. Lancet Neurol 6:45-55.

Jansen GA, Ofman R, Ferdinandusse S, Ijlst L, Muijsers AO, Skjeldal OH, Stokke O, Jakobs C, Besley GT, Wraith JE and Wanders RJ (1997): Refsum disease is caused by mutations in the phytanoyl-CoA hydroxylase gene. Nat Genet 17:190-193.

Jansen GA, Waterham HR and Wanders RJ (2004): Molecular basis of Refsum disease: sequence variations in phytanoyl-CoA hydroxylase (*PHYH*) and the PTS2 receptor (*PEX7*). Hum Mutat 23:209-218.

Jansson M, Darin N, Kyllerman M, Martinsson T, Wahlstrom J and Oldfors A (2000): Multiple mitochondrial DNA deletions in hereditary inclusion body myopathy. Acta Neuropathol 100:23-28.

Jiralerspong S, Liu Y, Montermini L, Stifani S and Pandolfo M (1997): Frataxin shows developmentally regulated tissue-specific expression in the mouse embryo. Neurobiol Dis 4:103-113.

Jonasson J, Juvonen V, Sistonen P, Ignatius J, Johansson D, Björck EJ, Wahlström J, Melberg A, Holmgren G, Forsgren L and Holmberg M (2000): Evidence for a common Spinocerebellar ataxia type 7 (SCA7) founder mutation in Scandinavia. Eur J Hum Genet 8:918-922.

Juvonen V, Hietala M, Päivärinta M, Rantamäki M, Hakamies L, Kaakkola S, Vierimaa O, Penttinen M and Savontaus ML (2000): Clinical and genetic findings in Finnish ataxia patients with the spinocerebellar ataxia 8 repeat expansion. Ann Neurol 48:354-361.

Juvonen V, Kulmala SM, Ignatius J, Penttinen M and Savontaus ML (2002): Dissecting the epidemiology of a trinucleotide repeat disease - example of FRDA in Finland. Hum Genet 110:36-40.

Juvonen V, Hietala M, Kairisto V and Savontaus ML (2005): The occurrence of dominant spinocerebellar ataxias among 251 Finnish ataxia patients and the role of predisposing large normal alleles in a genetically isolated population. Acta Neurol Scand 111:154-162.

Kaguni LS (2004): DNA polymerase gamma, the mitochondrial replicase. Annu Rev Biochem 73:293-320.

Kendall BE (1992): Disorders of lysosomes, peroxisomes, and mitochondria. AJNR Am J Neuroradiol 13:621-653.

Kerber KA, Jen JC, Perlman S and Baloh RW (2005): Late-onset pure cerebellar ataxia: differentiating those with and without identifiable mutations. J Neurol Sci 238:41-45.

Kijas AW, Harris JL, Harris JM and Lavin MF (2006): Aprataxin forms a discrete branch in the HIT (histidine triad) superfamily of proteins with both DNA/RNA binding and nucleotide hydrolase activities. J Biol Chem 281:13939-13948.

Klockhether T (2000): Clinical approach to ataxic patients. In: Handbook of ataxia disorders, pp. 101-114. Ed. T Klockhether, Marcel Dekker, Inc., New York.

Klockgether T, Schroth G, Diener HC and Dichgans J (1990): Idiopathic cerebellar ataxia of late onset: natural history and MRI morphology. J Neurol Neurosurg Psychiatry 53:297-305.

Koenig M and Dürr A (2000): Friedreich ataxia. In: Handbook of ataxia disorders, pp. 151-324. Ed. Thomas Klockgether, Marcel Dekker, Inc., New York.

Koide R, Ikeuchi T, Onodera O, Tanaka H, Igarashi S, Endo K, Takahashi H, Kondo R, Ishikawa A, Hayashi T, Saito M, Tomoda A, Miike T, Naito H, Ikuta F and Tsuji S (1994): Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). Nat Genet 6:9-13.

Koide R, Onodera O, Ikeuchi T, Kondo R, Tanaka H, Tokiguchi S, Tomoda A, Miike T, Isa F, Beppu H, Shimizu N, Watanabe Y, Horikawa Y, Shimohata T, Hirota K, Ishikawa A and Tsuji S (1997): Atrophy of the cerebellum and brainstem in dentatorubral pallidoluysian atrophy. Influence of CAG repeat size on MRI findings. Neurology 49:1605-1612.

Kollberg G, Moslemi AR, Darin N, Nennesmo I, Bjarnadottir I, Uvebrant P, Holme E, Melberg A, Tulinius M and Oldfors A (2006): *POLG1* mutations associated with progressive encephalopathy in childhood. J Neuropathol Exp Neurol 65:758-768.

Körkkö J, Annunen S, Pihlajamaa T, Prockop DJ and Ala-Kokko L (1998): Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci U S A 95:1681-1685.

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

Krishnan KJ, Reeve AK, Samuels DC, Chinnery PF, Blackwood JK, Taylor RW, Wanrooij S, Spelbrink JN, Lightowlers RN and Turnbull DM (2008): What causes mitochondrial DNA deletions in human cells? Nat Genet 40:275-279.

Lamantea E, Tiranti V, Bordoni A, Toscano A, Bono F, Servidei S, Papadimitriou A, Spelbrink H, Silvestri L, Casari G, Comi GP and Zeviani M (2002): Mutations of mitochondrial DNA polymerase gammaA are a frequent cause of autosomal dominant or recessive progressive external ophthalmoplegia. Ann Neurol 52:211-219.

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

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

Lodi R, Cooper JM, Bradley JL, Manners D, Styles P, Taylor DJ and Schapira AH (1999): Deficit of in vivo mitochondrial ATP production in patients with Friedreich ataxia. Proc Natl Acad Sci U S A 96:11492-11495.

Longley MJ, Graziewicz MA, Bienstock RJ and Copeland WC (2005): Consequences of mutations in human DNA polymerase gamma. Gene 354:125-131.

Lönnqvist T (1995): Infantile onset spinocerebellar ataxia (IOSCA). PhD-thesis, University of Helsinki. Helsinki University Print, Helsinki.

Lönnqvist T, Paetau A, Nikali K, von Boguslawski K and Pihko H (1998): Infantile onset spinocerebellar ataxia with sensory neuropathy (IOSCA): neuropathological features. J Neurol Sci 161:57-65.

Lorincz MT, Rainier S, Thomas D and Fink JK (2005): Cerebrotendinous xanthomatosis: possible higher prevalence than previously recognized. Arch Neurol 62:1459-1463.

Luo N and Kaguni LS (2005): Mutations in the spacer region of Drosophila mitochondrial DNA polymerase affect DNA binding, processivity, and the balance between Pol and Exo function. J Biol Chem 280:2491-2497.

Luoma P (2007): Mutations of mitochondrial DNA polymerase gamma: an important cause of neurological disorders. PhD-thesis, University of Helsinki. Helsinki University Print, Helsinki.

Luoma P, Melberg A, Rinne JO, Kaukonen JA, Nupponen NN, Chalmers RM, Oldfors A, Rautakorpi I, Peltonen L, Majamaa K, Somer H and Suomalainen A (2004): Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: clinical and molecular genetic study. Lancet 364:875-882.

Luoma PT, Luo N, Löscher WN, Farr CL, Horvath R, Wanschitz J, Kiechl S, Kaguni LS and Suomalainen A (2005): Functional defects due to spacer-region mutations of human mitochondrial DNA polymerase in a family with an ataxia-myopathy syndrome. Hum Mol Genet 14:1907-1920.

Macpherson J, Waghorn A, Hammans S and Jacobs P (2003): Observation of an excess of fragile-X premutations in a population of males referred with spinocerebellar ataxia. Hum Genet 112:619-620.

Mahjneh I, Anttonen AK, Somer M, Paetau A, Lehesjoki AE, Somer H and Udd B (2006): Myopathy is a prominent feature in Marinesco-Sjogren syndrome: A muscle computed tomography study. J Neurol 253:301-306.

Majamaa K, Moilanen JS, Uimonen S, Remes AM, Salmela PI, Karppa M, Majamaa-Voltti KA, Rusanen H, Sorri M, Peuhkurinen KJ and Hassinen IE (1998): Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. Am J Hum Genet 63:447-454.

Man PY, Griffiths PG, Brown DT, Howell N, Turnbull DM and Chinnery PF (2003): The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am J Hum Genet 72:333-339.

Mancuso M, Filosto M, Oh SJ and DiMauro S (2004): A novel polymerase gamma mutation in a family with ophthalmoplegia, neuropathy, and Parkinsonism. Arch Neurol 61:1777-1779.

Mancuso M, Filosto M, Choub A, Tentorio M, Broglio L, Padovani A and Siciliano G (2007): Mitochondrial DNA-related disorders. Biosci Rep 27:31-37.

Mariotti C, Fancellu R and Di Donato S (2005): An overview of the patient with ataxia. J Neurol 252:511-518.

Mattiazzi M, Vijayvergiya C, Gajewski CD, DeVivo DC, Lenaz G, Wiedmann M and Manfredi G (2004): The mtDNA T8993G (NARP) mutation results in an impairment of oxidative phosphorylation that can be improved by antioxidants. Hum Mol Genet 13:869-879.

McFarland R, Taylor RW and Turnbull DM (2002): The neurology of mitochondrial DNA disease. Lancet Neurol 1:343-351.

McFarland R, Hudson G, Taylor RW, Green SH, Hodges S, McKiernan PJ, Chinnery PF and Ramesh V (2008): Reversible valproate hepatotoxicity due to mutations in mitochondrial DNA polymerase gamma (POLG1). Arch Dis Child 93:151-153.

Mihalik SJ, Morrell JC, Kim D, Sacksteder KA, Watkins PA and Gould SJ (1997): Identification of *PAHX*, a Refsum disease gene. Nat Genet 17:185-189.

Milone M, Brunetti-Pierri N, Tang LY, Kumar N, Mezei MM, Josephs K, Powell S, Simpson E and Wong LJ (2008): Sensory ataxic neuropathy with ophthalmoparesis caused by POLG mutations. Neuromuscul Disord 18:626-632.

Moraes CT, DiMauro S, Zeviani M, Lombes A, Shanske S, Miranda AF, Nakase H, Bonilla E, Werneck LC, Servidei S, Nonaka I, Koga Y, Spiro AJ, Brownell KW, Schmidt B, Schotland DL, Zupanc M, DeVivo DC, Schon EA and Rowland LP (1989): Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome. N Engl J Med 320:1293-1299.

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

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

Mosconi L (2005): Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. Eur J Nucl Med Mol Imaging 32:486-510.

Moseley ML, Benzow KA, Schut LJ, Bird TD, Gomez CM, Barkhaus PE, Blindauer KA, Labuda M, Pandolfo M, Koob MD and Ranum LP (1998): Incidence of dominant spinocerebellar and Friedreich triplet repeats among 361 ataxia families. Neurology 51:1666-1671.

Mosesso P, Piane M, Palitti F, Pepe G, Penna S and Chessa L (2005): The novel human gene aprataxin is directly involved in DNA single-strand-break repair. Cell Mol Life Sci 62:485-491.

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

Narcisi TM, Shoulders CC, Chester SA, Read J, Brett DJ, Harrison GB, Grantham TT, Fox MF, Povey S, de Bruin TW, Erkelens DW, Muller DPR, Lloyd JK and Scott J. (1995): Mutations of the microsomal triglyceride-transfer-protein gene in abetalipoproteinemia. Am J Hum Genet 57:1298-1310.

Nardin RA and Johns DR (2001): Mitochondrial dysfunction and neuromuscular disease. Muscle Nerve 24:170-191.

Naviaux RK and Nguyen KV (2004): POLG mutations associated with Alpers' syndrome and mitochondrial DNA depletion. Ann Neurol 55:706-712.

Neudorfer O, Pastores GM, Zeng BJ, Gianutsos J, Zaroff CM and Kolodny EH (2005): Late-onset Tay-Sachs disease: phenotypic characterization and genotypic correlations in 21 affected patients. Genet Med 7:119-123.

Nguyen KV, Sharief FS, Chan SS, Copeland WC and Naviaux RK (2006): Molecular diagnosis of Alpers syndrome. J Hepatol 45:108-116.

Nikali K, Suomalainen A, Saharinen J, Kuokkanen M, Spelbrink JN, Lönnqvist T and Peltonen L (2005): Infantile onset spinocerebellar ataxia is caused by recessive mutations in mitochondrial proteins Twinkle and Twinky. Hum Mol Genet 14:2981-2990.

Nirenberg MJ, Libien J, Vonsattel JP and Fahn S (2007): Multiple system atrophy in a patient with the spinocerebellar ataxia 3 gene mutation. Mov Disord 22:251-254.

Norio R (2003): Finnish Disease Heritage I: characteristics, causes, background. Hum Genet 112:441-456.

Norremolle A, Nielsen JE, Sorensen SA and Hasholt L (1995): Elongated CAG repeats of the B37 gene in a Danish family with dentato-rubro-pallido-luysian atrophy. Hum Genet 95:313-318.

Nystuen A, Benke PJ, Merren J, Stone EM and Sheffield VC (1996): A cerebellar ataxia locus identified by DNA pooling to search for linkage disequilibrium in an isolated population from the Cayman Islands. Hum Mol Genet 5:525-531.

Ohashi K, Ishibashi S, Yamamoto M, Osuga J, Yazaki Y, Yukawa S and Yamada N (1998): A truncated species of apolipoprotein B (B-38.7) in a patient with homozygous hypobetalipoproteinemia associated with diabetes mellitus. Arterioscler Thromb Vasc Biol 18:1330-1334.

Oostra BA and Willemsen R (2009): FMR1: A gene with three faces. Biochim Biophys Acta Epub, DOI:10.1016/j.bbagen.2009.02.007.

Ormerod IE, Harding AE, Miller DH, Johnson G, MacManus D, du Boulay EP, Kendall BE, Moseley IF and McDonald WI (1994): Magnetic resonance imaging in degenerative ataxic disorders. J Neurol Neurosurg Psychiatry 57:51-57.

Orr HT and Zoghbi HY (2007): Trinucleotide repeat disorders. Annu Rev Neurosci 30:575-621.

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

Pandolfo M (2003): Friedreich ataxia. Semin Pediatr Neurol 10:163-172.

Pandolfo M (2008): Drug Insight: antioxidant therapy in inherited ataxias. Nat Clin Pract Neurol 4:86-96.

Pandolfo M and Pastore A (2009): The pathogenesis of Friedreich ataxia and the structure and function of frataxin. J Neurol 256 Suppl 1:9-17.

Pearce JMS (2004): Friedreich's ataxia. J Neurol Neurosurg Psychiatry 75:688.

Penney JB, Jr. (1995): Multiple systems atrophy and nonfamilial olivopontocerebellar atrophy are the same disease. Ann Neurol 37:553-554.

Perlman SL (2003): Diagnostic evaluation of ataxic patients. In: Genetics of movement disorders, pp. 253-272. Ed. S-M Pulst, Acamedic Press, Amsterdam, Boston.

Perlman SL (2004): Symptomatic and disease-modifying therapy for the progressive ataxias. Neurologist 10:275-289.

Peters N, Kamm C, Asmus F, Holinski-Feder E, Kraft E, Dichgans M, Bruning R, Gasser T and Botzel K (2006): Intrafamilial variability in fragile X-associated tremor/ataxia syndrome. Mov Disord 21:98-102.

Pineda M, Arpa J, Montero R, Aracil A, Domínguez F, Galván M, Mas A, Martorell L, Sierra C, Brandi N, García-Arumi E, Rissech M, Velasco D, Costa JA and Artuch R (2008): Idebenone treatment in paediatric and adult patients with Friedreich ataxia: Long-term follow-up. Eur J Paediatr Neurol 12:470-475.

Pulst S-T (2003): Inherited ataxias: an introduction. In: Genetics of movement disorders, pp. 19-34. Ed. S-M Pulst, Acamedic Press, Amsterdam, Boston.

Quinzii CM, Hirano M and DiMauro S (2007): CoQ10 deficiency diseases in adults. Mitochondrion 7 Suppl:S122-S126.

Ragno M, De Michele G, Cavalcanti F, Pianese L, Monticelli A, Curatola L, Bollettini F, Cocozza S, Caruso G, Santoro L and Filla A (1997): Broadened Friedreich's ataxia phenotype after gene cloning. Minimal GAA expansion causes late-onset spastic ataxia. Neurology 49:1617-1620.

Rahman S and Poulton J (2009): Diagnosis of mitochondrial DNA depletion syndromes. Arch Dis Child 94:3-5.

Rantamäki M, Wartiovaara A, Luoma P, Hakonen A, Majamaa K, Soini H, Finnilä S, Hietaharju A, Paetau A, Kääriäinen H and Udd B (2007): Aikuisiässä alkavan ataksian uusia perinnöllisiä syitä. Duodecim 123:2733-2740.

Remes AM, Hinttala R, Kärppä M, Soini H, Takalo R, Uusimaa J and Majamaa K (2008): Parkinsonism associated with the homozygous W748S mutation in the *POLG1* gene. Parkinsonism Relat Disord 14:652-654.

Riess O, Laccone FA, Gispert S, Schols L, Zuhlke C, Vieira-Saecker AM, Herlt S, Wessel K, Epplen JT, Weber BH, Kreuz F, Chahrokh-Zadeh S, Meindl A, Lunkes A, Aguiar J, Macek M, Jr., Krebsova A, Macek M, Sr., Burk K, Tinschert S, Schreyer I, Pulst SM and Auburger G (1997): SCA2 trinucleotide expansion in German SCA patients. Neurogenetics 1:59-64.

Ristow M, Pfister MF, Yee AJ, Schubert M, Michael L, Zhang CY, Ueki K, Michael MD, Lowell BB and Kahn CR (2000): Frataxin activates mitochondrial energy conversion and oxidative phosphorylation. Proc Natl Acad Sci U S A 97:12239-12243.

Rovio AT, Marchington DR, Donat S, Schuppe HC, Abel J, Fritsche E, Elliott DJ, Laippala P, Ahola AL, McNay D, Harrison RF, Hughes B, Barrett T, Bailey DM, Mehmet D, Jequier AM, Hargreave TB, Kao SH,

Cummins JM, Barton DE, Cooke HJ, Wei YH, Wichmann L, Poulton J and Jacobs HT (2001): Mutations at the mitochondrial DNA polymerase (POLG) locus associated with male infertility. Nat Genet 29:261-262.

Rustin P, Kleist-Retzow JC, Chantrel-Groussard K, Sidi D, Munnich A and Rotig A (1999): Effect of idebenone on cardiomyopathy in Friedreich's ataxia: a preliminary study. Lancet 354:477-479.

Rustin P, Rotig A, Munnich A and Sidi D (2002): Heart hypertrophy and function are improved by idebenone in Friedreich's ataxia. Free Radic Res 36:467-469.

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

Sarzi E, Bourdon A, Chretien D, Zarhrate M, Corcos J, Slama A, Cormier-Daire V, de LP, Munnich A and Rotig A (2007): Mitochondrial DNA depletion is a prevalent cause of multiple respiratory chain deficiency in childhood. J Pediatr 150:531-4, 534.

