Hereditary ataxias, epidemiological and genetic studies in a Norwegian population

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Preface

Our research is based on patients with hereditary ataxia. In Norway patients referred to our neurological clinics are older than 16 years and this work is therefore based on work with adult patients. In addition I have most of the time worked with the dominant forms as this thesis will be colored of. Paper 1 was a prevalence study on hereditary ataxias and in this study we included children. All results from patients younger than 16 years were therefore based upon collaboration with pediatricians. I have not examined the children in this study myself. Also the included work on A-T in this paper was done by Asbjørg Stray-Pedersen and based on the work she did during her thesis on these disorders. In the same paper the HSP part has already been defended by Anne Kjersti Erichsen in 2009 and this work is therefore not a part of my thesis.

Many recessive disorders have ataxia as an additional feature and the same goes for mitochondrial disorders. Only recessive disorders with ataxia as a main feature are included in my work.

Abbreviations

ADCA-**A**utosomal **D**ominant **C**erebellar **A**taxia

ARCA-**A**utosomal **R**ecessive **C**erebellar **A**taxia

FRDA-**Fr**ie**d**reich's **A**taxia

SCA-**S**pino**C**erebellar **A**taxia

AVED-**A**taxia with **V**itamin **E** **D**eficiency

AOA1-**A**taxia with **O**culomotor **A**praxia type **1**

SCAR1-**S**pino**C**erebellar**A**taxia **R**ecessive type **1**/AOA2-**A**taxia with **O**culomotor **A**praxia type **2**

A-T-**A**taxia-**T**elangiectasia

HSP-**H**ereditary **S**pastic **P**araparesis

MRI-**M**agnetic **R**esonance **I**maging

MSA-c-**M**ultippel **S**ystem **A**trophy **C**erebellum

DRPLA-**D**entato-**R**ubral-**P**allido-**L**uysian **A**trophy

HA-**H**ereditary **A**taxia

Publications included

1. Prevalence of hereditary ataxia and spastic paraplegia in southeast Norway: a population-based study
2. Ataxia with vitamin E deficiency in southeast Norway, case report
3. SCA14 in Norway, two families with autosomal dominant cerebellar ataxia and a novel mutation in the PRKCG gene
4. A late onset autosomal dominant ataxia maps to chromosome 10q26.3

Introduction and background

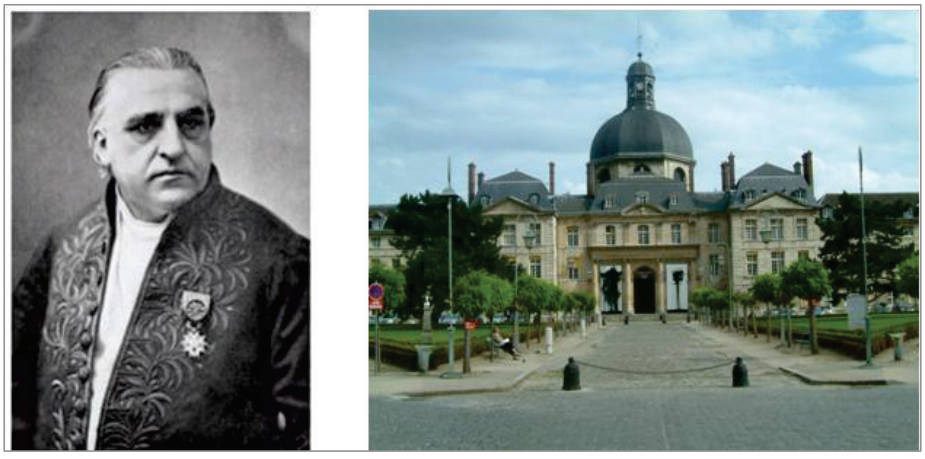
Definition

Ataxia means lack of order or in-coordination. It is one of the most common neurological symptoms and also described by J.M. Charcot (1825-1893) and his colleges at Hôpital de la Salpêtrière, Paris in the 17.century (Figure 1).

Ataxia may affect the arms, legs, truncus, speech, throat and eye movements. Ataxia can occur in all kind of disorders affecting the cerebellum and its pathways, the dorsal columns and the vestibular system. Four main groups of disorders can give the symptom ataxia:

1. Hereditary ataxias
2. Secondary ataxias
3. Sporadic ataxias
4. Mitochondrial disorders

Figure 1. *Jean Martin Charcot, the first professor in neurology working at Hôpital de la Salpêtrière in the late 17.century. The entrance of the Hôpital de la Salpêtrière in 2009.*



Hereditary ataxias

The hereditary ataxias (HA) are a group of rare monogenetic neurodegenerative disorders characterized by slowly progressive incoordination of gait, limbs, speech and eye movements which are inherited in an autosomal recessive, dominant or X-linked manner also called

autosomal recessive cerebellar ataxia (ARCA), autosomal dominant cerebellar ataxia (ADCA) or X-linked ataxias. Together with hereditary spastic paraplegias they are often called hereditary spinocerebellar disorders.