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Patanjali SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NG, Taylor AM, Arlett CF, Miki T, Weissman SM, Lovett M, Collins FS and Shiloh Y (1995a): A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268:1749-1753.

Savitsky K, Sfez S, Tagle DA, Ziv Y, Sartiel A, Collins FS, Shiloh Y and Rotman G (1995b): The complete sequence of the coding region of the *ATM* gene reveals similarity to cell cycle regulators in different species. Hum Mol Genet 4:2025-2032.

Schaefer AM, McFarland R, Blakely EL, He L, Whittaker RG, Taylor RW, Chinnery PF and Turnbull DM (2008): Prevalence of mitochondrial DNA disease in adults. Ann Neurol 63:35-39.

Schapira AH (2006): Mitochondrial disease. Lancet 368:70-82.

Schiffmann R and van der Knaap MS (2004): The latest on leukodystrophies. Curr Opin Neurol 17:187-192.

Schmitz-Hübsch T and Klockgether T (2008): An update on inherited ataxias. Curr Neurol Neurosci Rep 8:310-319.

Schöls L, Amoiridis G, Przuntek H, Frank G, Epplen JT and Epplen C (1997a): Friedreich's ataxia. Revision of the phenotype according to molecular genetics. Brain 120 (Pt 12):2131-2140.

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

Schöls L, Szymanski S, Peters S, Przuntek H, Epplen JT, Hardt C and Riess O (2000): Genetic background of apparently idiopathic sporadic cerebellar ataxia. Hum Genet 107:132-137.

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

Senderek J, Krieger M, Stendel C, Bergmann C, Moser M, Breitbach-Faller N, Rudnik-Schoneborn S, Blaschek A, Wolf NI, Harting I, North K, Smith J, Muntoni F, Brockington M, Quijano-Roy S, Renault F, Herrmann R, Hendershot LM, Schroder JM, Lochmuller H, Topaloglu H, Voit T, Weis J, Ebinger F and Zerres K (2005): Mutations in *SIL1* cause Marinesco-Sjogren syndrome, a cerebellar ataxia with cataract and myopathy. Nat Genet 37:1312-1314.

Servidei S, Zeviani M, Manfredi G, Ricci E, Silvestri G, Bertini E, Gellera C, Di Mauro S, Di Donato S and Tonali P (1991): Dominantly inherited mitochondrial myopathy with multiple deletions of mitochondrial DNA: clinical, morphologic, and biochemical studies. Neurology 41:1053-1059.

Shao J and Diamond MI (2007): Polyglutamine diseases: emerging concepts in pathogenesis and therapy. Hum Mol Genet 16 Spec No. 2:R115-R123.

Slavotinek A, Goldman J, Weisiger K, Kostiner D, Golabi M, Packman S, Wilcox W, Hoyme HE and Sherr E (2005): Marinesco-Sjogren syndrome in a male with mild dysmorphism. Am J Med Genet A 133:197-201.

Smeitink JA, Zeviani M, Turnbull DM and Jacobs HT (2006): Mitochondrial medicine: a metabolic perspective on the pathology of oxidative phosphorylation disorders. Cell Metab 3:9-13.

Soong BW and Liu RS (1998): Positron emission tomography in asymptomatic gene carriers of Machado-Joseph disease. J Neurol Neurosurg Psychiatry 64:499-504.

Soong BW and Paulson HL (2007): Spinocerebellar ataxias: an update. Curr Opin Neurol 20:438-446.

Spelbrink JN, Toivonen JM, Hakkaart GA, Kurkela JM, Cooper HM, Lehtinen SK, Lecrenier N, Back JW, Speijer D, Foury F and Jacobs HT (2000): In vivo functional analysis of the human mitochondrial DNA polymerase POLG expressed in cultured human cells. J Biol Chem 275:24818-24828.

Spinazzola A and Zeviani M (2007): Disorders of nuclear-mitochondrial intergenomic communication. Biosci Rep 27:39-51.

Stevenson VL and Playford ED (2007): Rehabilitation and MS. Int MS J 14:85-92.

Stewart JD, Tennant S, Powell H, Pyle A, Blakely EL, He L, Hudson G, Roberts M, du PD, Gow D, Mewasingh LD, Hanna MG, Omer S, Morris AA, Roxburgh R, Livingston JH, McFarland R, Turnbull DM, Chinnery PF and Taylor RW (2009): Novel *POLG1* mutations associated with neuromuscular and liver phenotypes in adults and children. J Med Genet 46:209-214.

Suomalainen A, Majander A, Haltia M, Somer H, Lönnqvist J, Savontaus ML and Peltonen L (1992): Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external ophthalmoplegia. J Clin Invest 90:61-66.

Suomalainen A, Majander A, Wallin M, Setälä K, Kontula K, Leinonen H, Salmi T, Paetau A, Haltia M, Valanne L, Lönnqvist J, Peltonen L and Somer H (1997): Autosomal dominant progressive external ophthalmoplegia with multiple deletions of mtDNA: clinical, biochemical, and molecular genetic features of the 10q-linked disease. Neurology 48:1244-1253.

Sutton IJ, Last JI, Ritchie SJ, Harrington HJ, Byrd PJ and Taylor AM (2004): Adult-onset ataxia telangiectasia due to ATM 5762ins137 mutation homozygosity. Ann Neurol 55:891-895.

Swanson PD (1995): Diagnosis of inherited metabolic disorders affecting the nervous system. J Neurol Neurosurg Psychiatry 59:460-470.

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

Takiyama Y, Sakoe K, Nakano I and Nishizawa M (1997): Machado-Joseph disease: cerebellar ataxia and autonomic dysfunction in a patient with the shortest known expanded allele (56 CAG repeat units) of the MJD1 gene. Neurology 49:604-606.

Tassone F, Iwahashi C and Hagerman PJ (2004): FMR1 RNA within the intranuclear inclusions of fragile X-associated tremor/ataxia syndrome (FXTAS). RNA Biol 1:103-105.

Tatuch Y, Christodoulou J, Feigenbaum A, Clarke JT, Wherret J, Smith C, Rudd N, Petrova-Benedict R and Robinson BH (1992): Heteroplasmic mtDNA mutation (T----G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. Am J Hum Genet 50:852-858.

Taylor AM and Byrd PJ (2005): Molecular pathology of ataxia telangiectasia. J Clin Pathol 58:1009-1015.

Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly Y, Gidlof S, Oldfors A, Wibom R, Tornell J, Jacobs HT and Larsson NG (2004): Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429:417-423.

Tyynismaa H and Suomalainen A (2009): Mouse models of mitochondrial DNA defects and their relevance for human disease. EMBO Rep 10:137-143.

Tzoulis C, Engelsen BA, Telstad W, Aasly J, Zeviani M, Winterthun S, Ferrari G, Aarseth JH and Bindoff LA (2006): The spectrum of clinical disease caused by the A467T and W748S POLG mutations: a study of 26 cases. Brain 129:1685-1692.

Ursic D, Chinchilla K, Finkel JS and Culbertson MR (2004): Multiple protein/protein and protein/RNA interactions suggest roles for yeast DNA/RNA helicase Sen1p in transcription, transcription-coupled DNA repair and RNA processing. Nucleic Acids Res 32:2441-2452.

Uusimaa J, Hinttala R, Rantala H, Päivärinta M, Herva R, Röyttä M, Soini H, Moilanen JS, Remes AM, Hassinen IE and Majamaa K (2008): Homozygous W748S mutation in the *POLG1* gene in patients with juvenile-onset Alpers syndrome and status epilepticus. Epilepsia 49:1038-1045.

van de Warrenburg BP, Sinke RJ and Kremer B (2005): Recent advances in hereditary spinocerebellar ataxias. J Neuropathol Exp Neurol 64:171-180.

van den Brink DM, Brites P, Haasjes J, Wierzbicki AS, Mitchell J, Lambert-Hamill M, de Belleroche J, Jansen GA, Waterham HR and Wanders RJ (2003): Identification of *PEX7* as the second gene involved in Refsum disease. Am J Hum Genet 72:471-477.

Van Esch H, Dom R, Bex D, Salden I, Caeckebeke J, Wibail A, Borghgraef M, Legius E, Fryns JP and Matthijs G (2005): Screening for *FMR-1* premutations in 122 older Flemish males presenting with ataxia. Eur J Hum Genet 13:121-123.

Van Goethem G (2006): Autosomal disorders of mitochondrial DNA maintenance. Acta Neurol Belg 106:66-72.

Van Goethem G, Dermaut B, Lofgren A, Martin JJ and Van Broeckhoven C (2001): Mutation of *POLG* is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. Nat Genet 28:211-212.

Van Goethem G, Martin JJ, Dermaut B, Lofgren A, Wibail A, Ververken D, Tack P, Dehaene I, Van Zandijcke M, Moonen M, Ceuterick C, De Jonghe P and Van Broeckhoven C (2003a): Recessive *POLG* mutations

presenting with sensory and ataxic neuropathy in compound heterozygote patients with progressive external ophthalmoplegia. Neuromuscul Disord 13:133-142.

Van Goethem G, Mercelis R, Löfgren A, Seneca S, Ceuterick C, Martin JJ and Van Broeckhoven C (2003b): Patient homozygous for a recessive *POLG* mutation presents with features of MERRF. Neurology 61:1811-1813.

Van Goethem G, Schwartz M, Lofgren A, Dermaut B, Van Broeckhoven C and Vissing J (2003c): Novel *POLG* mutations in progressive external ophthalmoplegia mimicking mitochondrial neurogastrointestinal encephalomyopathy. Eur J Hum Genet 11:547-549.

Vazquez-Memije ME, Shanske S, Santorelli FM, Kranz-Eble P, DeVivo DC and DiMauro S (1998): Comparative biochemical studies of ATPases in cells from patients with the T8993G or T8993C mitochondrial DNA mutations. J Inherit Metab Dis 21:829-836.

Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, Eussen BE, van Ommen G-J, Blonden LA, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA and Warren ST (1991): Identification of a gene (*FMR-1*) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905-914.

Wardle M, Majounie E, Muzaimi MB, Williams NM, Morris HR and Robertson NP (2009): The genetic aetiology of late-onset chronic progressive cerebellar ataxia : A population-based study. J Neurol Epub, DOI: 10.1007/s00415-009-0015-2.

Warner TT, Lennox GG, Janota I and Harding AE (1994): Autosomal-dominant dentatorubropallidoluysian atrophy in the United Kingdom. Mov Disord 9:289-296.

Wenning GK, Colosimo C, Geser F and Poewe W (2004): Multiple system atrophy. Lancet Neurol 3:93-103.

White SL, Collins VR, Wolfe R, Cleary MA, Shanske S, DiMauro S, Dahl HH and Thorburn DR (1999a): Genetic counseling and prenatal diagnosis for the mitochondrial DNA mutations at nucleotide 8993. Am J Hum Genet 65:474-482.

White SL, Shanske S, McGill JJ, Mountain H, Geraghty MT, DiMauro S, Dahl HH and Thorburn DR (1999b): Mitochondrial DNA mutations at nucleotide 8993 show a lack of tissue- or age-related variation. J Inherit Metab Dis 22:899-914.

Wild EJ and Tabrizi SJ (2007): Huntington's disease phenocopy syndromes. Curr Opin Neurol 20:681-687.

Wiltshire E, Davidzon G, DiMauro S, Akman HO, Sadleir L, Haas L, Zuccollo J, McEwen A and Thorburn DR (2008): Juvenile Alpers disease. Arch Neurol 65:121-124.

Winterthun S, Ferrari G, He L, Taylor RW, Zeviani M, Turnbull DM, Engelsen BA, Moen G and Bindoff LA (2005): Autosomal recessive mitochondrial ataxic syndrome due to mitochondrial polymerase gamma mutations. Neurology 64:1204-1208.

Wittenberger MD, Hagerman RJ, Sherman SL, McConkie-Rosell A, Welt CK, Rebar RW, Corrigan EC, Simpson JL and Nelson LM (2007): The *FMR1* premutation and reproduction. Fertil Steril 87:456-465.

Wong LJ, Naviaux RK, Brunetti-Pierri N, Zhang Q, Schmitt ES, Truong C, Milone M, Cohen BH, Wical B, Ganesh J, Basinger AA, Burton BK, Swoboda K, Gilbert DL, Vanderver A, Saneto RP, Maranda B, Arnold G, Abdenur JE, Waters PJ and Copeland WC (2008): Molecular and clinical genetics of mitochondrial diseases due to *POLG* mutations. Hum Mutat 29:E150-E172.

Worth PF (2004): Sorting out ataxia in adults. Practical Neurology 4:130-151.

Wüllner U, Klockgether T, Petersen D, Naegele T and Dichgans J (1993): Magnetic resonance imaging in hereditary and idiopathic ataxia. Neurology 43:318-325.

Wüllner U, Schmitz-Hübsch T, Abele M, Antony G, Bauer P and Eggert K (2007): Features of probable multiple system atrophy patients identified among 4770 patients with parkinsonism enrolled in the multicentre registry of the German Competence Network on Parkinson's disease. J Neural Transm 114:1161-1165.

Yuzaki M, Ohkoshi N, Kanazawa I, Kagawa Y and Ohta S (1989): Multiple deletions in mitochondrial DNA at direct repeats of non-D-loop regions in cases of familial mitochondrial myopathy. Biochem Biophys Res Commun 164:1352-1357.

Zeviani M and Di Donato S (2004): Mitochondrial disorders. Brain 127:2153-2172.

Zeviani M, Servidei S, Gellera C, Bertini E, DiMauro S and DiDonato S (1989): An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. Nature 339:309-311.

Adult-onset autosomal recessive ataxia with thalamic lesions in a Finnish family

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Article abstract—*Objective:* To describe an unusual kindred with adult-onset ataxia and thalamic lesions detected by brain MRI. *Methods:* The authors characterized clinical, laboratory, and pathologic features of the disease and sought linkage to previously recognized ataxia loci. *Results:* Two sisters and a brother developed progressive ataxia, dysarthria, mild cognitive impairment, and sensorimotor neuropathy at age 30, combined with epilepsy in one sibling. MRI showed symmetric thalamic lesions, changes in brainstem gray matter, and white matter changes in the cerebellum. Autopsy in one of the patients revealed neuronal degeneration with a peculiar vacuolar change in thalamus, probably representing transsynaptic degeneration in response to deafferentation. Neuronal and secondary tract degeneration was observed in the spinal cord, cerebellum, and brainstem suggesting a spinocerebellar degeneration. The disorder appears to be transmitted as an autosomal recessive trait. Genetic and sequence analysis of the *FRDA* gene and comprehensive laboratory examinations excluded Friedreich's ataxia and other similar recessive diseases. *Conclusion:* Adult-onset recessive ataxia with bilateral thalamic lesions in this family may represent a distinct hereditary spinocerebellar ataxia.

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Late-onset autosomal recessive ataxia is very rare; onset before age 25 used to be a principal feature of Friedreich's ataxia (FRDA1) in the classification by Harding.[1] The detection of a major mutation in the *frataxin (FRDA)* gene, an unstable $(GAA)_n$ trinucleotide repeat in intron 1, allows for rapid and accurate molecular diagnosis.[2] Recently, patients with adult-onset FRDA1 have also been identified by genetic testing of *FRDA*.[3]

The other well-defined recessive ataxias typically have an early onset. Disease-causing mutations have been identified in specific genes for ataxia with vitamin E deficiency (AVED)[4] and ataxia telangiectasia (A-T),[5] both of which are mainly childhood disorders. An autosomal recessive form of childhood-onset ataxia with spasticity (ARSACS), first reported in French–Canadian families from the Charlevoix–Saguenay region of Quebec, has been mapped to 13q11[6] with an allelic syndrome recently reported from Tunisia.[7] Early-onset cerebellar ataxia (EOCA) is genetically heterogenous, and the genetic defects underlying most forms are unknown.[8] However, some patients with EOCA have mutations in the *FRDA* gene and are diagnosed with Friedreich's ataxia with retained reflexes.[9] One form of EOCA, infantile-onset recessive ataxia, has been mapped to 10q23.3–10q24.1.[10] Other early-onset forms of recessive ataxia have been reported, each occurring with one of the following clinical features: hypogonadism, congenital or childhood deafness, optic atrophy, pigmentary retinopathy, extrapyramidal symptoms, cataracts, or mental retardation.[1]

Here we describe a kindred with five sibs, three of them affected by progressive ataxia starting at age 30. Clinical, biochemical, and molecular genetic investigations have ruled out FRDA1, ARSACS, and other known ataxias causing a similar symptomatology.

Patients and methods. Two sons were healthy, and one 45-year-old son and two daughters aged 36 and 42 years were affected (figure 1). The youngest patient died at the end of the study. Preliminary autopsy findings are included in this report, and a complete study will be published separately. Both parents and all four grandparents were born in the same rural area in Karelia, near the Finnish–Russian border.

The parents were examined by two neurologists (M.R. and B.U.); both were healthy. No other family members
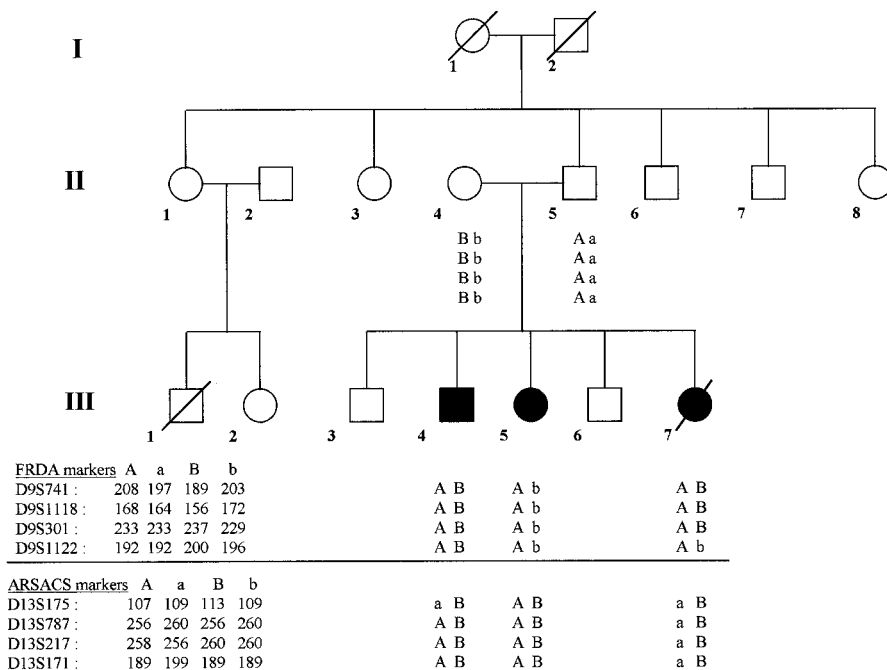
Figure 1. Family pedigree shows the haplotypes for FRDA and ARSACS loci segregating with parents and affected members. Filled symbols indicate affected family members.

FRDA markers	A	a	B	b		III-4	III-5	III-7
D9S741:	208	197	189	203		A B	A b	A B
D9S1118:	168	164	156	172		A B	A b	A B
D9S301:	233	233	237	229		A B	A b	A B
D9S1122:	192	192	200	196		A B	A b	A B

ARSACS markers	A	a	B	b		III-4	III-5	III-7
D13S175:	107	109	113	109		a B	A B	a B
D13S787:	256	260	256	260		A B	A B	a B
D13S217:	258	256	260	260		A B	A B	a B
D13S171:	189	199	189	189		A B	A B	a B

were affected by any similar disease. Two cousins had had neurologic problems (III-1 and III-2). One exhibited severe psychiatric symptoms that started at age 32 with alcoholism. He died suddenly at age 42, presumably from an epileptic seizure. No macroscopic changes in the brain were reported on autopsy. His 45-year-old sister had congenital cerebral palsy without subsequent progressive neurologic problems.

One of the affected individuals (III-4) refused further clinical examinations. However, he agreed to clinical examination by two neurologists (M.R. and B.U.). The two other patients (III-7, the proband, and III-5) underwent laboratory examinations of blood, urine, CSF, and fibroblast samples. Laboratory tests for lysosomal storage diseases included urinary screening for mucopolysaccharides and oligosaccharides by thin layer chromatography, and determination of the enzyme activity of arylsulfatase A, galactocerebrosidase, β-galactosidase, glucocerebrosidase, hexosaminidase A and B, and α-neuraminidase in fibroblasts. CT and MRI of the brain were performed. Neurophysiologic investigation included motor and antidromically determined sensory nerve conduction measurements using surface electrodes, sensory threshold measurements, electromyography (EMG), tibial and median nerve somatosensory-evoked potentials (SEP), brainstem auditory-evoked potentials, and reversal pattern visual-evoked potential (VEP), together with flash VEP, electroretinography and blink reflex, and EEG. Muscle and sural nerve biopsies and bone marrow aspiration were performed on Patient III-7. After her sudden death, autopsy and neuropathologic studies were conducted. Other examinations included neuropsychologic tests and ophthalmologic examinations, electrocardiography, thoracic and long-bone x-rays, abdominal ultrasonography, and a set of tests to examine functions of the autonomous nervous system.

DNA isolation. Seven members of the family were included in the genetic study (figure 1). Genomic DNA was isolated from venous blood or lymphoblastoid cell lines from all consenting individuals using standard methods.

All the samples were drawn in accordance with the Helsinki Declaration.

Genotyping. To determine linkage in the FRDA1 and ARSACS[6,7,11] regions in chromosome 9q13 and 13q11, we typed four microsatellite markers[12] flanking each candidate gene: *D9S741* (42.7 cM), *D19S1118* (58.3 cM), *D9S301* (66.3 cM), *D9S1122* (75.9 cM), *D13S175* (5.03 cM), *D13S787* (8.87 cM), *D13S217* (17.21 cM), and *D13S171* (25.08 cM). Genomic DNA samples were analyzed by PCR amplification. The forward primers were modified at the 5' end with a FAM, TET, or HEX fluorescent label. The PCR reactions were performed under the following conditions: 50 ng genomic DNA, 1 × Perkin–Elmer PCR buffer (Foster City, CA), 130 μM dNTP, 5 pmols of both the forward and the reverse primer, and 0.75 U Ampli Taq Gold™ polymerase (Perkin–Elmer) in a final volume of 15 μL. The cycling conditions were 94 °C for 10 minutes for one cycle, 94 °C for 30 seconds, 55 °C for 1 minute 15 seconds, and 72 °C for 1 minute, for 30 cycles (FAM and TET markers) or 35 cycles (HEX markers), and 72 °C for 10 minutes. Amplified products were separated by electrophoresis on 4.25% polyacrylamide-6 M urea gel using a 377 DNA sequencer (Perkin–Elmer/Applied Biosystems, Weiterstadt, Germany), and the results were processed with Genescan™ (version 2.0.2) and Genotyper™ (version 1.1) software (Perkin-Elmer/Applied Biosystems).

Mutation analyses. Detection of the (GAA)$_n$ expansion in intron 1 of the *FRDA* gene was performed as described,[2] except that the 5' and 3' primers were tailed with M13-forward (5'-TGTAAAACGACGGCCAGT-3') and M13-reverse (5'-CAGGAAACAGCTATGACC-3') tails for direct sequencing in both directions. PCR fragments were purified using the Exonuclease I/Shrimp Alkaline Phosphatase PCR Product Sequencing Kit (USB–Amersham Life Science, UK). Two μl of the PCR products was sequenced using the BigDye–Terminator AmpliTaq FS Cycle Sequencing Kit (Perkin–Elmer/Applied Biosystems), and results were analyzed with Sequence Navigator software (Perkin–Elmer/Applied Biosystems).

Table *Clinical findings of affected individuals in the pedigree*

Finding	Patient III-7	Patient III-5	Patient III-4
Sex/age, y at onset/age, y at study	F/29/36	F/30/41	M/30/45
Ataxia of limbs, gait, and stance	+++	+++	+++
Dysarthria	++	++	+++
Dysphagia	+	+	−
Nystagmus and jerky pursuit eye movements	+	+	+
Upward gaze paresis	−	+	+
Tendon reflexes			
Upper limbs	Normal	Normal	Decreased
Knee and ankle	Decreased	Absent	Absent
Babinski sign	+/+	−/−	−/−
Decreased vibration and joint position sense	+	+	+
Cognitive impairment	+	+	+
Epilepsy	+	−	−

+ Finding present; ++ prominent finding; +++ severe finding; − finding absent.

PCR was used for the amplification of $(CAG)_n$ repeats, and polyacrylamide gel electrophoresis for estimation of repeat numbers in the autosomal dominant SCA types 1, 2, 3, 6, 7, 8, 10, and 12 and dentatorubral-pallidolluysian atrophy (DRPLA) according to standard techniques previously described.[13-21] DNA mutation analyses of mitochondrial point mutations at positions 8344, 3243, and 8993 of the mtDNA associated with myoclonus epilepsy with ragged-red fibers (MERRF), mitochondrial encephalomyopathy-lactic acidosis, stroke-like symptoms (MELAS), neuropathy, ataxia with retinitis pigmentosa (NARP), and mitochondrial deletion screening were carried out from muscle samples of Patient III-7 according to methods described elsewhere.[22]

Case reports. *Patient III-7.* Her developmental milestones were normal. Neurologic symptoms started at age 29 with increasing difficulties in walking and balance. Her husband noticed decreased initiative and ability to concentrate. At age 30, severe gait ataxia was observed. MS was suspected, but CSF showed no signs of demyelinating disease. The patient had repeated grand-mal–type epileptic seizures, which were resistant to anticonvulsive medication. Dysarthria started after age 30, and the patient also reported slight dysphagia. In later years, the walking distance with a walker decreased to a few hundred meters, but the patient continued to be ambulatory until her sudden death at age 36, presumed to be caused by an epileptic seizure.

On clinical examination at age 36, the patient exhibited a grossly ataxic, wide-based gait while leaning on a walker. She had mild tremor of the head. Muscle strength and tone in the upper and lower extremities were normal, and there was no muscular atrophy. Finger-to-nose test was dysmetric, and diadochokinesia was clumsy. She had decreased fine motor movement in the fingers. Lower limbs showed marked dysmetria. Vibration and position senses were impaired, whereas her superficial sense was intact. Patellar and ankle reflexes were barely elicited, and Babinski sign was positive. Eye movements were full with gaze-evoked nystagmus and jerky pursuit movements. She

had diplopia when changing direction of gaze to the left. Her speech was mildly slurred. Mental alertness, interaction, orientation, and perception were good, but short-term memory was inaccurate.

Patient III-5. Early development and adolescence were normal. At approximately age 30, the patient noticed clumsiness in her legs while walking down stairs. Neurologic examination at age 36 showed pronounced ataxia. She also had mild dysphagia, and stress incontinence started at age 37. At age 42 she was using a walker, and her gait was ataxic, wide-based, but fairly rapid.

Patient III-4. Childhood development was normal. The patient performed military service at age 21 without problems. At approximately age 30, he started to show lack of initiative, tiredness, social withdrawal, slight balance problems, and clumsiness on walking. At age 35, he was referred to a mental health center. Lack of concentration and withdrawal were considered to suggest mental illness. Antipsychotics increased difficulty of movement, and he stopped taking them. The patient refused further neurologic examinations. Difficulty in walking worsened over time, his speech was slurred, and his hands were clumsy. The patient was aware of slight memory problems. He also had pollaciuria. Upon neurologic examination at age 44 he could stand without support, and walking was grossly ataxic.

Clinical findings are summarized in table 1.

Results. *Brain imaging findings.* The proband (III-7) underwent brain MRI three times, at ages 32, 33, and 35 (figure 2, A through B). Main findings included increased signal intensity lesions in T2-weighted series bilaterally in the thalamus and in the upper part of the medulla oblongata at the level of inferior olivary nuclei, primarily in the gray matter. In addition, bilateral high-intensity signals were seen in the cerebellar white matter. As with demyelination, these lesions were hypointensive in T1-weighted series. A small frontal cortical lesion observed on the first MRI was not visible in subsequent studies, but other findings persisted. No contrast enhancement was found. There
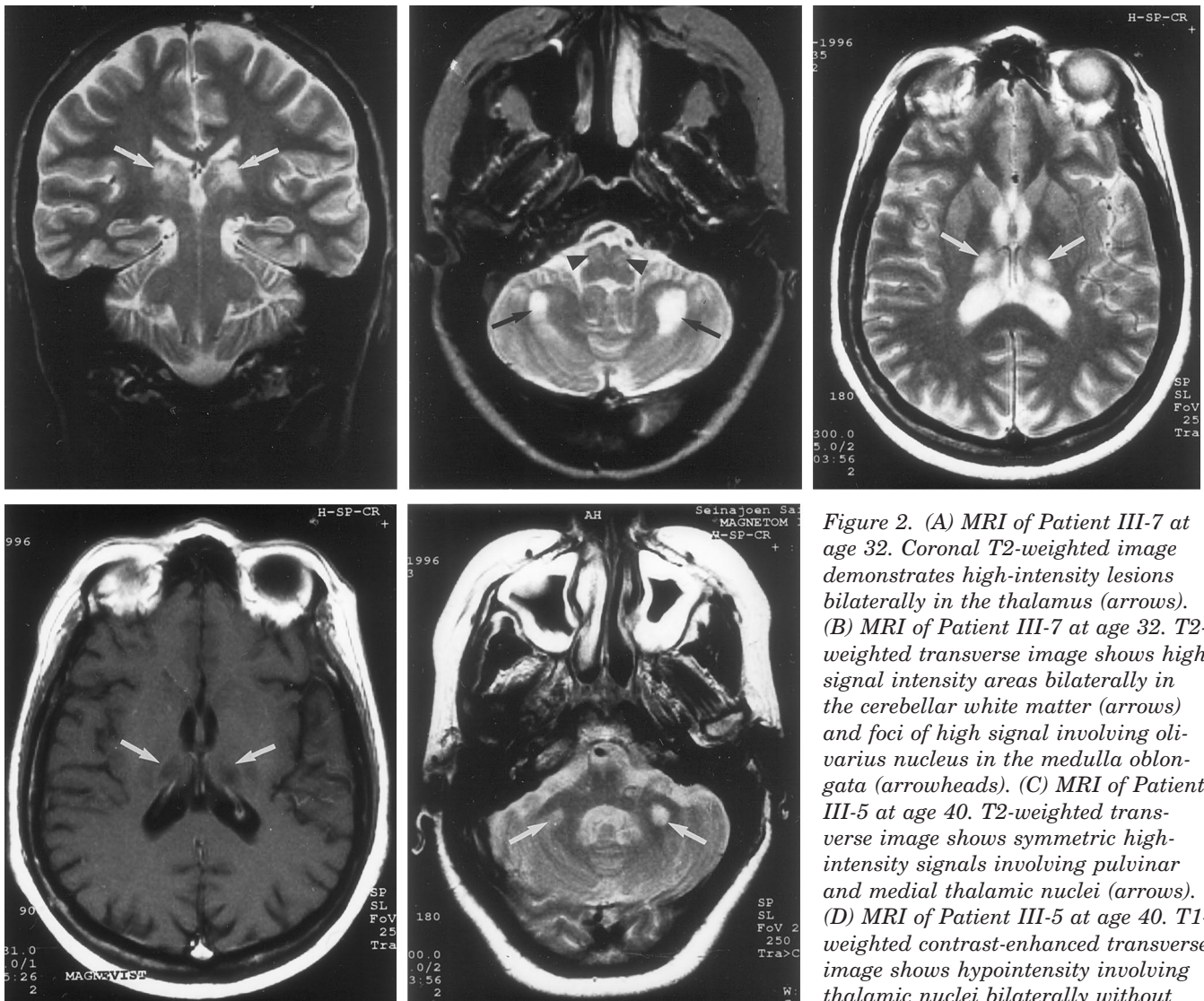
Figure 2. (A) MRI of Patient III-7 at age 32. Coronal T2-weighted image demonstrates high-intensity lesions bilaterally in the thalamus (arrows). (B) MRI of Patient III-7 at age 32. T2-weighted transverse image shows high signal intensity areas bilaterally in the cerebellar white matter (arrows) and foci of high signal involving olivarius nucleus in the medulla oblongata (arrowheads). (C) MRI of Patient III-5 at age 40. T2-weighted transverse image shows symmetric high-intensity signals involving pulvinar and medial thalamic nuclei (arrows). (D) MRI of Patient III-5 at age 40. T1-weighted contrast-enhanced transverse image shows hypointensity involving thalamic nuclei bilaterally without contrast enhancement (arrows). (E) MRI of Patient III-5 at age 40. T2-weighted transverse image demonstrates increased signal areas in middle cerebellar peduncles (arrows) and enlargement of fourth ventricle and pontine cysterns.

were minor infra- and supratentorial atrophic changes. Brain CT performed at age 32 was normal.

Patient III-5 underwent one brain MRI at age 40 (see figure 2, C through E). Symmetric thalamic and cerebellar hyperintensity signal abnormalities similar to those observed in her sister were detected in T2-weighted series, and these lesions were hypointensive in T1-weighted images. She had minor infra- and supratentorial atrophy. Symmetric low density areas in the thalamus and cerebellar white matter were also observed on brain CT performed at age 37.

Laboratory findings. Detailed blood, urine, and CSF tests were performed for the proband (III-7) and Patient III-5 without abnormal findings. Complete blood counts were normal. No acanthocytosis could be demonstrated in peripheral blood smears. Results relating to fasting glucose, glucose tolerance, electrolytes, creatinine, calcium, vitamin E, vitamin B_{12}, folic acid, creatine kinase, lactate dehydrogenase, serum electrophoresis, serum immunoglobulins, α-fetoprotein, very long chain fatty acids, phytanic acid, and cholestanol were normal. No significant abnormalities were demonstrated in liver and thyroid function tests, cholesterol and triglyceride levels, copper and ceruloplasmin values, or lactate and pyruvate levels. Blood ammonia and urinary organic and amino acids, CSF protein level, cell count, and glucose, lactate, and IgG indexes were all normal, as were laboratory tests for lysosomal disorders.

Results of basic cardiologic examinations, ECG, and chest x-rays were normal in Patients III-7 and III-5. Echocardiography in Patient III-7 showed minor mitral valve insufficiency but no signs of cardiomyopathy. Findings on abdominal ultrasonography and x-rays of long bones were also normal.

Neurophysiologic features. Sensory and motor conduction velocities were slightly reduced in Patients III-7 and III-5, and sensory action potentials in peroneal and sural nerves were absent or had markedly decreased amplitudes. The findings were consistent with a mainly sensory axonal neuropathy (findings summarized in table 2, available at www.neurology.org). On sensory threshold testing, Patient III-7 exhibited impaired vibration sense. Cold and heat thresholds were also abnormal, suggesting thin-fiber neuropathy.

Results of SEP tests of both patients demonstrated similar findings. Responses were diffusely delayed at the spinal level and missing or decreased at the cortical level, suggesting a central disorder in the somatosensory pathway. The central conduction time T12-P37 of the tibial SEP recorded from Patient III-7 was normal (18.3 msec on both sides). Tibial SEP P37 in Patient III-5 was remarkably prolonged (49.2 msec on the right side/49.3 msec on the left side), but because of missing spinal responses the exact level of the latency delay remains uncertain. Brainstem auditory-evoked potential stimulation of the right ear in Patient III-7 showed inconsistent brainstem responses. On the left, latencies were slightly prolonged, suggesting a disorder in the region of the pons. Latency of wave I was 1.7 msec, interpeak latency I-III 2.2 msec, and interpeak latency I-V 4.3 msec on the left side. Reversal pattern VEP, flash VEP, blink reflex, and electroretinograms were normal. EEG of Patient III-7 at age 33 revealed moderate diffuse background slowing. Autonomic function tests of Patient III-7 showed findings of the cardiovascular system compatible with mild autonomic dysfunction.

Specific studies. Ophthalmologic examination of Patients III-7 and III-5 revealed no retinopathy, optic nerve atrophy, or cataracts.

Extensive neuropsychologic examinations of Patient III-7 and III-5 showed cognitive and behavioral changes. The overall cognitive processing was at low–medium level: the full scale IQ of Patient III-7 was 88 (Wechsler Adult Intelligence Scale–Revised) and of Patient III-5 86 (Wechsler Adult Intelligence Scale), although the primary IQ level of both patients was supposed to be normal. Verbal and visual memory measured by Wechsler Memory Scale and visuomotor functions were impaired. Patient III-7 also had mild anomia and flattening of affect, and both patients had some loss of insight.

Morphologic studies and autopsy findings. Minor, nonspecific changes without significant neurogenic atrophy were observed on light and electron microscopic examination of muscle biopsy specimens, and there were no findings suggesting storage or mitochondrial diseases. Light and electron microscopy of distal sural nerve showed marked decrease of large myelinated fibers and some clusters of small regenerating fibers. No demyelination was observed. The pathologic findings were consistent with axonal neuropathy. No pathologic findings indicating storage disease were detected on bone marrow examination.

On autopsy the cerebral hemispheres were preserved, but degenerative pathology was observed in the thalamus, brainstem, cerebellum, and spinal cord. Slight degeneration and atrophy were seen in the dorsal root ganglia. The spinal nerve roots, predominantly the posterior, showed some fiber depletion. The posterior columns (especially the gracile), the posterior spinocerebellar tracts (to a lesser extent), and the corticospinal tracts (to an even milder extent) were atrophic. The dorsal nucleus (Clarke's column) showed a clear neuronal loss, which was not the case in the autonomic intermediolateral columns. In the cerebellum, the cortex was quite well preserved, except for a slight and patchy dropout of Purkinje cells. The most severe changes in the cerebellum were seen in the dentate: subtotal depletion of neurons, gliosis, and completely fiber-depleted hilus. In medulla oblongata, a similarly severe degeneration of the inferior olives was seen. The caudal

pontine basis was slightly atrophic, as were the corticospinal tracts, pontine, mesencephalic tegmentum, and periaqueductal gray matter. Inferior and superior cerebellar peduncles were atrophic, but middle cerebellar peduncles were only slightly atrophic. Substantia nigra was atrophic, especially at its pars lateralis. Some neuronal degeneration in the pulvinar, and especially in the dorsomedial thalamic nuclei, accompanied by a peculiar vacuolar change, was observed, and this morphologic change most probably represents a transsynaptic deafferentation-related atrophy. Basal ganglia, hippocampi, and white matter were well preserved. There were some focal laminar necrosis remnants in the cerebral cortex, but morphology was mostly normal. No signs of neuronal storage could be observed.