The hereditary ataxias have been difficult to classify and attempts to classify them have historically been based on clinical descriptions, neuropathological descriptions or image-forming techniques. At a time there were almost as many different types of hereditary ataxias as authors publishing them. The first neurologist who made a systematic classification system of hereditary ataxia was Anita Harding (1-4). She classified the ADCA clinically into four main groups due to additional features (Table 1) (3).

Table 1. *Harding's classification system for ADCA, based on clinical features in addition to the cerebellar ataxia*

ADCA type	Clinical features in addition to the cerebellar symptoms
ADCA1	Ophtalmoplegia/optic atrophy/dementia/extrapyramidal signs and symptoms
ADCA2	Pigmentary retinopathy and/or opthalmoplegia/extrapyramidal features
ADCA3	Pure, often late onset (over 50 years)
ADCA4	Myoclonus, deafness

ADCA-autosomal dominant cerebellar ataxia

With increased knowledge of molecular diagnoses from the early nineteen-ninety the classification changed from clinical descriptions to exact genetic diagnoses. A genetic classification system is therefore nowadays common and accepted. This means they are classified after main mode of inheritance and then if possible after the gene/locus causing the disorder. ADCA is commonly denoted **S**pino**C**erebellar **A**taxia and then numbered chronologically following publication date (**SCA**1-36). The same system is being proposed with ARCA, **S**pino**C**erebellar **A**taxia **R**ecessive **SCAR**1-10 or ARCA1-2, but unfortunately this easily understood classification system is not in use and incomplete (5-17). For the recessive ataxias it is today more common to use the well-established names after main clinical feature, protein dysfunction, geographical origin or after the scientist who first described the phenotype, e.g.; Friedreich's ataxia, ataxia with oculomotor apraxia (AOA1 and 2), ataxia telangiectasia (A-T), ataxia with vitamin E deficiency (AVED) and autosomal dominant spastic ataxia of Charlevoix-Saguenay (ARSACS).

Supplementary investigations with neuroimaging, neurophysiologic investigations, genetic tests and biomarkers are often needed to exclude other causes of ataxia and in order to give an

exact genetic diagnosis. Main diagnoses to be considered before a hereditary ataxia diagnosis can be made are listed in Table 2 and discussed briefly in the chapter non-hereditary neurodegenerative ataxias (8;9;11-13;18-26) .

Table 2. *Main diagnoses to be considered when investigating a patient with progressive ataxia*

Group of disorder	Disorders affecting cerebellum and/ or its pathways	Clinical clues
Acquired ataxias/ secondary ataxias	Paraneoplastic disorders, multiple sclerosis, vascular malformations, ischemia, bleeding, infections (i.e. HIV, syphilis, borrelia etc.), primary or metastatic tumors, toxic substances (alcohol, phenytoin), vitamin deficiencies (B12, vitamin E)	Additional investigation with brain MRI, lumbar puncture, blood samples will give important clues to the diagnoses. No typical family history. May not be progressive.
Non-hereditary neurodegenerative ataxias	Sporadic ataxia, MSA-c	Late onset (>50 years), MSA-c often with autonomic symptoms. No family history. Progressive.
Primary hereditary ataxias	**Autosomal recessive, dominant and X-linked hereditary ataxias**	**Other family members affected with a similar disorder, autosomal recessive forms have often earlier onset compared to the dominant forms. Progressive or episodic forms.**
Mitochondrial disorders*	POLG related disorders (i.e.: SCAE, SANDO, MEMSA), IOSCA, MERRF, NARP Kearne-Sayre syndrome Maternally inherited Leigh syndrome Leigh syndrome, LBSL	Ataxia is seen in many mitochondrial disorders. Mitochondrial disorders have to be considered when ataxia is accompanied by other symptoms and signs. SCAE is a frequent MIRAS in Norway. These disorders are progressive, often multi-system disorders /giving other neurological manifestations in addition to ataxia.
Metabolic disorders with ataxia	Disorders affecting the ammonium, amino acid, pyruvate, lactate or the fatty acid metabolism, such as; GM1 and GM2 gangliosidosis, Niemann-Pick, Refsums disease, abetalipoptroteinemias, acanthocytosis, Cerebrotendinous Xanthomatosis	Often early onset, ataxia is often an additional symptom and not the only symptom. The progression can be more aggressive compared to the primary hereditary ataxias.

*- only mitochondrial disorders were ataxia is a common feature is mentioned.

MSA-c= multiple system atrophy cerebellum, MIRAS= mitochondrial recessive ataxia syndrome, SCAE=spinocerebellar ataxia with epilepsy, SANDO=sensory ataxia neuropathy dysarthria and ophthalmoplegia, MEMSA=Myoclonic epilepsy myopathy sensory ataxia, IOSCA= infantile-onset spinocerebellar ataxia, MERRF= myoclonic epilepsy with ragged red fibers, NARP=neuropathy-ataxia retinitis pigmentosa syndrome, LBSL=leucencephalopathy with brainstem and spinal cord involvement, dilated cardiomyopathy with ataxia, GM gangliosidosis-lysosomal storage disorders characterized by generalized accumulation of gangliosidosis due to deficiency of different enzymes

Non-hereditary neurodegenerative ataxias/sporadic ataxia and isolated cases

Many patients have progressive balance difficulties, dysarthria, dysphagia and visual symptoms with features of a cerebellar disease without other family members having the same symptoms (19;27). Isolated subjects can hide a monogenetic disorder, because no family members report the same disorder, due to complicating factors to the inheritance pattern listed in Table 3. As we learn more about the inheritance patterns for monogenetic disorders, it is becoming clear that exceptions to the simple Mendelian rules of inheritance are relatively common. Sporadic ataxia is often defined as a non-genetic ataxia, but there is no distinct definition for sporadic ataxia in the literature. Some publications include MSA, late onset idiopathic cerebellar ataxias and secondary ataxias (27). Others use more stringent criteria with only adult onset sporadic ataxia with unknown etiology and MSA-c (19), as we do in our work. The sporadic ataxias can be divided in pure or complex forms. Pure forms, known as late onset idiopathic cerebellar ataxias, have mainly pure cerebellar symptoms and signs. The complex form, known as MSA-c, may in addition to the cerebellar signs have brainstem, Parkinson-like and autonomic symptoms. Disability may be greater and the disease progress faster with the MSA-c. Brain imaging typically shows cerebellar atrophy in sporadic ataxia with or without atrophy of the brainstem without other findings for a secondary ataxia.
The etiology of these disorders is unknown, but also among sporadic ataxia there will be hidden isolated subjects with hereditary ataxia, because molecular testing for all HA genes has until today not been feasible due to the high expenses and the time-consuming process it is to sequence all genes.

Table 3. *A list over complicating factors to the general inheritance patterns*

1. Incomplete penetrance/age-dependent penetrance
2. Small family size, early death of an affected parent, adoption or not reported correct biological father may hide the inheritance pattern in the family
3. Unstable alleles/ anticipation
4. De novo mutation
5. Variable expressivity
6. Sex related genetic effects (i.e. imprinting)

Epidemiology

Previous studies (Table 4) estimate the prevalence of hereditary ataxia between 1.6-18/100 000, ADCA between 1.6-3.7/100 000 with spinocerebellar ataxia (SCA) 1, 2 and 3 as the most common subtypes worldwide (6;27-43). Figure 2 shows the distribution of the most common SCA worldwide (15;25). There are less data on the prevalence of ARCA, which is estimated to be between 1/100 000 to 4.7/100 000 (28-31;35;37;44). Friedreich's ataxia (FRDA) is reported to be the most prevalent recessive form in central Europe, however with an uneven distribution worldwide. Its estimated prevalence ranges from being non-existent to 4.7/100,000 (2;31;32;35;36;39;44-48). Ataxia telangiectasia (A-T) is estimated as high as 1 per 40.000-100.000 live births in the USA (49) and is the only form of hereditary ataxia recently genetically described in Norway (50-52). Ataxia with vitamin E deficiency (AVED) and ataxia with ocular apraxia type 1 (AOA1) and type 2 (AOA2) are rare and to date the prevalence is unknown (8;39;53).

One of the first epidemiological studies on hereditary ataxias was conducted by a Norwegian neurologist, Håvard Skre, on the west coast of Norway in 1974 and 1975. He estimated, on clinical criteria, the prevalence of ADCA at 3.2/100 000, and ARCA at 3.0/100 000 (30;31). No studies have been performed since then, even though we today have molecular diagnostics and a new classification system for these disorders. Our neighbour countries have published data on SCA8 (Finland) and SCA6 (Sweden) (46;54;55).

Despite all these studies there are huge differences in prevalence of hereditary ataxias worldwide and the occurrence of the different forms. This is mainly due to differences in methods, inclusions criteria and founder effects. SCA8 in Finland, SCA3 in Portugal, AVED in Italy and northern Africa and ARSACS in Canada are all good examples of founder effects in these countries (25;55-58). No studies of the occurrences of the different forms existed from Norway before we started our work.

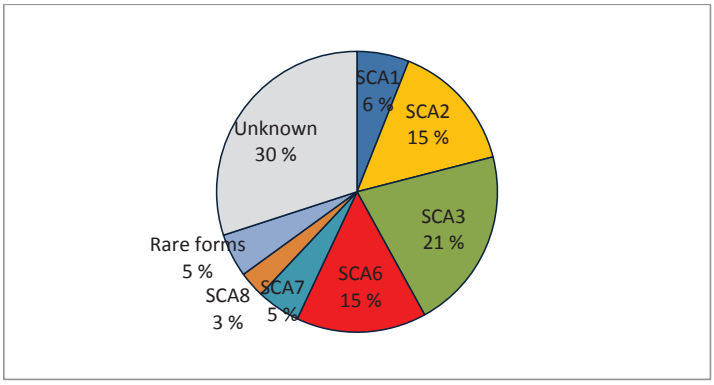
Table 4. *Prevalence studies previous to our study*