Genetic analyses. Three members of the family presented here had the disease, whereas no symptoms were observed in the parents or the other two healthy siblings (figure 1). Both sexes were affected without major variations in the phenotype. Genealogic studies revealed no links among parents over the past 150 years, but the fact that they were born in the same rural community increases the probability of distant common ancestry. An autosomal dominant genetic defect with incomplete penetrance or maternal inheritance were considered unlikely because of the absence of phenotypic variation, including age at onset, and no signs of disease, even to a lesser extent, in the healthy family members. Taken together, these findings are most consistent with an autosomal recessive inheritance pattern.

DNA analyses for the major mutations in autosomal dominant SCA types 1, 2, 3, 6, 7, 8, 10, and 12, DRPLA, mitochondrial deletions, and point mutations associated with MERRF, MELAS, and NARP were all negative.

In addition, we considered linkage to the two autosomal recessive ataxia loci currently characterized, *FRDA* in 9q13 and *SACS* in 13q11. Although all three patients were heterozygous for the four polymorphic markers flanking the *FRDA* locus, the two patients (III-7 and III-5) tested for the FRDA1-associated (GAA)$_n$ expansion were homozygous for the same (GAA)$_{10}$ allele. This suggested the possibility of a homozygous mutation in *FRDA* other than the (GAA)$_n$ expansion. To exclude such a mutation, we sequenced the entire region, coding and flanking intronic sequences. No mutations were identified. Haplotypes constructed with four polymorphic markers in the ARSACS and FRDA1 regions show that both parental chromosomes are inherited among the patients for both disease loci (see figure 1), thus excluding linkage.

Discussion. Here we describe a family with progressive adult-onset spinocerebellar ataxia in three affected siblings. Clinical findings include gait ataxia, dysarthria, nystagmus, peripheral neuropathy, mild impairment in cognition, and, in one patient, epileptic seizures. Bilateral symmetric thalamic lesions constitute a very specific finding on brain MRI. Other MRI findings are cerebellar white matter changes and mild infra- and supratentorial atrophy. The presentation of the disorder in the family is most compatible with an autosomal recessive mode of inheritance.

Some features may suggest a diagnosis of FRDA.[23]

These include ataxia, dysarthria, loss of deep sensation, and absent or mild cerebellar atrophy. FRDA1 may also be associated with cardiomyopathy or diabetes, but these were not present in this family. All patients had cognitive impairment and one had epilepsy, which are not features of FRDA1. Unique MRI and morphologic findings of bilateral thalamic lesions have not been reported in FRDA1 or any other recessive ataxia. An FRDA1-associated mutant $(GAA)_n$ expansion in *FRDA* was excluded as the underlying mutation.

Gene sequencing and linkage analysis exclude other mutations of the *FRDA* gene. We also considered linkage to the other characterized recessive ataxia locus, *SACS*, in 13q11. Nonidentical sharing of parental chromosomes in the patients shown by segregation of haplotypes excluded linkage to the ARSACS region.

Recessive ataxia caused by abnormal vitamin E metabolism,[4] abetalipoproteinemia,[24] and A-T[5] were excluded by normal blood biochemistry results. Two other syndromes associated with defective DNA repair and neurodegeneration, xeroderma pigmentosum and Cockayne's syndrome, were excluded on the basis of clinical features.[25]

Symptoms of inherited metabolic disorders usually start in early infancy or childhood, and many of these disorders were previously considered to be limited to childhood. However, some of them should be considered in the differential diagnosis of adults. In these disorders, ataxia may be one symptom in addition to other CNS manifestations such as pyramidal symptoms, extrapyramidal symptoms, and dementia. In our patients, GM2 gangliosidosis,[24] GM1 gangliosidosis,[24] Gaucher disease,[24] Krabbe disease,[24] metachromatic leukodystrophy,[24] and sialidosis[26] were excluded by means of normal fibroblast enzyme assay results. Niemann–Pick disease type C was ruled out by normal bone marrow findings and normal morphology of visceral autopsy specimens.[24] Urinary screening for glycosaminoglycans and oligosaccharides was also negative, ruling out mucopolysaccharidoses, disorders of glycoprotein degradation, and sialuria (Salla disease).[27] Normal serum very long chain fatty acids and phytanic acid excluded adrenoleukodystrophy[28] and Refsum disease.[24] Wilson disease, disorders of amino acid metabolism, and cerebrotendinous xanthomatosis were excluded by biochemical results.[24] No accumulation suggesting polyglucosan body disease was observed on sural nerve biopsy,[29] and the adult form of ceroid lipofuscinosis does not fit well clinically because no lipopigment was observed in neurons at brain autopsy.[30] Lack of increase in serum lactate, pyruvate, and cerebrospinal lactate levels, absence of ragged-red and cytochrome c oxidase-negative fibers on muscle biopsy, lack of known point mutations (MELAS, MERRF, NARP), and mtDNA deletions in muscle biopsy specimen make mitochondrial diseases unlikely.[31] Autopsy findings are not compatible with typical Leigh's disease.[32]

Brain MRI findings in our patients are very characteristic, although not pathognomic. Unspecific white and gray matter abnormalities have been shown in many neurometabolic diseases such as lysosomal and peroxisomal storage disorders.[33] MRI findings of mitochondrial diseases include leukodystrophic changes and deep gray nuclear changes in brainstem, thalamus, and basal ganglia, in addition to cerebral and cerebellar atrophy.[34] Abnormal signal intensity of transverse pontine fibers has been reported in patients with SCA2 and other spinocerebellar ataxias.[35-37] High-intensity signals have been detected on T2-weighted images of the brainstem and thalamus, in addition to diffuse, high-intensity areas in cerebral white matter, in some patients with DRPLA.[38] MRI showed mild infratentorial atrophy and vague supratentorial atrophy.

With the exception of FRDA1, adult- and late-onset recessive ataxias are poorly described and understood. A new type was recently reported,[39] but the disease was different in terms of conjunctival telangiectasias as a pathognomonic feature and elevated serum creatine kinase, gamma globulin, and α-fetoprotein levels as abnormal laboratory findings. Two reports on testing large groups of patients with recessive or sporadic ataxia for the main mutation in the *FRDA* gene have recently been published.[3,40] Mutant $(GAA)_n$ expansion in *FRDA* was excluded in 25% and approximately 50% of the patients in both these studies.[3,40]

References

1. Harding AE. The hereditary ataxias and related disorders. Edinburgh: Churchill Livingstone, 1984.
2. Campuzano V, Montermini L, Moltò MD, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 1996;271:1423–1427.
3. Dürr A, Cossee M, Agid Y, et al. Clinical and genetic abnormalities in patients with Friedreich's ataxia. N Engl J Med 1996;335:1169–1175.
4. Ouachi K, Arita M, Kayden H, et al. Ataxia with isolated vitamin E deficiency is caused by mutations in alpha-tocopherol transfer protein. Nat Genet 1995;9:141–145.
5. Concannon P, Gatti RA. Diversity of ATM gene mutations detected in patients with ataxia-telangiectasia. Hum Mutat 1997;10:100–107.
6. Engert JC, Berube P, Mercier J, et al. ARSACS, a spastic ataxia common in northeastern Quebec, is caused by mutations in a new gene encoding an 11.5-kb ORF. Nat Genet 2000;24:120–125.
7. Mrissa N, Belal S, Ben Hamida C, et al. Linkage to chromosome 13q11–12 of an autosomal recessive cerebellar ataxia in a Tunisian family. Neurology 2000;54:1408–1414.
8. Klockgether T, Petersen D, Grodd W, Dichgans J. Early onset cerebellar ataxia with retained tendon reflexes: clinical, electrophysiological and MRI observations in comparison with Friedreich's ataxia. Brain 1991;114:1559–1573.
9. Geschwind DH, Perlman S, Grody WW, et al. Friedreich's ataxia GAA repeat expansion in patients with recessive or sporadic ataxia. Neurology 1997;49:1004–1009.

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

11. Bouchard JP, Richter A, Mathieu J, et al. Autosomal recessive spastic ataxia of Charlevoix–Saguenay. Neuromuscul Disord 1998;8:474–479.

12. Lindqvist A-KB, Magnusson PKE, Balciuniene J, et al. Chromosome-specific panels of tri- and tetranucleotide microsatellite markers for multiplex fluorescent detection of automated genotyping: evaluation of their utility in pathology and forensics. Genome Res 1996;6:1170–1176.

13. Orr HT, Chung MY, Banfi S, et al. Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. Nat Genet 1993;4:221–226.

14. Pulst SM, Nechiporuk A, Nechiporuk T, et al. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat Genet 1996;14:269–276.

15. Kawaguchi Y, Okamoto T, Taniwaki M, et al. CAG expansions in a novel gene for Machado–Joseph disease at chromosome 14q32.1. Nat Genet 1994;8:221–228.

16. Zhuchenko O, Bailey J, Bonnen P, et al. Autosomal dominant cerebellar ataxia (SCA 6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. Nat Genet 1997;15:62–69.

17. David G, Abbas N, Stevanin G, et al. Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. Nat Genet 1997;17:65–70.

18. Koob MD, Moseley ML, Schut LJ, et al. An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). Nat Genet 1999;21:379–384.

19. Matsuura T, Yamagata T, Burgess DL, et al. Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. Nat Genet 2000;26:191–194.

20. Holmes SE, O'Hearn EE, McInnis MG, et al. Expansion of a novel CAG trinucleotide repeat in the 5' region of PPP2R2B is associated with SCA12. Nat Genet 1999;23:391–392.

21. Koide R, Ikeuchi T, Onodera O, et al. Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). Nat Genet 1994;6:9–13.

22. Moraes CT, DiMauro S, Zeviani M, et al. Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns–Sayre syndrome. N Engl J Med 1989;320:1293–1299.

23. Filla A, De Michele G, Cavalcanti F, et al. The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich ataxia. Am J Hum Genet 1996;59:554–560.

24. Swanson PD. Diagnosis of inherited metabolic disorders affecting the nervous system. J Neurol Neurosurg Psychiatry 1995;59:460–470.

25. Chu G, Mayne L. Xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy: do the genes explain the diseases? Trends Genet 1996;12:187–192.

26. Johnson WG. Disorders of glycoprotein degradation: sialidosis, fucosidosis, alpha-mannosidosis, beta-mannosidosis, and aspartyl-glycosaminuria. In: Rosenberg RN, Prusiner SB, DiMauro S, Barchi R, eds. The molecular and genetic basic of neurological disease. Boston: Butterworth–Heineman, 1995: 335–369.

27. Romppanen J, Mononen I. Age-related reference values for urinary excretion of sialic acid and deoxysialic acid: application to diagnosis of storage disorders of free sialic acid. Clin Chem 1995;41:544–547.

28. Moser HW. Adrenoleukodystrophy: phenotype, genetics, pathogenesis and therapy. Brain 1997;120:1485–1508.

29. Bruno C, Servidei S, Shanske S, et al. Glycogen branching enzyme deficiency in adult polyglucosan body disease. Ann Neurol 1993;33:88–93.

30. Martin J-J. Adult type of neuronal ceroid-lipofuscinosis. J Inher Metab Dis 1993;16:237–240.

31. DiMauro S, Moraes CT. Mitochondrial encephalomyopathies. Arch Neurol 1993;50:1197–1208.

32. Chalmers RM, Lamont PJ, Nelson I, et al. A mitochondrial DNA tRNA(Val) point mutation associated with adult-onset Leigh syndrome. Neurology 1997;49:589–592.

33. Kendall BE. Disorders of lysosomes, peroxisomes, and mitochondria. Am J Neuroradiol 1992;13:621–653.

34. Wray SH, Provenzale JM, Johns DR, Thulborn KR. MR of the brain in mitochondrial myopathy. Am J Neuroradiol 1995;16:1167–1173.

35. Giuffrida S, Saponara R, Restivo DA, et al. Supratentorial atrophy in spinocerebellar ataxia type 2: MRI study of 20 patients. J Neurol 1999;246:383–388.

36. Wüllner U, Klockgether T, Petersen D, Naegele T, Dichgans J. Magnetic resonance imaging in hereditary and idiopathic ataxia. Neurology 1993;43:318–325.

37. Murata Y, Yamaguchi S, Kawakami H, et al. Characteristic magnetic resonance imaging findings in Machado–Joseph disease. Arch Neurol 1998;55:33–37.

38. Koide R, Onodera O, Ikeuchi T, et al. Atrophy of the cerebellum and brainstem in dentatorubral pallidoluysian atrophy: influence of CAG repeat size on MRI findings. Neurology 1997;49:1605–1612.

39. Watanabe M, Sugai Y, Concannon P, et al. Familial spinocerebellar ataxia with cerebellar atrophy, peripheral neuropathy, and elevated level of serum creatine kinase, γ-globulin, and α-fetoprotein. Ann Neurol 1998;44:265–269.

40. Pandolfo M. A reappraisal of the clinical features of Friedreich ataxia: what indications for a molecular test? Neurology 1999; 52(suppl 2):A260. Abstract.

POLG mutations in neurodegenerative disorders with ataxia but no muscle involvement

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Abstract—Objective: To identify *POLG* mutations in patients with sensory ataxia and CNS features. Methods: The authors characterized clinical, laboratory, and molecular genetic features in eight patients from five European families. The authors conducted sequencing of coding exons of *POLG, C10orf2* (Twinkle), and *ANT1* and analyzed muscle mitochondrial DNA (mtDNA), including Southern blot analysis and long-range PCR. Results: Ataxia occurred in combination with various CNS features, including myoclonus, epilepsy, cognitive decline, nystagmus, dysarthria, thalamic and cerebellar white matter lesions on MRI, and neuronal loss in discrete gray nuclei on autopsy. Gastrointestinal dysmotility, weight loss, cardiomyopathy, and valproate-induced hepatotoxicity occurred less frequently. Two patients died without preceding signs of progressive external ophthalmoplegia. In muscle, typical findings of mitochondrial disease, such as ragged red fibers and Southern blot mtDNA abnormalities, were absent. *POLG* mutations were present in eight patients, including two isolated cases, and one Finnish and two unrelated Belgian families contained in total six patients. All *POLG* mutations were recessive, occurring in a homozygous state in seven patients and in a compound heterozygous state in one patient. The novel W748S mutation was identified in five patients from three unrelated families. Conclusions: The clinical spectrum of recessive *POLG* mutations is expanded by sensory ataxic neuropathy, combined with variable features of involvement of CNS and other organs. Progressive external ophthalmoplegia, myopathy, ragged red fibers, and Southern blot abnormalities of muscle mitochondrial DNA also are not mandatory features associated with *POLG* mutations.

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Mitochondrial DNA (mtDNA) maintenance disorders are characterized by somatic depletion[1] or multiple deletions of mtDNA in different, mainly postmitotic tissues.[2] The majority of disorders characterized by multiple mtDNA deletions present with progressive external ophthalmoplegia (PEO),[2] but several other phenotypes have been reported.[3-9] In PEO, four nuclear genes associated with multiple mtDNA deletions have been identified. In autosomal dominant PEO (adPEO; MIM 157640), mutations were demonstrated in the genes *ANT1*,[10] *C10orf2*,[11] and *POLG*.[12] In autosomal recessive PEO (arPEO), mutations were identified in *ECGF1*[13] and *POLG*.[12] *ANT1* (MIM 103220) encodes adenine nucleotide translocator 1. *C10orf2* (MIM 606075) encodes the mitochondrial helicase Twinkle. *POLG* (MIM 174763) encodes DNA polymerase gamma subunit 1 (synonyms, mitochondrial DNA polymerase catalytic subunit or PolG-α [Swiss-Prot ID, P54098; EC 2.7.7.7]). *ECGF1* (MIM 131222) encodes thymidine phosphorylase, and mutations are associated with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE; MIM 603041), an arPEO variant characterized by gastrointestinal dysmotility[14] and leukodystrophy.[15]

POLG mutations are the most frequent cause of multiple mtDNA deletions in PEO, both in families with dominant or recessive inheritance[16] and in isolated patients. Isolated patients are most frequently compound heterozygous for two distinct recessive *POLG* mutations.[17,18]
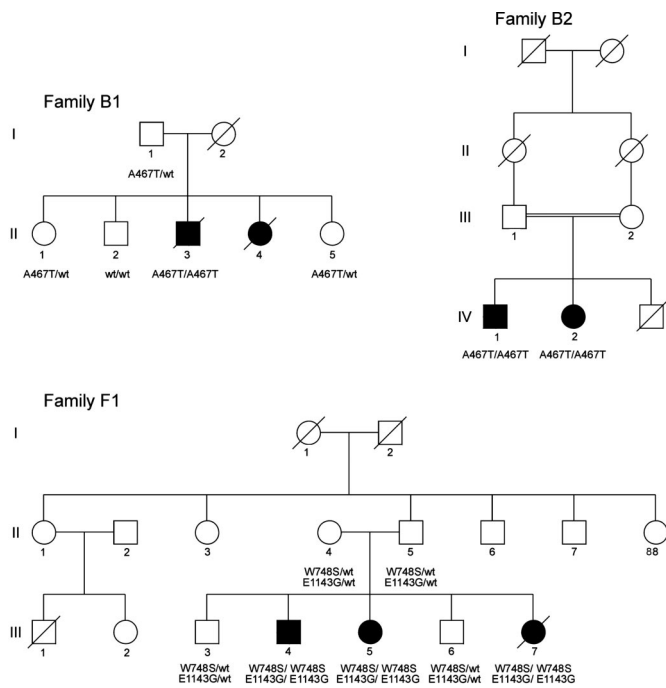
Additional material related to this article can be found on the Neurology Web site. Go to www.neurology.org and scroll down the Table of Contents for the October 12 issue to find the title link for this article.

Figure 1. Segregation analysis of POLG *mutations in Belgian Families B1 and B2 and in Finnish Family F1. Filled symbols represent affected individuals. All available family members were clinically examined, including the deceased affected individual, F1.III.7.*

We previously reported that sensory ataxia or other features of sensory neuropathy can precede PEO associated with recessive *POLG* mutations.[19] Here we report *POLG* mutations in patients initially seeking treatment for sensory ataxia or CNS abnormalities and who subsequently do not develop PEO or only discrete and asymptomatic ophthalmoparesis.