Author	Country	Published year	Population	Included diagnoses	All diagnoses	HA	ADCA	ARCA/FRDA
Leone	Italy	1990	3 617 915	FRDA	1.2	NA	NA	NA /1.2
Polo	Spain	1991	510 000	HSP,HA,spo	20.2	8.2	1.2	7.0/4.7
Filla	Italy	1992	335 211	HSP and HA	7.5	4.8	0	4.8/2.1
Hirayama	Japan	1994	123 000 000	HA,spo,MSA,HSP	4.53	1.5	NA	NA/0.1
Lopez-Arlandis	Spain	1995	3 898 241	FRDA	3.98**	NA	NA	3.98**
Silva	Portugal	1997	1 600 000	HSP,HA	6.4	4.4	0.8	2.4/2.4
Mori	Japan	2001	613 439	HA, spo,MSA,HSP	17.8	NA	3.36¤	NA/0
van de Warrenburg	Netherland	2002	15 863 950	ADCA	3.0*	NA	3.0*	NA
Zhao	Singapore	2002	3 500 000	ADCA	¤¤	NA	3.7	NA
Juvonen	Finland	2002	5 180 000	FRDA	***	NA	NA	***
Sasaki	Japan	2003	NA	HA,spo,MSA,HSP	15.68	NA	NA	NA/0
Zortea	Italy	2004	845 203	HA	9.33	9.3	2.4±	NA/0.6
Muzaimi	Wales	2004	570 000	HA, spo>20years	10.2	1.8	NA	NA
Infante	Spain	2005	527 000	ADCA	1.6	NA	1.6	NA
Tsuji	Japan	2007	NA	HA,spo,MSA,HSP	18.5	30.3%†	27.0%†	1.8%†

The table shows prevalence studies published from 1990 to 2007. Prevalence studies with a population less than 200 000 are not included in this table. *extrapolation, **Hardy Weinberg's principle, ***carrier frequency 1/500. 7 subjects/5.18 mill, ¤SCA1,3,6 and DRPLA, ¤¤1/27.000, ± SCA 1,2,3,6,7,8,12,17,DRPLA , †% of the prevalence for all included diagnosis

ADCA=autosomal dominant cerebellar ataxia, ARCA=autosomal recessive cerebellar ataxia, FRDA= Friedreich's ataxia, spo=sporadic ataxia, MSA=multi system atrophy, HSP=hereditary spastic paraparesis.

Figure 2. *Worldwide distribution of the most common SCA subtypes*



Genetic classification

Hereditary ataxias can be divided according to the mode of inheritance; dominant, recessive and X-linked (Fragile X premutations and X-linked sideroblastic anemia and ataxia, will not be discussed here) and further subdivided into the exact disease causing gene. The number of ARCA and ADCA is increasing every year and over 40 hereditary ataxias genes are already known.

Autosomal dominant cerebellar ataxias-ADCA

The dominant ataxias can be divided in two forms; the episodic forms and the progressive forms.

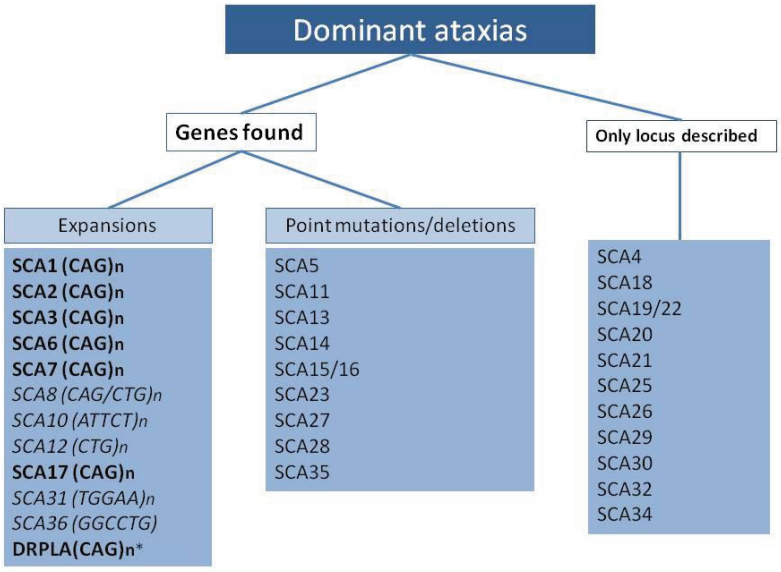
The progressive forms are characterized by cerebellar ataxia resulting in unsteady gait and often poor coordination of hands, legs, speech and eye movements. Additional signs and symptoms may occur, such as cognitive impairment, tremor, polyneuropathy, epilepsy and myoclonus. Thirty-three different genetic loci have been mapped to different forms of ADCA. They are referred as spinocerebellar ataxia (SCA) in the genetic nomenclature and given names chronologically from SCA1 found in 1993 (59) and up to SCA35 and 36 described in 2011 (60-63). DRPLA has never got a SCA number, but classified under the progressive ADCA.

Seven forms are described for the episodic forms and chronologically called episodic ataxias (EA) 1-7(10).