Methods. *Clinical investigations.* In seven of eight genotyped patients, clinical examination was recently performed, including testing of strength, tone, muscle bulk, eyelid elevation, and extraocular movements. If possible, video-oculography was performed. Unaffected family members, included in the segregation analyses (figure 1), were also examined. In seven of eight patients, electrophysiologic examination was performed, and the eighth patient had a nerve and muscle biopsy. Muscle biopsy was also performed in four of five probands. Laboratory investigations are presented in the patient reports and listed in table E-2 (available on the *Neurology* Web site at www.neurology.org). Clinical data from previous examinations and findings of deceased Patient B1.II.4 were extracted from hospital records or were collected from a previous study (Family F1).[20]

DNA analysis. Genomic DNA was isolated from total blood using standard DNA extraction procedures. Mutation analyses were performed using direct sequencing of PCR amplicons of coding exons of *POLG* on an ABI 3100 or ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA).[12] In index patients carrying *POLG* mutations, mutations in *ANT1* and *C10orf2* were subsequently excluded.[21] One hundred sixty-eight Belgian and 70 Finnish control individuals were examined for the presence of the c.2243G→C transition (W748S) and the c.3428A→G transition (E1143G). We used pyrosequencing[22] to examine Belgian control subjects and direct PCR sequencing for Finnish control subjects, including 46 individuals from the genetic isolate of North Karelia in Finland, from which family F1 originated.

Mitochondrial DNA analysis. DNA extraction from muscle biopsy specimens and Southern blot analysis of mtDNA were performed as described previously[23] with linearization of mtDNA by

*Bam*HI or *Pvu*II restriction enzymes and with a fragment of mtDNA (nucleotides 1 to 740) as a hybridization probe. The radioactive signals were detected with the Typhoon 9400 Imager (Amersham Biosciences, Piscataway, NJ). Quantification of mtDNA amounts compared with a nuclear 18S ribosomal RNA gene was done with ImageQuant 5.1 software (Amersham Biosciences) to detect possible mtDNA depletion. The same Southern blots were hybridized with a nuclear 18S ribosomal RNA gene probe. The amount of mtDNA signal on each lane was compared with that of the nuclear gene in two different experiments.

Long-range PCR analysis was performed using a pair of oligonucleotides that primed on 8.3-kb separated regions of mtDNA, namely, at nucleotide positions 8,238 to 8,262 (forward) and 16,496 to 16,465 (reverse). PCR amplification was done using Expand Long PCR long template PCR system (Roche, Basel, Switzerland) according to the manufacturer's instructions, but with 10 ng of total DNA template, with buffer system 1, and either 8 minutes or 3 minutes of extension time. The short extension time selectively amplifies smaller-sized deletion-containing mtDNA fragments, whereas long time also allows amplification of the wild-type template.

As controls, we used diagnostic muscle DNA samples from healthy siblings of patients with *POLG* mutations or *POLG* patients' leukocyte DNA. As controls for PCR reactions, we also used a muscle sample of a patient with a dominant *POLG* mutation (Y955C), known to harbor multiple mtDNA deletions, and a fibroblast DNA sample from a patient with Kearns–Sayre syndrome carrying a single mtDNA deletion in muscle.

Brain autopsy studies. Fixation was performed with formalin according to standard procedures. On frozen sections, cresyl violet staining was used to assess neuronal loss, and the Spielmeyer method was used to observe myelin pallor. On paraffin-embedded sections, H-E staining and cresyl violet staining were performed to study neuronal populations. The Bodian method was used to evaluate axons, and the Klüver–Barrera method was used to observe myelin. Immunohistochemistry was performed using markers of hyperphosphorylated tau (AT8), Aβ amyloid (4G8), glial fibrillary acidic protein (GFAP), ubiquitin, and the macrophages/microglia marker CD68.

Results. The clinical findings are summarized in table E-1 (available on the *Neurology* Web site at www.neurology.org). Laboratory features are summarized in table E-2. Patient reports are presented in detail below.

Patient reports. Patients B1.II.3 and B1.II.4. At age 18 years, B1.II.3 was evaluated for status epilepticus lasting 8 days, followed by a "Todd paralysis" of the left arm and face. Five years later, he had an acute psychiatric illness, hyperventilation, gastrointestinal symptoms, gait unsteadiness, and disturbed limb coordination. Between 32 and 35 years of age, he lost 13 kg of weight. On examination, he had sensory gait ataxia, limb ataxia, areflexia, generalized dystrophy, and loss of vibration and static joint position sense at the distal lower limbs, severe dysarthria, and a left-sided Babinski sign. Romberg test was positive. On funduscopy, optic disks were pale without pigmentary retinopathy. At age 38 years, he had intestinal pseudo-obstruction, anorexia, and further weight loss. A few weeks later, he developed stupor (Glasgow coma scale, 5/15), hyperventilation, myoclonic jerks, and seizures necessitating intensive care and artificial ventilation. Because of lactic acidosis, sodium valproate was changed to phenytoin and levetiracetam, but seizures and myoclonus persisted. Postpyloric enteric feeding failed because of severe gastroparesis and intestinal pseudo-obstruction, and weight further decreased to 35 kg. Numerous medical complications preceded death at age 39 years. Blepharoptosis and ophthalmoparesis were never noted.

From onset, EEGs repeatedly showed slowed background activity. Nerve conduction studies and EMG, first performed at age 34 years, were consistent with a predom-
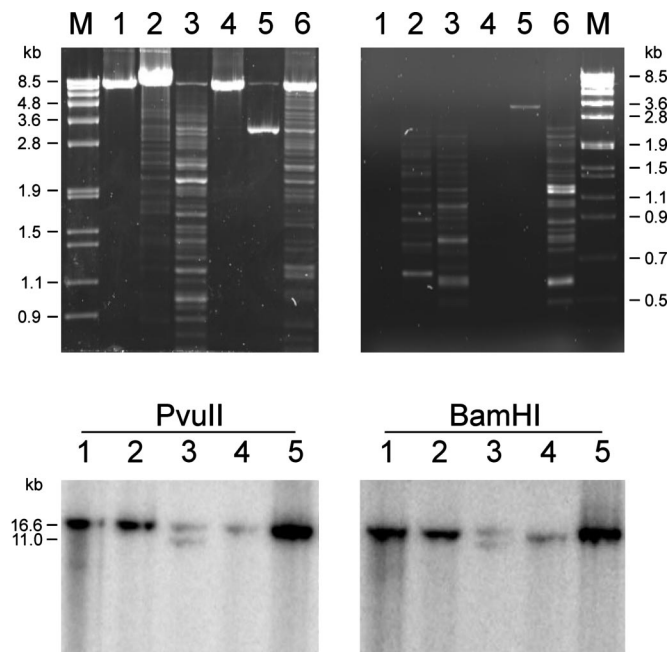
Figure 2. Mitochondrial DNA analysis. Top, PCR analysis. Mitochondrial DNA (mtDNA) fragment between the mtDNA nucleotides 8.2 kb and 16.5 kb was amplified from total muscle DNA. Left, 8-minute PCR extension time, allowing amplification of full-length fragments. Single product of 8.3 kb, from wild-type mtDNA is present in lane 1, leukocyte DNA of Patient F2.II.1, and in lane 4, loaded with DNA from 58-year-old control muscle. Additional products corresponding to multiple deleted mtDNA molecules can be seen in lanes 2 and 3 of two ataxia patients (lane 2, F1.III.7; lane 3, B1.II.3), and in lane 6, muscle DNA of a multiple-deletion control progressive external ophthalmoplegia (PEO) patient with a dominant Y955C mutation in POLG. Lane 5, as a second disease control, shows a single mutant product from an isolated patient harboring a single 5-kb mtDNA deletion. M denotes marker VII (Roche Applied Sciences, Penzberg, Germany); kb denotes the kilobase sizes of marker fragments. Right, Same PCR as on the left, but with 3-minute extension time, favoring the amplification of short mutant mtDNA. Multiple products corresponding to mtDNA deletions are present in samples of F1.III.7 and B1.II.3 (lanes 2 and 3), as well as the multiple mtDNA deletion control Y955C POLG mutant (lane 6). Only the mutant molecule containing a single deletion can be seen in lane 5. No amplification product was obtained from leukocytes of ataxia Patient F2.II.1 or from the healthy control muscle DNA. Bottom, Southern hybridization analysis of mtDNA. Samples: lane 1, muscle DNA of Patient B1.II.3; lane 2, muscle DNA of Patient F1.III.7; lane 3, fibroblast DNA of a disease control individual with a single mtDNA deletion; lane 4, control lymphoblast DNA sample; lane 5, control myoblast DNA sample; kb, denoting kilobases, is the molecular size marker. As indicated, total DNA was digested with either PvuII or BamHI restriction enzymes, linearizing mtDNA. The blot was hybridized with a short cloned mtDNA fragment (mtDNA nucleotides 1 to 740). In lane 1, a faint "smear" signal is present below the wild-type mtDNA signal. In lanes 2, 4, and 5, just wild-type mtDNA signal is present. Lane 3 shows two signals: one from wild-type mtDNA and one from mtDNA molecule containing a 5-kb single deletion.

inant sensory axonal peripheral neuropathy. On several occasions, CSF analysis revealed elevated protein at 129 to 183 mg/dL but normal lactate. Total and unconjugated serum bilirubin levels were slightly elevated, but liver enzymes were always normal. Serum lactate was initially normal but was elevated at 8.1 mmol/L (normal values < 2.0) with sodium valproate treatment. Creatine kinase (CK) was mildly elevated at 342 U/L (normal values < 170). Brain MRI remained normal, even at age 39 years, and SPECT was normal at age 34 years. Echocardiography demonstrated dilated cardiomyopathy. There was no hearing loss on audiometry. At age 34 years, a muscle biopsy was performed, and succinate dehydrogenase (SDH) stain showed no abnormalities. However, electron microscopy demonstrated some fibers with subsarcolemmal accumulation of mitochondria containing paracrystalline inclusions. Diagnostic mtDNA analysis revealed no pathogenic mtDNA point mutations.[24] There was a faint smear but no visible deletions on Southern analysis of muscle mtDNA (figure 2).

The sister (Patient B1.II.4) was examined at age 17 years for an acute encephalopathy, myoclonus, partial epileptic seizures, and left hemianopsia, followed by cortical blindness, stupor, Cheyne–Stokes respiration pattern, and sudden death. DNA of this patient was not available for molecular genetic studies. Three siblings appeared unaffected on clinical examination at ages 43, 41, and 33 years, as well as the father (age 67 years). The mother, who died before this study was performed, was reportedly unaffected. The pedigree is presented in figure 1.

Patients B2.IV.1 and B2.IV.2. The patients are a 49-year-old Belgian man and his 47-year-old sister. Their unaffected younger brother died at age 38 years from leishmaniasis. Paternal and maternal grandmothers were sisters (see figure 1). Clinical features were almost identical in both siblings (see tables E-1 and E-2 on the *Neurology* Web site), but the sister (B2.IV.2) was more severely affected. From adolescence, both patients experienced gradually increasing gait unsteadiness, which was worse in the dark. In her fourth decade, the sister had frequent falls, and she became wheelchair dependent in the fifth decade. The brother had mild cognitive decline in the fifth decade. He underwent cataract surgery. Both siblings had an ataxic gait. Heel-to-shin testing was more ataxic than finger-to-nose testing, which markedly deteriorated with eye closure. Deep tendon reflexes were absent. Vibration and position sense were absent at the lower limbs early on, whereas light touch sensation was decreased at a later stage. Both had pes cavus. Dysarthria was only present in the sister. There was no blepharoptosis. Mild and asymptomatic upward gaze paresis appeared at age 48 years in Patient B2.IV.1, whereas in Patient B.IV.2, from age 40 years, smooth pursuit ocular movements became saccadic, and there was a horizontal and vertical gaze-evoked nystagmus. Muscle strength was always preserved. Electrophysiologic examinations in both patients showed signs of axonal generalized peripheral neuropathy, predominantly involving sensory nerves. Needle EMG was normal (B2.IV.2) or showed minor neurogenic abnormalities in distal lower-extremity muscles (B2.IV.1). Visual evoked potential (VEP) and electroretinography (ERG) were normal (B2.IV.2). Oculography confirmed gaze-evoked nystagmus and saccadic pursuit movements and was suggestive of

brainstem dysfunction (B2.IV.2; age 40 years). Brain MRI showed symmetric cerebellar white matter lesions similar to those observed in Family F1[20] (B2.IV.2; age 49 years).

Patient UK1.II.1. This patient of British origin noticed minor tremor of the hands from age 12 years. In his late 20s, he experienced tingling in both hands, followed by tingling in the legs. In the early fourth decade, he became unsteady at walking, especially in the dark. From his early 40s, his speech became slurred. When relaxed, he noticed abnormal movements of the toes. His son died suddenly at age 30 years, without obvious cause.

On examination at age 52 years, he had an ataxic gait, and Romberg test was positive. There was dysarthria and bilateral ophthalmoparesis. In the limbs, muscle tone and strength were normal. There was generalized areflexia. Pin-prick sensation was reduced in a stocking distribution; proprioception was reduced bilaterally to the ankle; and vibration sensation was reduced bilaterally to the iliac crests.

Brain MRI showed minor atrophy. CSF protein was 75 mg/dL. Sural nerve biopsy showed marked decrease of myelinated fibers of all diameters but no active demyelination. Pathologic findings were consistent with axonal neuropathy. Muscle biopsy, performed at age 52 years, showed no abnormalities on histochemical staining for oxidative enzymes.

Patients F1.III.4, 5, and 7. The pedigree is presented in figure 1. The detailed clinical and laboratory features of these patients of Finnish (Karelian) origin have been reported previously.[20] Briefly, they had a combination of adult-onset progressive ataxia with severe gait ataxia, dysarthria, mild cognitive impairment, and, in the case of Patients F1.III.4 and F1.III.7, epilepsy. Patient F1.III.7 unexpectedly died at age 36 years. She had no ophthalmoparesis, but her two affected siblings developed mild upward gaze paresis in the fifth decade. Blepharoptosis was absent on the original study in all three patients.[20] Electrophysiology was consistent with a predominantly sensory axonal neuropathy. Brain MRI showed symmetric thalamic lesions and white matter changes in the cerebellum. Southern analysis on muscle DNA had not revealed large mtDNA deletions (see figure 2), and pathogenic mtDNA point mutations were not observed.[20] After we detected *POLG* mutations in this pedigree, the authors re-examined the two surviving patients, F1.III.4 and F1.III.5, with particular attention to external eye muscle function and other muscular features. In both patients, mild blepharoptosis and ophthalmoparesis had now appeared between ages 46 and 48 years. Patient F1.III.4 also had mild diffuse muscle weakness and atrophy, particularly at distal lower limbs. In both patients, ataxia of gait and stance had progressed, necessitating help or a walker. At age 47 years, needle EMG and nerve conduction velocity studies were consistent with moderate to severe axonal sensorimotor neuropathy, whereas CK was normal (Patient F1.III.4).

Patient F2.II.1. This 33-year-old Finnish man is one of three children from unrelated parents originating from North Karelia and Central Lapland. He was not closely related to Family F1. He reported balance problems in two maternal second cousins. He had plurimetabolic syndrome. At age 30 years, he noted disturbed balance, and ataxia and severe axonal neuropathy were diagnosed. On examination at age 32 years, he had slowed ocular pursuit movements but with full range of motion. There were

spontaneous slow lateral eye movements but no nystagmus. He had no blepharoptosis. He had severe gait ataxia, imbalance, and trunk ataxia, as well as clumsiness of the hands. Achilles tendon reflexes were absent. Vibration sensation was decreased at the lower limbs, and pain and touch sensations were decreased in a stocking-like distribution to knee level at the lower extremities. His speech was dysarthric. He was obese. Brain MRI showed bilateral symmetric intense signals in the cerebellar white matter on T2-weighted images. Electrophysiology revealed severe sensory and moderate chronic motor axonopathy at the lower limbs and moderate sensory neuropathy at the upper limbs. Muscle biopsy showed mild neurogenic atrophy, no ragged red fibers, and 1:100s cytochrome c oxidase-negative fibers. Analysis of muscle mtDNA revealed no pathogenic mutations.[24] Biochemical analysis of respiratory chain function was normal.
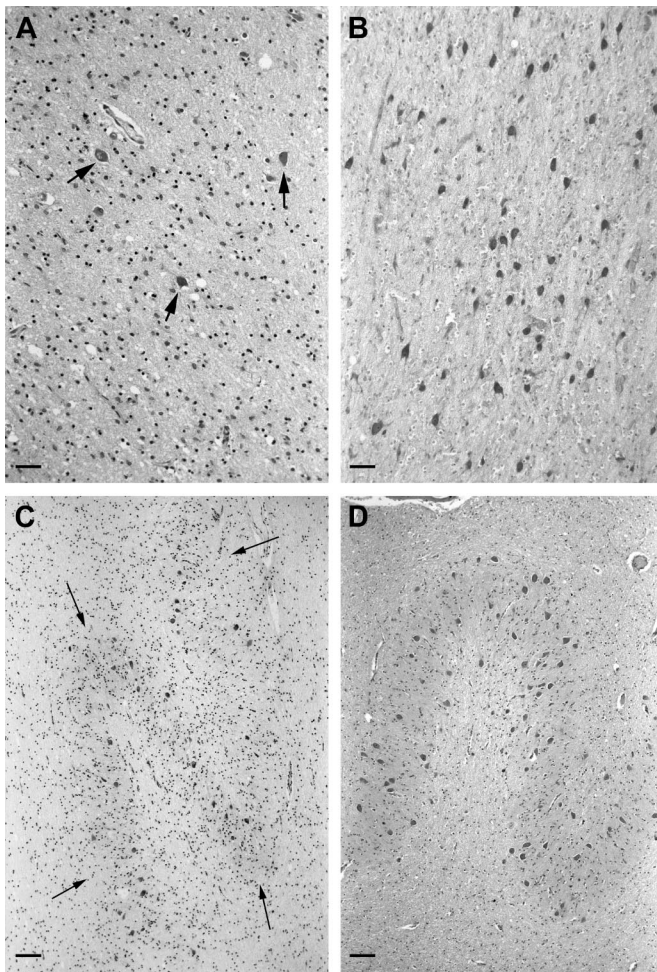
Morphologic studies and autopsy findings. In muscle biopsy, no findings suggestive of mitochondrial disease were detected on light microscopy, including histochemical SDH and cytochrome c oxidase stains (Patients B.II.3, UK1.II.1, F1.III.7, and F.II.2.1).

On autopsy of Patient B1.II.3, cerebellum and cerebral hemispheres were preserved. All gray nuclei appeared paler than normal, particularly in the substantia nigra and locus ceruleus. Unfortunately, because of incomplete autopsy, the peripheral nerves, dorsal root ganglia, spinal cord, medulla oblongata, and lower part of the pons were unavailable for this study. Frozen material was not available for histochemical studies and DNA extraction.[25]

On light microscopy, optic nerves and cerebral and cerebellar cortex were normal. Caudate nucleus, putamen, and pallidum were normal. In the thalamus, there was mild neuronal loss in some nuclei belonging to the lateral formation and in the magnocellular part of the centromedian nucleus, whereas the large association nuclei (pulvinar and dorsomedial nucleus) were normal. Neuronal loss was present, most prominent in the subthalamic nucleus and in the cerebellar dentate nucleus (figure 3). The locus ceruleus was also affected, whereas the compact zone of the substantia nigra was barely affected. Gliosis, strongly reactive to GFAP antibodies, was present in the white matter, particularly at the level of the subcortical U fibers and in subependymal and subpial regions. Myelin was normally stained. Moderate amounts of CD68^{+} cells were present in the perivascular spaces in the white matter.

Genetic findings. POLG analysis. In all eight patients, we sequenced *POLG* because of the presence of a sensory ataxic neuropathy. Mutations in *POLG* are presented in the table.

Four Finnish ataxia patients (F1.III.4, 5, and 7 and F2.II.1) carried a single base change in *POLG*, namely, c.2243G→C, predicting a W748S substitution. This mutation occurred in the homozygous state in all patients and in the heterozygous state in unaffected siblings and parents of Family F1 (see figure 1). The British ataxia patient was compound heterozygous for W748S and for the previously reported A467T mutation.[12] Interestingly, in addition to the W748S mutation, all four Finnish patients and the British patient also carried the previously reported c.3428A→G mutation, predicting an E1143G substitution (http://www.ncbi.nlm.nih.gov/SNP/). E1143G occurred in the homozygous state in the Finnish patients and in the

rived from normal mtDNA in control samples, in muscle of Patient F1.III.7, and in leukocytes of Patient F2.II.1. Long-range PCR, which selectively amplified short deleted molecules, revealed clearly different-sized products in muscle biopsy samples of Patients B1.II.3 and F1.III.7, whereas control subjects only showed the 16.5-kb mtDNA fragment or no PCR product (see figure 2). mtDNA depletion was not present in any of the patients.

Discussion. We report a neurodegenerative phenotype of recessive *POLG* mutations. We present eight patients, from five unrelated families, with adult- or juvenile-onset sensory ataxic neuropathy in combination with various CNS abnormalities. Notably, there was no apparent skeletal muscle involvement, and on muscle biopsy samples, there were no signs of mitochondrial disease on morphologic, biochemical, or routine mtDNA analyses.

The presenting and major feature in all patients was ataxia caused by axonal sensory neuropathy. Ataxia occurred in combination with CNS abnormalities, including myoclonus, seizures, transient hemiparesis, cognitive decline, dysarthria, and nystagmus. In some patients, MRI showed lesions in the thalamus and/or cerebellar white matter. Brain autopsy findings, unfortunately incomplete for Patient B1.II.3, were not typical of primary mtDNA disorders but showed prominent neuronal loss in gray nuclei, including the subthalamic and cerebellar dentate nucleus.[26] For Patient F1.III.7, a more complete autopsy had also shown atrophy in dorsal root ganglia, posterior columns, and, to a lesser extent, in posterior spinocerebellar and corticospinal tracts. There was neuronal loss in the dorsal nucleus (Clarke column), and, contrary to Patient B1.II.3, in the pulvinar and the dorsomedial thalamic nuclei. There was also degeneration of inferior olives.[20] The involvement pattern differs from other spinocerebellar ataxias but resembles findings in system atrophies.[27]

Reports on patients with *POLG* mutations have mentioned associated CNS features and neuropathy in some instances.[16,19,28-30] However, PEO was a con-

Figure 3. Neuropathology. (A) Subthalamic nucleus of Patient B1.II.3: severe neuronal losses and astrocytic gliosis. A few neurons are remaining, some of which are arrowed. (B) Subthalamic nucleus of control individual. (C) Cerebellar nucleus dentatus of Patient B1.II.3: neuronal losses and astrocytic gliosis. The thin arrows point to the curves of the dentate nucleus, and similar curves can easily be seen in the control nucleus (D). (D) Cerebellar nucleus dentatus of control individual (formalin fixation, paraffin slides, cresyl violet staining; scale bar = 100 μm).

heterozygous state in the British patient. W748S was absent in 168 Belgian and 70 Finnish control subjects, of which 46 were of North Karelian origin. Eleven of 168 Belgian and 3 of 70 Finnish control subjects were heterozygous for E1143G, and control subjects homozygous for E1143G were not encountered. This implies allele G frequencies of 3.3% (11/336) in the Belgian population and of 2.1% (3/140) in the Finnish population. One North Karelian control subject carried E1143G, corresponding with a 1.0% (1/92) allele G frequency in the genetic isolate.

Three Belgian patients were homozygous for the previously reported c.1399G→A mutation, predicting an A467T substitution.[12] The isolated British patient UK1.II.1 was heterozygous for A467T.

Mutations in *ANT1* and *C10orf2* were absent in four index patients (not tested on isolated Patient F2.II.1).

Mitochondrial DNA analysis. Southern blot hybridization of total muscle DNA showed the 16.5-kb signal de-

sistent feature, except for one teenager with myoclonus.[29] In our present study, two of eight patients died at ages 36 and 39 years without extraocular muscle involvement. In the other six patients, PEO was absent at clinical presentation, although minor signs of incipient PEO appeared in a late disease stage in five. Hence, PEO represents merely one variant of the clinical phenotypic expression of *POLG* mutations; therefore, *POLG* could be designated as an "ataxia gene" and a "PEO gene."

One isolated patient had the clinical triad of sensory ataxic neuropathy, dysarthria, and ophthalmoplegia (SANDO), previously claimed as a unique mitochondrial disorder.[31] Further studies are necessary to test the hypothesis that SANDO could be a specific phenotype of mutations in *POLG*.[19]

The large differences in phenotypic expression of recessive *POLG* mutations are puzzling. Although PEO and sensory ataxia are common, no single clinical feature is shared by all patients.[32] Differences between our own series and those reported by others[16-18,33] could be attributed to clinical selection bias. Several speculations could be made about molecular causes of the observed clinical heterogeneity. Data on familial patients suggest that clinical variability is larger between families than within families (e.g., Families B1 and B2 in this article and the family in Van Goethem et al.,[29] which all contain individuals homozygous for A467T). In our view, the contribution of the genetic background would explain best the observed clinical similarities in siblings.

In view of this considerable heterogeneity, what are the clinical clues to mutated *POLG*? Clinical features are confined to energy-demanding postmitotic tissues, with the exception of the liver. Hepatocytes have a low mitotic index, and they may become affected with sodium valproate treatment (e.g., Patient B1.II.3 and Van Goethem et al.[29]). Because substantial tissue accumulation of mtDNA deletions putatively causes pathology,[34] the absence of muscle mtDNA deletions on diagnostic Southern analysis correlates with the absence of clinical and morphologic abnormalities in the biopsied muscle. However, long-range PCR did show low levels of multiple mtDNA deletions in muscle, suggesting that the proportion of deleted mtDNA in muscle can be estimated as <5%.

In all patients (two isolated patients and six patients belonging to three unrelated families), we demonstrated recessive missense mutations in *POLG*. Seven patients (six familial, one isolated) carried homozygous mutations. One isolated patient was compound heterozygous. A467T is a known mutation,[12] whereas W748S is a novel pathogenic mutation, and E1143G is most likely a low frequency polymorphism. W748S and E1143 always occurred together (five patients from three unrelated families). In four Finnish patients, the novel W748S mutation occurred in the homozygous state, and in a British patient, it occurred in the compound heterozygous state with A467T. In Family F1, segrega-

tion analysis indicated recessive inheritance. Interestingly, all five W748S patients carried a second missense mutation, namely, E1143G. E1143G was previously designated as a polymorphism because of its high frequency (2.9%) in a North American control population (http://www.ncbi.nlm.nih.gov/SNP/). We measured a comparable frequency in the Belgian and Finnish control population. Control individuals homozygous for E1143G were not encountered. In Families F1 and F2, haplotype analysis using five informative polymorphic DNA markers surrounding *POLG*[29] revealed a common haplotype suggestive of a common ancestral origin of the W748S/E1143G alleles (data not shown). W748S and E1143G alter evolutionary conserved amino acid residues. Secondary structure prediction (program PHDSec, EMBL, Heidelberg, Germany) suggests that W748 is one residue out of a highly conserved block of six amino acids forming a β-sheet in the spacer region of the enzyme. This so-called "palm" region is presumably involved in primer-template interaction of the DNA polymerase.[35,36] The contribution of E1143G to the phenotype remains unclear. Because E1143G accompanied W748S in three of three unrelated families, we cannot conclude that W748S alone is sufficient to cause disease in homozygous or compound heterozygous patients. This situation parallels the finding of *POLG* mutations T251I and P587L in cis in arPEO patients of different ethnic origins.[18,28] Such data may suggest the concept of a recessive haplotype.[37] Therefore, segregation analysis of novel low-frequency mutations remains crucial for the determination of their pathogenic nature because in vivo functional studies on DNA polymerase gamma are still unavailable, and phenotypic expression is absent in patients' mitotic cells, including cultured fibroblasts, lymphocytes, or myoblasts.

The current study presents three novel patients homozygous for A467T, which is common to Belgian arPEO patients[32] and originates from a common ancestral chromosome in the Belgian population.[29] This mutation is also retrieved in the compound heterozygous state in a patient of British origin, and previously it was reported in an Italian PEO patient.[17] We had predicted a homozygous appearance of A467T in 36×10^{-6} Belgian individuals.[12] The present findings support our suggestion that a subgroup of patients homozygous for A467T could have clinical features different from PEO.[29]

References

1. Moraes CT, Shanske S, Tritschler HJ, et al. mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. Am J Hum Genet 1991;48:492–501.
2. Zeviani M, Servidei S, Gellera C, Bertini E, DiMauro S, DiDonato S. An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. Nature 1989;339:309–311.
3. Yuzaki M, Ohkoshi N, Kanazawa I, Kagawa Y, Ohta S. Multiple deletions in mitochondrial DNA at direct repeats of non-D-loop regions in cases of familial mitochondrial myopathy. Biochem Biophys Res Commun 1989;164:1352–1357.
4. Cormier V, Rotig A, Tardieu M, Colonna M, Saudubray JM, Munnich A. Autosomal dominant deletions of the mitochondrial genome in a case of progressive encephalomyopathy. Am J Hum Genet 1991;48:643–648.
5. Ohno K, Tanaka M, Sahashi K, et al. Mitochondrial DNA deletions in inherited recurrent myoglobinuria. Ann Neurol 1991;29:364–369.
6. Suomalainen A, Paetau A, Leinonen H, Majander A, Peltonen L, Somer H. Inherited idiopathic dilated cardiomyopathy with multiple deletions of mitochondrial DNA. Lancet 1992;340:1319–1320.
7. Bohlega S, Tanji K, Santorelli FM, Hirano M, al Jishi A, DiMauro S. Multiple mitochondrial DNA deletions associated with autosomal recessive ophthalmoplegia and severe cardiomyopathy. Neurology 1996;46:1329–1334.
8. Blumenthal DT, Shanske S, Schochet SS, et al. Myoclonus epilepsy with ragged red fibers and multiple mtDNA deletions. Neurology 1998;50:524–525.
9. Paul R, Desnuelle C, Pouget J, et al. Importance of searching for associated mitochondrial DNA alterations in patients with multiple deletions. Eur J Hum Genet 2000;8:331–338.
10. Kaukonen J, Juselius JK, Tiranti V, et al. Role of adenine nucleotide translocator 1 in mtDNA maintenance. Science 2000;289:782–785.
11. Spelbrink JN, Li FY, Tiranti V, et al. Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. Nat Genet 2001;28:223–231.
12. Van Goethem G, Dermaut B, Lofgren A, Martin JJ, Van Broeckhoven C. Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. Nat Genet 2001;28:211–212.
13. Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. Science 1999;283:689–692.
14. Hirano M, Silvestri G, Blake DM, et al. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical, biochemical, and genetic features of an autosomal recessive mitochondrial disorder. Neurology 1994;44:721–727.
15. Nishino I, Spinazzola A, Papadimitriou A, et al. Mitochondrial neurogastrointestinal encephalomyopathy: an autosomal recessive disorder due to thymidine phosphorylase mutations. Ann Neurol 2000;47:792–800.
16. Lamantea E, Tiranti V, Bordoni A, et al. Mutations of mitochondrial DNA polymerase gammaA are a frequent cause of autosomal dominant or recessive progressive external ophthalmoplegia. Ann Neurol 2002;52:211–219.
17. Agostino A, Valletta L, Chinnery PF, et al. Mutations of ANT1, Twinkle, and POLG1 in sporadic progressive external ophthalmoplegia (PEO). Neurology 2003;60:1354–1356.
18. Di Fonzo A, Bordoni A, Crimi M, et al. POLG mutations in sporadic mitochondrial disorders with multiple mtDNA deletions. Hum Mutat 2003;22:498–499.
19. Van Goethem G, Martin JJ, Dermaut B, et al. Recessive POLG mutations presenting with sensory and ataxic neuropathy in compound heterozygote patients with progressive external ophthalmoplegia. Neuromuscul Disord 2003;13:133–142.
20. Rantamaki M, Krahe R, Paetau A, Cormand B, Mononen I, Udd B. Adult-onset autosomal recessive ataxia with thalamic lesions in a Finnish family. Neurology 2001;57:1043–1049.
21. Van Goethem G, Lofgren A, Dermaut B, Ceuterick C, Martin JJ, Van Broeckhoven C. Digenic progressive external ophthalmoplegia in a sporadic patient: recessive mutations in POLG and C10orf2/Twinkle. Hum Mutat 2003;22:175–176.
22. Alderborn A, Kristofferson A, Hammerling U. Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing. Genome Res 2000;10:1249–1258.
23. Suomalainen A, Majander A, Haltia M, et al. Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external ophthalmoplegia. J Clin Invest 1992;90:61–66.
24. Kogelnik AM, Lott MT, Brown MD, Navathe SB, Wallace DC. MITOMAP: a human mitochondrial genome database. Nucleic Acids Res 1996;24:177–179.
25. Cottrell DA, Ince PG, Blakely EL, et al. Neuropathological and histochemical changes in a multiple mitochondrial DNA deletion disorder. J Neuropathol Exp Neurol 2000;59:621–627.
26. Sparaco M, Bonilla E, DiMauro S, Powers JM. Neuropathology of mitochondrial encephalomyopathies due to mitochondrial DNA defects. J Neuropathol Exp Neurol 1993;52:1–10.
27. Martin JJ. Thalamic degenerations. In: de Jong JMBV, ed. Handbook of Clinical Neurology, vol. 16/60, Hereditary Neuropathies and Spinocerebellar Atrophies. Amsterdam: Elsevier Science Publishers, 1991:701–715.
28. Van Goethem G, Schwartz M, Lofgren A, Dermaut B, Van Broeckhoven C, Vissing J. Novel POLG mutations in progressive external ophthalmoplegia mimicking mitochondrial neurogastrointestinal encephalomyopathy. Eur J Hum Genet 2003;11:547–549.
29. Van Goethem G, Mercelis R, Lofgren A, et al. Patient homozygous for a recessive POLG mutation presents with features of MERRF. Neurology 2003;61:1811–1813.
30. Van Goethem G, Martin JJ, Lofgren A, et al. Unusual presentation and clinical variability in Belgian pedigrees with progressive external ophthalmoplegia and multiple deletions of mitochondrial DNA. Eur J Neurol 1997;4:476–484.
31. Fadic R, Russell JA, Vedanarayanan VV, Lehar M, Kuncl RW, Johns DR. Sensory ataxic neuropathy as the presenting feature of a novel mitochondrial disease. Neurology 1997;49:239–245.
32. Van Goethem G, Martin JJ, Van Broeckhoven C. Progressive external ophthalmoplegia characterized by multiple deletions of mitochondrial DNA: unraveling the pathogenesis of human mitochondrial DNA instability and the initiation of a genetic classification. Neuromolecular Med 2003;3:129–146.
33. Filosto M, Mancuso M, Nishigaki Y, et al. Clinical and genetic heterogeneity in progressive external ophthalmoplegia due to mutations in polymerase gamma. Arch Neurol 2003;60:1279–1284.
34. Moraes CT, Ricci E, Petruzzella V, et al. Molecular analysis of the muscle pathology associated with mitochondrial DNA deletions. Nat Genet 1992;1:359–367.
35. Ye F, Carrodeguas JA, Bogenhagen DF. The gamma subfamily of DNA polymerases: cloning of a developmentally regulated cDNA encoding Xenopus laevis mitochondrial DNA polymerase gamma. Nucleic Acids Res 1996;24:1481–1488.
36. Kaguni LS, Clayton DA. Template-directed pausing in in vitro DNA synthesis by DNA polymerase a from Drosophila melanogaster embryos. Proc Natl Acad Sci USA 1982;79:983–987.
37. Parton MJ, Broom W, Andersen PM, et al. D90A-SOD1 mediated amyotrophic lateral sclerosis: a single founder for all cases with evidence for a Cis-acting disease modifier in the recessive haplotype. Hum Mutat 2002;20:473.

Short Report

Do carriers of POLG mutation W748S have disease manifestations?

Rantamäki M, Luoma P, Virta JJ, Rinne JO, Paetau A, Suomalainen A, Udd B. Do carriers of POLG mutation W748S have disease manifestations?
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Mitochondrial recessive ataxia syndrome (MIRAS) is a common cause of autosomal recessive juvenile- or adult-onset ataxia, at least in Scandinavia. MIRAS patients are homozygous or compound heterozygous for POLG mutations W748S and A467T. Because many first-degree relatives of MIRAS patients in the studied families have reported neurological symptoms and some recent studies have suggested dominant negative effect of these mutations, a careful family study of heterozygotes was needed. We investigated all available members of the original large MIRAS family with W748S mutation. Neurological symptoms and signs were present in a number of carriers, but clearly defined neurological diseases did not segregate consistently with the mutation. Sensory polyneuropathy as a subclinical finding was observed in the majority of carriers examined. By positron emission tomography, cerebral glucose metabolism was moderately reduced in two out of four heterozygotes compared with severe reduction in one MIRAS patient. In conclusion, W748S heterozygotes showed no clinically manifesting phenotype.

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Both dominant and recessive mutations in the *POLG1* gene coding for the catalytic subunit of the mitochondrial DNA polymerase γ have been shown to be an important cause of human diseases and neurodegeneration (1–5). Recently, we and others identified a new neurodegenerative disorder, mitochondrial recessive ataxia syndrome (MIRAS), in patients homozygous or compound heterozygous for two POLG mutations W748S and A467T (6–8). Typically, MIRAS patients present with adult- or juvenile-onset ataxia combined with dysarthria, sensory neuropathy, late cognitive impairment, oculomotor defects, myoclonus, tremor, psychiatric symptoms and seizures (6, 8–10). Carrier frequency for W748S was found to be very high in the general population in Finland (1:125), for A467T in Sweden (1:200) and for both mutations in Norway (1:100 each), indicating that MIRAS is the most common inherited ataxia in these countries (8, 10, 11). Haplotype analysis of the *POLG1* locus showed that patients from Europe, Australia, New Zealand and the United States

harbouring W748S and A467T mutations originated from common ancient European founders (8, 12). On the founder haplotype, W748S always cosegregated in *cis* with E1143G (6, 8). The latter was previously considered a polymorphism, but its functional significance remains unsettled (4, 7, 13–16).