Spinocerebellar Ataxia –SCA and DRPLA

SCA1-36 and DRPLA comprise a wide spectrum of diseases with different genetic mechanisms causing the disorders (63). Trinucleotid expansions are the mechanism behind SCA1-3, 6, 7, 17 and DRPLA, also called polyglutamine expansions SCAs. More recently expansions in non-coding regions were found to be the genetic mechanism behind SCA8, 10, 12, 31 and 36. The third main genetic mechanism is conventional mutations, causing SCA5, 11, 13, 14, 15, 23, 27, 28 and 35. Figure 3 presents an overview (63;64). When the ITPR1gene was found, SCA15 and 16 appeared to be the same disorder which is today called SCA15/16 (65-67). The same is probably true for SCA19 and 22. The latter are located on the same chromosomal region, but the gene is not yet found (68). SCA24 proved to be a recessive disorder and is therefore withdrawn from the SCA list. SCA9 and SCA33 (63) is not yet published, but the numbers are reserved.

Figure 3. An overview of the different spinocerebellar ataxias (SCA) divided into different genetic groups



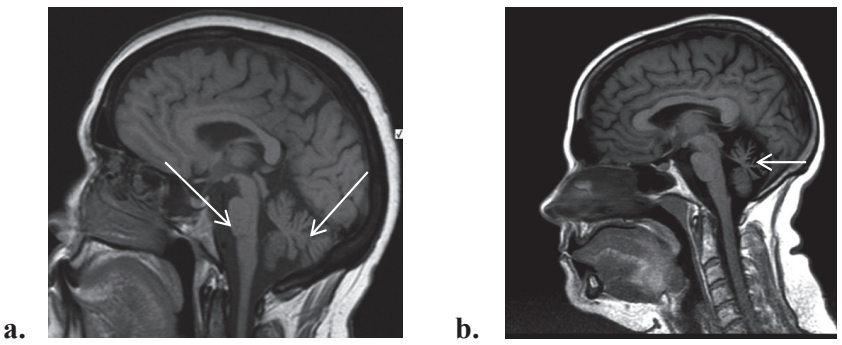
Data from August 2011, *without a SCA number. SCA9 and SCA33 are reserved, SCA24 is reclassified to SCAR4. Bold; Coding polyglutamine expansions, italic; non-coding expansions.

Polyglutamine expansion SCAs

These disorders (SCA1,2,3,7,17 and DRPLA) are characterized by variable degrees of degeneration and dysfunction of the cerebellum and the brainstem with variable age of onset, but as a main rule often onset in the third or fourth decades (4;6;12-15;21;69). These are neurodegenerative disorders leading to death by brainstem failure and can be referred to as ADCAI according to Harding's classification system (Table 1). Cerebral MRI most often reveals severe brainstem atrophy in addition to atrophy of the cerebellum (6) (Figure 4). Anticipation is observed in SCA with CAG trinucleotide repeats. Anticipation refers to earlier onset and increasing severity of the disease from generation to generation due to higher number of CAG repeats. Anticipation is severe in SCA7 trough paternal transmission and reported with infantile aggressive forms (70;71). These disorders manifest above a threshold of CAG repeats, which differs in each gene. It is difficult on clinical basis alone to give an exact diagnose because of locus heterogeneity, i.e., mutations in many different genes cause

the same phenotype. There are some clinical clues which may be of help in finding the genetic diagnosis given in Table 5 and in Appendix 1 (6;13;20;21;63;64). Gait difficulties are the main clinical feature in most of these patients, but double vision, dysarthria, impaired writing, episodic vertigo can precede the atactic gait in some patients. SCA6 differs from the other polyglutamine SCA in many respects. Clinically it is characterized by later age of onset, 5-6th decades, is often slowly progressive, episodic symptoms can be observed initially and the atrophy is mainly in the cerebellum. SCA6 is therefore often referred to as ADCAIII (Table 1). In SCA7 retinopathy in addition to the cerebellar signs is seen and therefore easy to divide from the other polyglutamine SCAs. SCA7 can be classified as ADCAII.

Figure 4 a. *SCA1 patient with cerebellar atrophy and thinning of the brainstem.* **b.** *Patient with SCA14 with vermian atrophy without other cerebral changes.*



Both patients from our cohort

Non-coding expansion SCAs

Untranslated repeat expansions causes gait ataxia in SCA8, 10, 12, 31 and 36 (61;64). They give all a milder phenotype with a wide range of onset compared to the coding expansion disorders and less brain stem involvement. SCA8 was the first published repeat expansion SCA in an untranslated region. It is characterized by repeat instability and with no correlation between repeat length and penetrance and the diagnose SCA8 remains therefore controversial (6;72). Many of these disorders seem less frequent compared to the coding expansion SCAs and the relative frequencies are yet not established (6;64). In Table 5 and Appendix 1 there are some more details about the disorders (6;25;63;73).

Conventional mutation or rearrangements SCAs

These disorders comprise SCA 4, 11,13,14,15,23,27,28 and 35. It seems that hereditary ataxias due to conventional mutations or rearrangements are less frequent than polyglutamine expansions, have a milder disease progression, often earlier age of onset, but with a wide range of disease onset and little or no brainstem involvement. These disorders typically show cerebellar atrophy in variable degrees on brain MRI (Figure 4). The disorders do not, as a main rule, influence the life expectancy. There are often lacks of phenotype-genotype correlations among these disorders and therefore often difficult on clinical grounds alone to give a certain SCA diagnose, but some symptoms and finding can differ and these are listed in Table 5 and Appendix 1 (6;25;63;73).

Episodic ataxias

Episodic ataxias (EA) are characterized by intermittent symptoms or episodes with cerebellar dysfunction (dysarthria, dizziness, ringing in the ears, unsteadiness) that can vary in duration, lasting from minutes to days with subtle symptoms/findings between attacks (cerebellar findings in EA2 and myotonia in EA1). They are also referred to as ion-channel disorders, because mutations in ion-channels or related genes are found (10;25;63). These disorders have a higher frequency of epilepsy and migraine. Attacks can be as frequent as several times a week, but may occur much less frequently. Precipitating causes are often attributed to; alcohol, caffeine, stress and physical exercise. On the other hand rest and sleep may contribute to reverse or to stop the attacks. The attacks may respond to antiepileptic drug, especially for episodic ataxia type 2. Acetazolamide should be tried when an episodic ataxia is suspected. EA can be difficult to diagnose, because symptoms and signs are absent or sparse between the attacks particularly in the beginning of the disease course.
EA1 and 2 are the most common forms, EA3-7 only published in few families. The mutations found in EA2 are in the same gene (CACNA1a) as found in SCA6 and in some forms of hemiplegic migraine.

Autosomal recessive cerebellar ataxias-ARCA

There are no good definitions for these disorders which comprise a huge group of neurodegenerative disorders affecting the cerebellum and its pathways and often also the peripheral nervous system. Many of these ataxias are rare and reports from few families are published. Some of these disorders are described with loci (63). The numbers of ARCA is

growing every year, but still the main causes of ARCA worldwide include Friedreich's ataxia, ataxia telangiectasia, AVED and ataxia with oculomotor apraxia type 1 and 2 (8). Non-coding expansions are the genetic mechanism behind Friedreich's ataxia, conventional mutations in the other described ARCAs. Most of the autosomal recessive ataxias have an early onset and as a main rule before the age of 20 years (Table 5), but with many exceptions (25;63). The typically neuropathologically findings are degeneration of the cerebellum and/or the dorsal column, brainstem and spinocerebellar long tracts. The symptoms and signs are unsteady gait, poor balance, coordination problems, dysarthria, dysphagia, pyramidal signs, polyneuropathy and/or postural tremor. Some forms have in addition to these symptoms other organ manifestations, such as diabetes mellitus, cardiomyopathy and scoliosis seen in FRDA. The most common ARCA are listed in Table 5 and described briefly in Appendix 2 (8;25;63;73-75).

Table 5. *Genetic classification of the different ADCA and ARCA with chromosome position, gene (when known), age of onset and main clinical symptoms and signs.*

NAME	***LOCUS/GENE***	***ONSET (YEARS)***	**SYMPTOMS AND SIGNS IN ADDITION TO THE ATAXIA**
Dominant			
*SCA1***	*6p23/**ATXN1***	*30-40*	*Pyramidal and extra-pyramidal signs, mild cognitive impairment. Brainstem atrophy. Early swallowing problems*
*SCA2***	*12q24/**ATXN2***	*20-40*	*Pyramidal signs, optic atrophy, chorea, dystonia. Slow eye movements. Brainstem atrophy*
*SCA3***	*14q32.12/**ATXN3***	*1-60*	*"Machado-Joseph disorder", pyramidal and extra-pyramidal signs, dementia, ophtalmoplegia, bulging eyes. Myokymias.Brainstem atrophy.*
SCA4	16q22.1	30-50	Extra-pyramidal signs, sensory neuropathy
SCA5	11q13/**spectrin-SPTBN2**	10-50	Pyramidal and bulbar signs
*SCA6***	*19p13/**CACNA1A***	*20-65*	*Sensory neuropathy, can be episodic in the beginning, same gene (see EA2)*
SCA7	*3p14.2/**ATXN7***	*1-45*	*Retina/macular degeneration. Pyramidal and extra pyramidal signs, brainstem atrophy*
SCA8	*13q21/**ATXN 8***	*18-65*	*Tremor, mild spasticity and sensory neuropathy*
SCA9	Reserved		
SCA10	*22q13.31/**ATXN10***	*14-44*	*Epilepsy and dementia*
SCA11	15q15.2/ **TTBK2**	20-40	Slight pyramidal signs
SCA12	*5q32/**PPP2R2B***	*8-55*	*Extra-pyramidal signs, tremor, dementia*
SCA13	19q13.33/**KCNC3**	10-60	Pyramidal signs, mental retardation
SCA14**	19q13.4/**PRKCG**	10-59	Slight extra pyramidal and pyramidal features, myoclonus, slow progression
SCA15/16	3p26 / **ITPR1**	Early	Hyperreflexia, tremor, slow progression.
SCA17	*6q27/**TATABP***	*19-45*	*Dystonia, bradykinesia, dementia, chorea like, brainstem atrophy*
SCA18	7q22-q32	10-30	Axonal sensory neuropathy, pyramidal signs, tremor
SCA19/22?	1p21-q21		Postural tremor, cognitive decline, myoclonus
SCA20	11p13-q11	19-64	Palatal tremor and myoclonus, tremor, nucleus dentatus calcification seen in some subjects on MRI of the brain, spasmodic cough, dysphonia
SCA21	7p21.3-p15.1	6-30	Postural tremor, extra-pyramidal signs, cognitive decline
SCA22/19?	1p21-q21	10-46	Mild ataxia with cognitive decline
SCA23	20p13/**PDYN**	43-56	Slow progression, neuropathy, dysarthria
SCA24-**reclassified**	1p36	20-30	Pyramidal signs, myoclonus, reduced joint sense, pes cavus. Renamed to; SCAR4-spinocerebellær ataxia recessive 4
SCA25	2p21-p15	1-39	Areflexia , many with sensory neuropathy, gastric problems in some subjects
SCA26	19p13.3	26-60	Mild, late onset, a Norwegian family published
SCA27**	*13q34/**FGF14***	15-20	Cognitive impairment, tremor exacerbated by physical exercise and emotional stress, dyskinesia, neuropathy
SCA28	*18p11.21/**AFG3L2***	12-36	Pyramidal signs, ptosis, opthtalmoparesis, early onset
SCA29	*3p26*	Early childhood	Non-progressive, some subjects with cognitive impairment, nystagmus
SCA30	4q34.3-q35.1	*~50*	Late onset, relatively pure ataxia
SCA31	*16q21-22/ **BEAN***	*20-72*	*Some subjects have sensory hearing loss, nystagmus*
SCA32	*7q32-q33*	*Variable*	Mental impairment, azospermia (infertile men)
SCA33	*No information*		
SCA34	6p12.3-q16.1	*Early onset*	Sparse information yet, erythrokeratodermia
SCA35	20p13/**TGM6**	~40	Dystonia, tremor in some subjects, pyramidal signs
SCA36	*20p13/**NOP56***	*43-58*	*Tongue fasciculations and atrophy, muscle faciculations and atrophy,*
DRPLA	*12p13/**DRPLA***	*10-70*	*Choreoathetose, dementia, myoclonus, epilepsy*