Recent observations have raised the question of a possible dominant effect in heterozygotes. Significantly more severe phenotypes and poorer survival were observed in patients with compound heterozygosity of W748S and A467T compared with patients being homozygous for either A467T or W748S, suggesting a possible codominant negative effect (10). Clinical and functional studies have indicated that A467T can also behave as a dominant mutation causing a mild phenotype with late-onset ptosis (17). In addition, a recent study identified one W748S carrier, who developed epilepsy, ataxia, mild parkinsonism and peripheral neuropathy in late adulthood and another carrier with epilepsy and peripheral neuropathy, suggesting the possibility of a dominant effect also with W748S (10, 16).

Subjects and methods

Forty-one members belonging to the original MIRAS family (6, 9) were included: 36 individuals were available for clinical examination and reports of five deceased individuals were extracted from hospital records. Blood samples from 30 family members were analysed for the W748S mutation (i.e. the 2243G>C substitution). Approval for the study was given by the Institutional Review Board of the Hospital District of Southern Ostrobothnia. Numbering of the individuals is presented in the pedigree (Fig. 1).

Motor and sensory nerve conduction velocity (NCV) studies were performed in seven heterozygous individuals (Table 2). During the study, one carrier (III-11) died. Autopsy and neuropathological examination were conducted. One MIRAS patient (III-12), four carriers (III-8, III-23, IV-5 and IV-12), and six control individuals (two con-

trols were non-carrier members of this family: IV-6 and IV-13) were studied with F-18 fluorodeoxyglucose positron emission tomography (FDG-PET) and magnetic resonance imaging (MRI) with methods previously described (18–20).

Results

Genetic analyses

Heterozygous W748S mutation was detected in 18 of 30 individuals and was absent in 12 of 30 family members (Fig. 1). In addition, individual III-17 was an obligate carrier as his son (IV-12) was heterozygote by genotyping. Five individuals (III-21, III-22, IV-1, IV-2 and IV-3) were not genotyped as their respective parents of the family were non-carriers. Individuals IV-10 and IV-11 were obligate carriers. In four deceased individuals, the genetic status remained unknown.

Clinical findings

Clinical examination of family members showed no neurological findings suggesting central or peripheral nerve system disease, e.g. ataxic disorder or peripheral neuropathy. Medical history summary of 20 carriers is presented in Table 1a. Summaries of deceased individuals with unknown genetic status are presented in Table 1b and of 17 non-carriers in Table 1c. More details of medical histories and clinical examinations are available as supplemental data (Supplementary Material online).

Clinical findings in the homozygous MIRAS patient (III-12), the participant in the PET study, have previously been described (6, 9). In short, from the age of 30 years, she developed a combination of progressive gait ataxia, dysarthria and mild cognitive impairment. In the fifth decade, mild ophthalmoparesis and ptosis appeared. On examination at 48 years of age, ataxia of gait and stance was severe. She needed personal assistance and a walker.
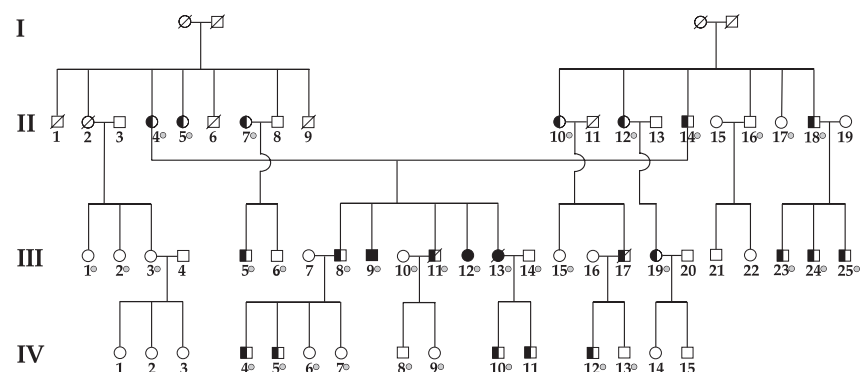

Fig. 1. Pedigree of the studied family shows segregation of *POLG1* mutation. Filled symbols indicate mitochondrial recessive ataxia syndrome (MIRAS) patients harbouring homozygous W748S mutation and semifilled symbols indicate heterozygote carriers. Filled circles beside numbers indicate available samples for DNA study.

Table 1a. Clinical findings in W748S mutation carriers

Individual no	Age at study (years)	Neurological symptoms and signs	Other diagnoses
II-4	77	Alzheimer's disease	Type 2 diabetes
II-5	74	Alzheimer's disease	Chronic psychosis and type 2 diabetes
II-7	69	–	–
II-10	84	–	Hypertension and osteoarthritis
II-12	82	–	Hypertension
II-14	80	–	Gastric and colon carcinomas, hypertension, and paroxysmal atrial fibrillation
II-18	68	–	Paroxysmal atrial fibrillation
III-5	44	Epilepsy	Hypertension
III-8	56	Migraine	Lower back and neck pain
III-11	Died at 49 years	Transient imbalance and dysarthric speech	Depression and alcoholism
III-17	Died at 38 years	Alcohol withdrawal seizures and delirium episodes	Personality disorder and chronic alcohol abuse
III-19	59	–	–
III-23	41	–	Type 1 diabetes
III-24	37	–	–
III-25	31	–	–
IV-4	31	–	Colitis ulcerosa
IV-5	29	–	–
IV-10	23	–	Depression
IV-11	19	–	Depression
IV-12	32	Ictal amnesia with alcohol intake. Incidental benign pineal cyst on magnetic resonance imaging	–

Table 1b. Disorders in deceased individuals with unknown genetic status

Individual no	Age at death (years)	Neurological symptoms and signs	Other diagnoses
II-1	70	Spinal injury	Psychotic features and cardiac failure
II-2	74	Alzheimer's disease and migraine	Colon carcinoma, persistent atrial fibrillation and depression
II-6	70	–	Psychotic features
II-9	12	Traumatic intracranial haemorrhage	–

Electrophysiological findings

All seven examined carriers showed normal motor and sensory NCVs (Table 2). Distal sensory and motor amplitude response latencies were within normal limits, but five out of seven carriers showed slightly low distal sensory amplitudes (SD values between −1.0 and −2.5) (21).

Neuropathology findings

III-11 (W748S carrier, sudden death at 49 years) The cerebral hemispheres were preserved and no degenerative atrophic changes in the thalamus, brainstem or spinal cord were detected. The only slightly pathologic finding was a minimal dropout of Purkinje cells in the cerebellar cortex. Distinct spinocerebellar pathology as we reported in a homozygous MIRAS patient (III-13) (9) was not seen in this patient.

PET and MRI findings

III-12 (homozygous MIRAS patient, age 48 years) Cerebral glucose metabolism was reduced to 55–75% in various brain areas examined. Greatest declines were seen in the thalamus (55% of the control value), cerebellum (59%) and occipital cortex (75%) (Fig. 2). T2-weighted MRI showed increased signal lesions bilaterally in the cerebellar white matter, in the thalamus and in the upper part of medulla oblongata. Moderate cerebellar atrophy and minor supratentorial cortical atrophic changes were observed (Table 3).

Table 1c. Occurrence of symptoms and findings in non-carriers

Individual no	Age at study (years)	Neurological symptoms and signs	Other diagnoses
II-16	77	–	Cholelithiasis and cholecystectomy
II-17	72	–	Asthma
III-1	60	Migraine and essential tremor	Arthritis
III-2	58	Cervical dystonia and migraine	–
III-3	55	Hypophyseal adenoma, essential tremor and migraine	–
III-6	41	Syncope and presyncopal symptoms	–
III-15	58	Congenital cerebral palsy	Breast cancer
III-21	49	Migraine	–
III-22	49	–	Fibromyalgia
IV-1	27	Essential tremor	–
IV-2	27	Essential tremor	–
IV-3	20	–	–
IV-6	24	–	–
IV-7	21	–	–
IV-8	29	Dysarthric speech with alcohol intake	–
IV-9	26	–	–
IV-13	28	Incidental benign pineal cyst on magnetic resonance imaging	

III-8 (W748S carrier, age 56 years)

Cerebral glucose metabolism was reduced to 80–95%. Greatest declines were found in the temporal cortex (80%), cerebellum (81%) and hippocampus (83%) (Fig. 2). On MRI, only three small subcortical ischaemic lesions in the parietal and frontal white matter were observed, but no atrophic changes were seen.

Table 2. Results of sensory and motor nerve conduction studies

Subject	Age at examination (years)	Nerve	Side	Type	Distal amplitude	SD	Velocity m/s	SD
II-7	68	Suralis	Right	S	4.8 µV	−1.1	48	−0.1
		Suralis	Left	S	4.7 µV	−1.2	45	−0.7
		Deep peroneal	Right	M	3.1 mV	−0.6	43	−0.9
II-12	82 years	Suralis	Right	S	1.7 µV	−2.5	49	0.1
		Suralis	Left	S	3.9 µV	−1.0	51	0.4
		Deep peroneal	Right	M	2.4 mV	−0.8	53	2.6
		Deep peroneal	Left	M	3.2 mV	−0.3	52	2.3
II-14	80	Suralis	Right	S	3.7 µV	−1.2	43	−1.0
		Suralis	Left	S	2.1 µV	−2.2	48	0.3
		Deep peroneal	Right	M	1.4 mV	−1.5	38	−1.1
		Deep peroneal	Left	M	4.9 mV	0.4	40	−0.4
II-18	68	Suralis	Right	S	11.6 µV	0.5	44	−0.3
		Suralis	Left	S	13.8 µV	0.8	41	−0.9
		Deep peroneal	Right	M	2.7 mV	−0.8	41	−0.3
		Deep peroneal	Left	M	1.6 mV	−1.5	40	−0.4
III-8	54	Superficial peroneal	Right	S	2.8 µV	borderline[a]	38	Normal for age[a]
		Superficial peroneal	Left	S	1.8 µV	low borderline[a]	38	Normal for age[a]
		Deep peroneal	Right	M	3.9 mV	−0.4	40	0.0
		Deep peroneal	Left	M	3.1 mV	−0.8	38	−0.7
III-25	31	Suralis	Right	S	18.1 µV	−0.1	48	−0.0
		Suralis	Left	S	11.0 µV	−1.0	45	−0.7
		Deep peroneal	Right	M	8.6 mV	1.0	49	0.5
		Deep peroneal	Left	M	2.1 mV	−1.8	52	1.4
IV-12	32	Suralis	Right	S	20.0 µV	0.2	49	0.6
		Suralis	Left	S	21.0 µV	0.2	49	0.6
		Deep peroneal	Right	M	8.3 mV	0.9	47	1.0
		Deep peroneal	Left	M	6.2 mV	0.2	51	1.7

M, motor; S, sensory; SD, standard deviation within normal population standardized with age, gender, length and temperature (21): <2.0 = normal, 2.0–2.5 = mild, 2.5–3.0 = moderate, >3.0 = severe.
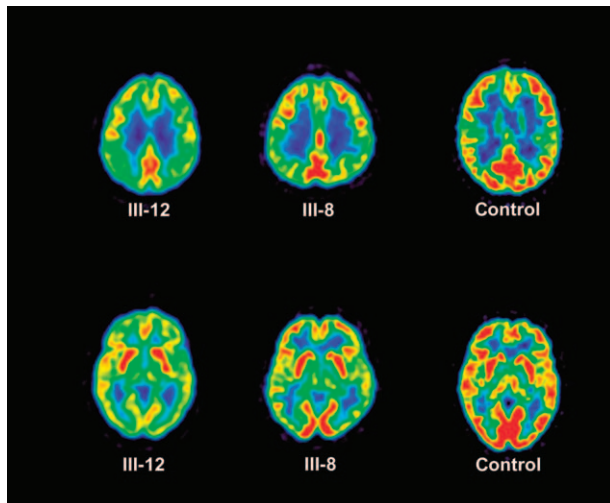[a]Superficial nerve conduction values were judged by an experienced neurophysiologist.

Fig. 2. Fluorodeoxyglucose positron emission tomography (FDG-PET) scans in mitochondrial recessive ataxia syndrome (MIRAS) patient (left, subject III-12) in one W748S mutation carrier (middle, subject III-8) and in a control subject (right). The upper panel represents parietal/posterior temporal level and the lower panel represents the basal ganglia level. Note the widespread cortical hypometabolism in the MIRAS patient. In the carrier, parietal and temporal hypometabolism can be observed.

III-23 (W748S carrier, age 41 years)
Considerable decline (64–79%) in all brain regions, most prominent in the medial frontal cortex (64%) and thalamus (65%). Brain MRI was normal.

In two other carriers (IV-5, age 29 years; IV-12, age 32 years), no significant changes were detected either in PET or MRI.

Discussion

Three individuals with Alzheimer disease and the majority of individuals with psychiatric syndromes were clearly clustered in the maternal branch of the family, whereas the paternal branch showed no increased prevalence of neurological problems. This asymmetric segregation of neurological symptoms in the family as well as lack of complete cosegregation with the W748S status suggest that the disorders probably were mediated by other inherited genetic factors in the maternal branch of the pedigree. Episodic symptoms such as migraine-like headache and epilepsy can present in MIRAS (10) but are also fairly common symptoms in the general population (22). In this family, migraine was more frequent in the non-carrier group, and epilepsy occurred in one heterozygous individual only.

Electrophysiological studies showed slightly low or borderline low distal sensory amplitudes in a majority of examined carriers. In lack of other known causes for incipient axonal sensory neuropathy, this finding may indicate a subclinical manifestation in carriers. Autopsy findings in one carrier reported here showed only slight loss of Purkinje cells in the cerebellar cortex. These subtle atrophic changes in the cerebellar cortex were considered to be caused by chronic alcohol abuse (23). However, involvement of heterozygous POLG mutation cannot be excluded.

FDG-PET imaging in the MIRAS patient showed reduced glucose metabolism in all examined brain regions. In two out of four carriers, decreased glucose metabolism levels were detected. They had no deficits in their cognitive capacity. One

Table 3. Regional cerebral metabolism rates (rCMRs; μmol/ml/min) in one MIRAS patient, four W748S mutation carriers and controls

Brain regions	MIRAS patient		Heterozygous carriers								Controls $n = 6$	
Age (years)	48		38.75 ± 12.2 (mean ± SD)								40.8 ± 12.8 (mean ± SD)	
Individuals	III-12		III-8		III-23		IV-5		IV-12			
	rCMR		rCMR		rCMR		rCMR		rCMR			
	rate	%	rate	%	rate	%	rate	%	rate	%	mean rCMR	SD
Caudate nucleus	0.30	67%	0.39	86%	0.33	73%	0.49	108%	0.42	92%	0.45	0.07
Cerebellum	0.20	59%	0.28	81%	0.25	73%	0.34	98%	0.32	91%	0.35	0.06
Prefrontal cortex	0.28	64%	0.38	86%	0.31	71%	0.49	113%	0.46	104%	0.44	0.08
Hippocampus	0.22	75%	0.24	83%	0.22	76%	0.30	103%	0.27	94%	0.29	0.04
Medial frontal cortex	0.28	70%	0.35	86%	0.26	64%	0.44	110%	0.42	103%	0.40	0.07
Putamen	0.34	72%	0.41	87%	0.34	71%	0.53	112%	0.46	97%	0.48	0.08
Sensori-motor cortex	0.29	75%	0.36	95%	0.27	70%	0.43	112%	0.42	108%	0.38	0.06
Lateral temporal cortex	0.27	69%	0.32	80%	0.29	72%	0.41	104%	0.39	99%	0.39	0.06
Thalamus	0.23	55%	0.35	85%	0.27	65%	0.49	118%	0.43	105%	0.41	0.06
Occipital cortex	0.25	57%	0.39	87%	0.35	79%	0.50	113%	0.43	97%	0.44	0.04

MIRAS, mitochondrial recessive ataxia syndrome; %, percentage of the control mean; SD, standard deviation.

of them (III-23) had type I diabetes but was in completely euglycaemic state during the PET scan. Because previous studies have shown both increased and decreased glucose metabolism values in diabetics (24), the exact cause of this finding cannot be determined. The other carrier (III-8) had mild migraine. The limited PET study allows us to hypothesize that carriers may at older age show reduced glucose utilization in their brain that exceeds the reduction upon normal ageing, which might be an early sign of neurodegeneration and implicate further studies.

A definite clinically manifest phenotype associated with W748S heterozygosity cannot be determined. Axonal sensory neuropathy was a frequent subclinical finding. Moreover, subclinical central nervous system abnormalities as shown by PET results and susceptibility to develop neurological symptoms when combined with environmental toxic factors or other genetic factors cannot be excluded.

Supplementary material

Data of clinical findings

Supplementary materials are available as part of the online article at http://www.blackwell-synergy.com

Acknowledgements

References

1. Kaguni LS. DNA polymerase-γ, the mitochondrial replicase. Annu Rev Biochem 2004: 73: 293–320.
2. Hudson G, Chinnery PF. Mitochondrial DNA polymerase-γ and human disease. Hum Mol Genet 2006: 15: 244–252.
3. Van Goethem G. Autosomal disorders of mitochondrial DNA maintenance. Acta Neurol Belg 2006: 106: 66–72.
4. Horvath R, Hudson G, Ferrari G et al. Phenotypic spectrum associated with mutations of mitochondrial polymerase γ gene. Brain 2006: 129: 1674–1684.
5. Luoma P, Melberg A, Rinne JO et al. Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: clinical and molecular genetic study. Lancet 2004: 364: 875–882.
6. Van Goethem G, Luoma P, Rantamäki M et al. POLG mutations in neurodegenerative disorders with ataxia but no muscle involvement. Neurology 2004: 63: 1251–1257.
7. Winterthun S, Ferrari G, He L et al. Autosomal recessive mitochondrial ataxic syndrome due to mitochondrial polymerase γ mutations. Neurology 2005: 64: 1204–1208.
8. Hakonen AH, Heiskanen S, Juvonen V et al. Mitochondrial DNA polymerase W748S mutation: a common cause of autosomal recessive ataxia with ancient European origin. Am J Hum Genet 2005: 77: 430–441.
9. Rantamäki M, Krahe R, Paetau A, Cormand B, Mononen I, Udd B. Adult-onset autosomal recessive ataxia with thalamic lesions in a Finnish family. Neurology 2001: 57: 1043–1049.
10. Tzoulis C, Engelsen BA, Telstad W et al. The spectrum of clinical disease caused by the A467T and W748S POLG mutations: a study of 26 cases. Brain 2006: 129: 1685–1692.
11. Kollberg G, Moslemi A-R, Darin N et al. POLG1 mutations associated with progressive encephalopathy in childhood. J Neuropathol Exp Neurol 2006: 65: 758–768.
12. Hakonen AH, Davidzon G, Salemi R et al. Abundance of the POLG disease mutations in Europe, Australia, New Zealand, and the United States explained by single ancient European founders. Eur J Hum Genet 2007: 15: 779–783.
13. Hisama FM, Mancuso M, Filosto M, DiMauro S. Progressive external ophthalmoplegia: a new family with tremor and peripheral neuropathy. Am J Med Genet 2005: 135: 217–219 (Letter).
14. Chan SS, Longley MJ, Copeland WC. Modulation of the W748S in DNA polymerase γ by the E1143G polymorphism in mitochondrial disorders. Hum Mol Genet 2006: 15: 3473–3483.
15. Craig K, Ferrari G, Tiangyou W et al. The A467T and W748S substitutions are a rare cause of adult-onset ataxia in Europe. Brain 2007: 130: E69 (Letter).
16. Bindoff L. Reply. Brain 2007: 130: E70 (Letter).
17. Luoma PT, Luo N, Löscher WN et al. Functional defects due to spacer-region mutations of human mitochondrial DNA polymerase in a family with an ataxia-myopathy syndrome. Hum Mol Genet 2005: 14: 1907–1920.
18. Hamacher K, Coenen HH, Stocklin G. Efficient stereospecific synthesis of no-carrier-added 2-[18F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med 1986: 27: 235–238.
19. Patlak CS, Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. J Cereb Blood Flow Metab 1985: 5: 584–590.
20. Järvenpää T, Räihä I, Kaprio J et al. Regional cerebral glucose metabolism in monozygotic twins discordant for Alzheimer's disease. Dement Geriatr Cogn Disord 2003: 16: 245–252.
21. Falck B, Andreassen S, Groth T et al. The development of a multicenter database for reference values in clinical neurophysiology: principles and examples. Comput Methods Programs Biomed 1991: 34: 145–162.
22. Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the 'common' neurologic disorders? Neurology 2007: 68: 326–337.
23. Harper C. The neurotoxicity of alcohol. Hum Exp Toxicol 2007: 26: 251–257.
24. Sabri O, Hellwig D, Schreckenberger M et al. Influence of diabetes mellitus on regional cerebral glucose metabolism and regional blood flow. Nucl Med Commun 2002: 21: 19–29.

6. Bachoud-Levi AC, Remy P, Nguyen J-P, et al. Motor and cognitive improvements in patients with Huntington's disease after neural transplantation. Lancet 2000;356:1975–1979.
7. Gaura V, Bachoud-Levi AC, Ribeiro MJ, et al. Striatal neural grafting improves cortical metabolism in Huntington's disease patients. Brain 2004;127:65–72.
8. Bachoud-Levi A, Hantraye P, Peschanski M, et al. Fetal neural grafts for Huntington's disease: a prospective view. Mov Disord 2002;17:439–444.
9. Freeman TB, Cicchetti F, Hauser RA, et al. Transplanted fetal striatum in Huntington's disease: phenotypic development and lack of pathology. Proc Natl Acad Sci U S A 2000;97:13877–13822.

Adult-Onset Ataxia and Polyneuropathy Caused by Mitochondrial 8993T→C Mutation

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The 8993T→C mutation in mitochondrial DNA (mtDNA) has been described previously to be associated with infantile- or childhood-onset phenotypes, ranging from Leigh's syndrome to neurogenic weakness, ataxia, and retinitis pigmentosa syndrome. We report a kindred with adult-onset slowly progressive ataxia and polyneuropathy and with the heteroplasmic 8993T→C mutation. Our findings suggest that the 8993T→C mtDNA mutation should be considered in the differential diagnosis of nondominant adult-onset ataxia and axonal neuropathy.

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Adult- or late-onset nondominant ataxia with axonal polyneuropathy is a diagnostic challenge for the clinician. After exclusion of Friedreich's ataxia (FRDA), many rare genetic and metabolic disorders remain to be considered.[1] Ataxia can be a prominent feature in the myoclonic epilepsy with ragged-red fibers syndrome (MERRF) caused by the 8344A→G mitochondrial DNA (mtDNA) mutation[2] and in syndromes associated with mtDNA deletions.[2] Incomplete penetrance in some of the dominant spinocerebellar ataxias (SCAs) may also be considered.[3] We describe here a kindred of four siblings. The second child had died in the 1960s and Friedreich's ataxia was determined at autopsy. The three other siblings developed ataxia and polyneuropathy in adulthood, but no expansions were detected in the FRDA gene. Because the neurological disease segregated to all four children with highly variable severity, a mtDNA mutation was suspected. Screening for the common mtDNA point mutations (3243A→G, 8344A→G, 8993T→G) and for mtDNA deletions proved to be negative, and therefore sequencing of the entire mtDNA coding region was considered necessary.

Patients and Methods

The parents of the affected siblings were healthy. The father had died at age 90 years and the mother was still alive at age 89 years, both without neuromuscular symptoms (Fig).

Patient III-6

The proband is a 52-year-old woman. Childhood development was normal. At age 28 years, she noticed slight balance problems during her first pregnancy. She had repeated atonic seizures or drop attacks despite anticonvulsive medication. Neurological examination at age 36 years showed mild gait ataxia, absent ankle reflexes, positive Babinski signs, and pes cavus. She was diagnosed with hereditary spinocerebellar ataxia on the basis of the family history.

At age 51 years, her gait was grossly ataxic and she used a walker. She had mild limb ataxia and dysarthria. There was mild muscle weakness and atrophy. She showed gaze-evoked nystagmus, but eye movements were unrestricted and fundoscopy was normal. Memory and cognitive functions were normal. Cerebrospinal fluid examination, electroencephalogram, and visual-evoked and brainstem auditory-evoked potentials were normal. Tibial nerve somatosensory-evoked potential (SEP) responses were delayed at the spinal level and with decreased amplitudes on right side at the cortical level, suggesting a lesion in the central somatosensory pathway. Electrophysiological studies were compatible with axonal sensorimotor polyneuropathy (nerve conduction velocity upper limb: sensory 51m/sec, motor 56m/sec: lower limb sensory 40m/sec, motor 36m/sec). Histological examination of muscle showed mild chronic neurogenic changes. Enzyme histochemistry was normal and no cytochrome c oxidase-negative fibers or ragged red fibers were detected. Serum creatine kinase (CK) was mildly elevated and lactate was normal. Brain magnetic resonance imaging (MRI) showed mild supratentorial and infratentorial atrophy. DNA analyses for FRDA, Charcot-Marie-Tooth disease type 1A, dentatorubral-pallidoluysian atrophy, autosomal dominant SCA types 1, 2,

3, 6, 7, 8, 10, 12, and 17, mitochondrial deletions and the common point mutations associated with MERRF, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), and neuropathy, ataxia, and retinitis pigmentosa (NARP/8993T→G) were all negative.

Patient III-3

Patient III-3 is a 65-year-old man. He had noticed diminished physical endurance compared with his peers during military service at age 20 years. Slight balance difficulties and paresthesias of legs manifested at the age of 57 years. On neurological examination, his gait was normal, but he had decreased vibration sensation in lower limbs and ankle reflexes were absent. Electrophysiological examination showed severe axonal sensorimotor polyneuropathy (nerve conduction velocity upper limb: sensory 46m/sec, motor 56m/sec: lower limb sensory no potential, motor 39m/sec).

Difficulty in walking progressed and he was compelled to use sticks outdoors. He suffered from sudden loss of muscle tone control, probably representing mild astatic seizures. Clinical examination at age 64 years showed wide-based walking and mild distal leg muscle weakness, but no overt ataxia nor dysarthria. Babinski sign was indifferent. Brain MRI showed slight periventricular white matter abnormalities and mild atrophy in cerebellar vermis. Tibial nerve SEP test was abnormal with delayed responses at cortical level on the right side and no response on the left side. Serum lactate and CK levels were normal. Muscle histology was normal.

Patient III-4

The elder sister had normal perinatal history except for diagnosis of clubfoot. She developed slowly and her gait remained unsteady. Neurological examination at age 12 years showed ataxic gait with mild limb dysmetria and high arched feet. Patellar and ankle reflexes were diminished and muscle strength in lower extremities was reduced. Babinski sign was positive. Her mental impairment was considered moderate.

At age 22 years, walking and stance were impossible because of severe ataxia. She developed repeated seizures and a febrile illness. She became progressively lethargic, lost consciousness, and died. Autopsy showed marked gliosis of brain and the findings were considered compatible with Friedreich's ataxia.

Patient III-5

Patient III-5 is a 58-year-old man. He had noticed excessive tiredness of legs during military service at age 20 years. Manifest muscle fatigue and balance difficulties occurred at age 45 years. At age 50 years, he walked wide-based with slight steppage gait. Muscle histology and electrophysiological studies confirmed axonal sensorimotor neuropathy. Brain computed tomography scan was normal.

On examination at age 58 years, his gait was ataxic, but he could walk without support. His speech was dysarthric and pursuit eye movements were jerky. Limb movements were slightly dysmetric.
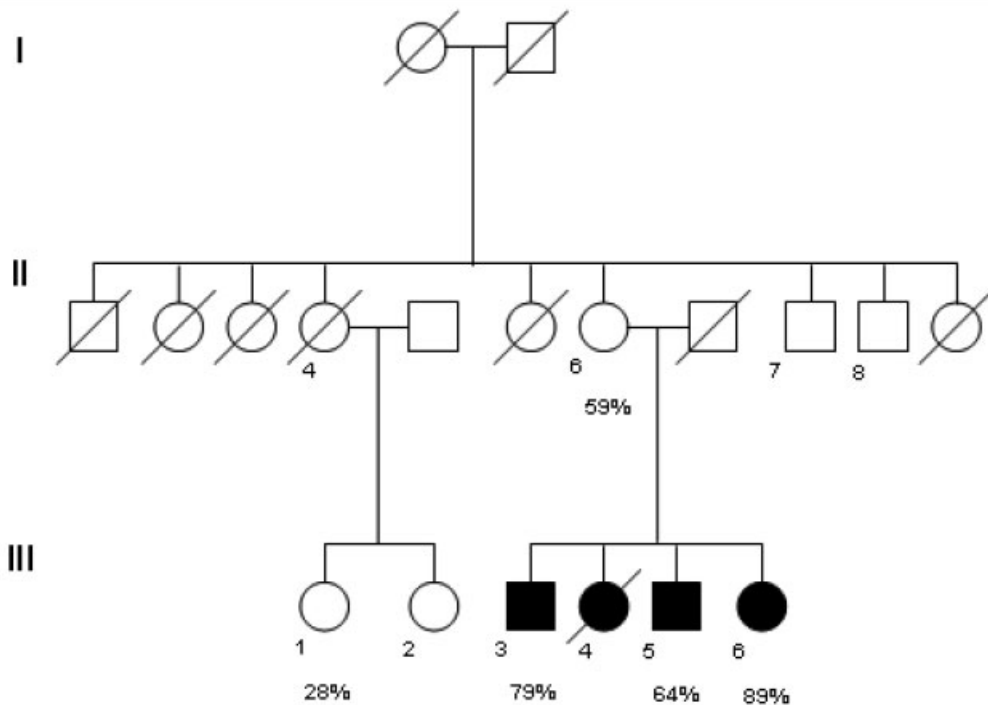
Fig. Pedigree of the family with the 8993T→C mutation in mtDNA. The proportion of the mutant genome in blood DNA is shown. Filled symbols indicate affected individuals. None of individuals II-4, II-6, II-7, or II-8 had reported neurological symptoms. Patient III-1 has been diagnosed with multiple sclerosis in accordance with the Poser's criteria.

Methods

Mitochondrial DNA coding region spanning the nucleotides 577 to 16090 was amplified in 63 partially overlapping fragments, and these fragments then were subjected to conformation sensitive gel electrophoresis (CSGE) as described earlier.[4,5] Fragments that differed in mobility in CSGE were sequenced (ABI PRISM 377 Sequencer using DYEnamic ET Terminator Cycle Sequencing Kit; Amersham Pharmacia Biotech Inc., Piscataway, NJ) after purification with exonuclease I and shrimp alkaline phosphatase.[6] Sequences were compared to the human mtDNA Cambridge reference sequence.[7]

Heteroplasmy of 8993T→C was determined by restriction fragment analysis of a fragment that was amplified in the presence of ^{35}S-dATP (Perkin-Elmer, Wellesley, MA). The 8993T→C mutation creates a restriction site for Eco52I. After an overnight incubation with this enzyme at 37°C, the fragments were electrophoresed on 6% polyacrylamide gel and exposed on film. The intensities of the fragments (Quantity One; Bio-Rad, Hercules, CA) were used to calculate heteroplasmy.

Results

Sequencing of the entire mtDNA coding region in the proband revealed six polymorphisms that differed from the Cambridge reference sequence (750A→G, 1438A→G, 3010G→A, 4769A→G, 8860A→G, and 15326A→G) and that were compatible with mtDNA haplogroup H. In addition, the proband was found to harbor 8993T→C mutation in the ATPase6 gene. The mutation was heteroplasmic in blood and the proportion of the mutant genome was 89%. The mutation was found in blood DNA in all family members (see Fig). Interestingly, the mutation was also found at a low heteroplasmy in a maternal cousin, who had been diagnosed with relapsing remitting multiple sclerosis at age 35 years.

Discussion

We found the 8993T→C mtDNA mutation in a family with adult-onset slowly progressive ataxia and polyneuropathy. This mutation previously has been associated with Leigh's syndrome and other infantile or childhood-onset syndromic diseases, but not with adult-onset ataxia. Mutations in position 8993 are associated either with NARP or with Leigh's syndrome phenotypes.[8,9] Different phenotypes may occur within the same family.[10,11] Patients with the more common mutation 8993T→G are considered to have an earlier onset, more rapid progression, and more severe outcome than those with 8993T→C.[12] Together these two mutations are among the most common mtDNA mutations in childhood mitochondrial diseases.[13,14] Among adults, the most common mtDNA mutations are the MELAS mutation 3243A→G[15] and the two LHON mutations 11778G→A and 3460G→A.[16]

However, mtDNA mutations are only rarely causes of adult-onset ataxia.[15,17]

Three siblings were clinically examined. Two of them presented with an adult or late-onset slowly progressive gait ataxia and dysarthria, and all three had peripheral axonal neuropathy. Ataxia was considered both cerebellar and sensory in origin. Patient III-4 had suffered from an early-onset disease well in line with previously reported clinical features associated with the 8993T→C mutation, and she had been diagnosed with Friedreich's ataxia on the basis of autopsy findings. Other causes had not been considered in the family before her sister proved to be negative for the FRDA expansion. However, adult-onset axonal polyneuropathy combined with slowly progressive ataxic gait and pyramidal signs has not previously been described in patients with 8993T→C. The correct diagnosis may easily be missed, when these clinical features occur in a sporadic patient without other findings suggestive of a mitochondrial disease. The low heteroplasmy of 8993T→C in the maternal cousin primarily suggests an independent cause of her multiple sclerosis disease.

Adult-onset nondominant ataxias are less well known than the dominant ones and only a few autosomal recessive ataxias have been genetically characterized so far.[18] Mitochondrial diseases contribute to the differential diagnosis of adult-onset nondominant ataxias. However, patients with adult mitochondrial disease may, in addition, present with myopathy, hearing loss, seizures, polyneuropathy, pigmentary retinopathy, or, more rarely, movement disorders.[2] Interestingly, homozygous mutations recently have been found in the polymerase gamma (POLG) gene in patients with an ataxic syndrome.[19,20] Because Friedreich's ataxia may also be considered a mitochondrial disorder, it seems that mitochondrial dysfunction has a central role in the pathogenesis of many inherited ataxias. The family reported here supports this concept and suggests that the 8993T→C mtDNA mutation should be considered in the differential diagnosis of disorders with adult-onset ataxia and/or axonal neuropathy.

References

1. Rantamäki M, Krahe R, Paetau A, et al. Adult-onset autosomal recessive ataxia with thalamic lesions in a Finnish family. Neurology 2001;57:1043–1049.
2. Zeviani M, Di Donato S. Mitochondrial disorders. Brain 2004; 127:2153–2172.

3. Schöls L, Bauer P, Schmidt T, et al. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 2004;3:291–304.

4. Körkkö J, Annunen S, Pihlajamaa T, et al. Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 1998;95:1681–1685.

5. Finnilä S, Hassinen IE, Ala-Kokko L, Majamaa K. Phylogenetic network of the mtDNA haplogroup U in Northern Finland based on sequence analysis of the complete coding region by conformation-sensitive gel electrophoresis. Am J Hum Genet 2000;66:1017–1026.

6. Werle E, Schneider C, Renner M, et al. Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acids Res 1994;22:4354–4355.

7. Andrews RM, Kubacka I, Chinnery PF, et al. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 1999;23:147.

8. Holt IJ, Harding AE, Petty RKH, Morgan-Hugles JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 1990;46:428–433.

9. de Vries DD, van Engelen BGM, Gabreëls FJM, et al. A second missense mutation in the mitochondrial ATPase 6 gene in Leigh's syndrome. Ann Neurol 1993;34:410–412.

10. Suzuki Y, Wada T, Sakai T, et al. Phenotypic variability in a family with a mitochondrial DNA T8993C mutation. Pediatr Neurol 1998;19:283–286.

11. Sciacco M, Prelle A, D'Adda E, et al. Familiar mtDNA T8993C transition causing both the NARP and the MILS phenotype in the same generation. A morphological, genetic and spectroscopic study. J Neurol 2003;250:1498–1500.

12. Santorelli FM, Mak SC, Văzquez-Memije ME, et al. Clinical heterogeneity associated with the mitochondrial DNA T8993C point mutation. Pediatr Res 1996;39:914–917.

13. Darin N, Oldfors A, Moslemi A-R, et al. The incidence of mitochondrial encephalomyopathies in childhood: clinical features and morphological, biochemical, and DNA abnormalities. Ann Neurol 2001;49:377–383.

14. Skladal D, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial respiratory chain disorders in children. Brain 2003;126:1905–1912.

15. Majamaa K, Moilanen JS, Uimonen S, et al. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. Am J Hum Genet 1998;63: 447–454.

16. Man PY, Griffiths PG, Brown DT, et al. The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am J Hum Genet 2003;72:333–339.

17. Chinnery PF, Brown DT, Archibald K, et al. Spinocerebellar ataxia and the A3243G and A8344G mtDNA mutations. J Med Genet 2002;39:e22.

18. Di Donato S, Cellera C, Mariotti C. The complex clinical and genetic classification of inherited ataxias. II. Autosomal recessive ataxias. Neurol Sci 2001;22:219–228.

19. Van Goethem G, Luoma P, Rantamäki M, et al. POLG mutations in neurodegenerative disorders with ataxia but no muscle involvement. Neurology 2004;63:1251–1257.

20. Winterthun S, Ferrari G, He L, et al. Autosomal recessive mitochondrial ataxic syndrome due to mitochondrial polymerase γ mutations. Neurology 2005;64:1204–1208.