Episodic ataxia			
EA1	12p13.32/**KCNA1**	<10-20	Myokymia, hand, head and foot movements during attacks. Attacks lasting typically seconds-minutes
EA2**	19p13.2/ **CACNA1A**	<10	Nystagmus, tinnitus. Attacks followed by fatigue. Migraine. Attacks last often many minutes to hours.
EA3	1q42	30-60	Short-lasting attacks with vertigo, tinnitus, diplopia, nystagmus, myokymia
EA4	Probably the same as EA3	20-50	Headache, tinnitus, diplopia, vomiting, nausea, myokymia
EA5	2q23.3/**CACNB4**	20-60	Epilepsy, vertigo, attacks may last for hours. Nystagmus
EA6	5p13.2/**SLC1A3**	<10	Vertigo, diplopia, nausea, hemiplegic migraine, some headache headache, dysarthria
EA7	19q13	<20	Vertigo, weakness, dysarthria
Recessive			
*FRDA1***	*9q11-q13/**frataxin***	*1-60*	*Neuropathy, multi-organ disorder. Loss of position sense. Cerebellar symptoms are a late sign. The ataxia seen is mostly due to dorsal columns degeneration.*
FRDA2	9p23-p11	1-20	FRDA-like. Neuropathy, multi-organ disorder
AVED**	8q12.3/**α-tocopherolTP**	<20	FRDA-like. Areflexia, neuropathy, weakness, tilting of the head
A-T**	11q22.3/**ATM**	<3	Oculomotoric apraxia, multi-organ disorder, elevated α-foetoprotein, increased risk for cancers and infections
AOA1	9p13/**aprataxin**	1-6	Oculomotor apraxia, neuropathy, choreoathetose, hypoalbuminemia, hypercholesterolemia
AOA2/ SCAR1**	9q34/**senataxin**	10-25	Nystagmus, sensory neuropathy, oculomotor apraxia, elevated α-foetoprotein , chorea/dystonia and/or tremor
SCAN1	14q31/**TDP1**	13-15	Axonal sensori-motoric neuropathy, hypoalbuminemia, hypercholesterolemia. Muscle atrophy
ARCA1/SCAR8	6q/**SYNE1**	17-46	Pure, some with increased reflexes, slow progression, dysarthria.
ARSACS	13q12/**SACS**	5-15	Spastic ataxia, neuropathy, retinal striation in some subjects
ARCA2/SCAR9	1q44.2/**CABC1**	Childhood	Pure recessive ataxia with some pyramidal signs, cognitive decline reported in some subjects.
SCAE**	15q26.1/**POLG1**	Early childhood	Neuropathy, epilepsy, classified with mitochondrial disorders*
MSS	5q31/**SIL1**	Early childhood	Congenital cataract, weakness, myopathy, delayed psychomotor development, short stature, hypogonadism
PHARC**	20p11.21-1/**ABHD12**	Teenage	Hearing loss, polyneuropathy, retinitis pigmentosa, pyramidal signs, cataract

Ataxias with expansions are marked in Italic
DRPLA=dentatorubral-pallidoluysian atrophy, EA=episodic ataxia, FRDA=Friedreich's ataxia, AVED=ataxia with vitamin E deficiency, A-T=ataxia telangiectasia, AOA=ataxia with oculomotor apraxia, SCAN1=spinocerebellar ataxia plus neuropathy type 1, SCAE=spinocerebellar ataxia with epilepsy, ARSACS=autosomal recessive spastic ataxia Charlevoix-Saguenay, MSS=Marinesco-Sjøgrens syndrome, PHARC= polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract

For more clinical details for SCA1-32 and ARCA see appendix 1 and 2.

*Mitochondrial disorders not included in this table, see Table 2.

** To the author's knowledge found in Norway

Pathophysiology

Many pathophysiological mechanisms are found to cause hereditary ataxias (6;13;15;20;76-79) including altered gene expression, synaptic transmission and intracellular signaling pathways. Other pathways involved are altered mitochondrial function, RNA metabolism and phosphorylation-dependent intracellular signaling.

The cell mainly suffering from these alterations is the Purkinje cell in the cerebellum which degenerates. The inherited ataxias are often caused by mutations in seemingly unrelated genes causing either gain-of-function or loss-of-function of the protein. Molecular pathways that are essential for Purkinje cells function and survival are of particular importance.

Altered gene expression

In SCA1, 2,3,6,7 and DRPLA gain of function is one of the many mechanisms involved. In many of these disorders toxic accumulation of protein aggregates in the cell is found leading to cell death.

Chromatin remodeling as an altered transcription of proteins is seen as one of probably many pathophysiological mechanism in SCA7, 8 and FRDA. In SCA1 both gain and loss of function of the protein are seen altering RNA splicing and transcription. In addition accumulation of toxic proteins and altered levels of the β-III spectrin protein is found in SCA1 mouse models. In SCA6, mutations in a calcium channel unit are found. Probably the toxic gain of function mainly explains the pathology seen in SCA6 rather than the altered synaptic transmission also seen in this disorder.

Altered synaptic transmission and intracellular pathways

Many of the SCAs have mutations that give rise to altered synaptic transmission and intracellular signaling pathways. The Purkinje cells seem particularly vulnerable to altered levels of calcium and glutamate. Mutations in the inositol-triphosphate receptor seen in SCA15 seem to alter the output of calcium from the endoplasmatic reticulum and decrease the levels of calcium inside the cell. β-III spectrin protein stabilizes a glutamate transporter protein on the cell surface and mutations in the β-III spectrin gene seen in SCA5 decrease reuptake of glutamate from the synapsis and strengthens the glutaminergic signaling. In SCA6 accumulation of mutant calcium channels will influence the calcium transport in the cell membrane. Also mutations in other ion-channels proteins seem important for synaptic

transmission, for instance the mutations in potassium and sodium channel genes seen in SCA13 and SCA27.
The mechanisms mentioned in this chapter are not well understood and whether these pathways function independently or are interconnected remains to be further explored (80).

Genetic testing and how to diagnose hereditary ataxias

Diagnoses are based on the patients' medical history, family history and a complete neurological evaluation including a MRI scan of the brain (Figure 4), blood samples, neurophysiological investigations and if necessary lumbar puncture (5;8-20;25;45;81). It is much easier to give a hereditary ataxia diagnosis when a family history is established. In sporadic cases clinicians must carefully exclude all known secondary causes to a progressive ataxia. As a rule more than one family member should be examined because of the inter- and intrafamiliar heterogeneity in these disorders.

Molecular tests are now available for many types of hereditary ataxias (82). International guidelines are to test for SCA1, 2,3,6,7 and 8 in dominant cases because these genotypes represent over 50% of the affected families. It is also recommended to test for FRDA in recessive/isolated cases (6;8;9). It is further recommended to test for the different recessive forms when having positive biomarkers (see discussion) or if the phenotype is compatible with the genotype according to the neurological examination and the supplementary investigations (blood samples, MRI of the brain and medulla and neurography). As a main rule ARCA or another recessive disorder with an atypical phenotype are more likely than idiopathic sporadic ataxia when onset occurs before the age of 20 years (8;81).

Aims of the study

The primary aims of this study were:

1. To find the prevalence of HA in a defined population
2. To find the occurrence of known described phenotypes and genotypes in this population
3. Explore the Norwegian ataxia population and investigate carefully selected phenotypes and determine the genotypes
4. The ultimate goal was to identify a new phenotype in the Norwegian ataxia population and find a new gene/locus explaining this phenotype.

The secondary aims of this study were:

1. Propose rational diagnostic and follow-up strategies, based on the prevalence and occurrences of each form.

Material and methods

Patients and control material

To identify all patients with hereditary ataxia we used several search strategies:

1. We established a tight collaboration with colleagues in hospitals and departments all over southeast Norway (genetic, pediatric and neurological departments)
2. We searched in hospital journal archives back to 1992
3. We had a tight collaboration with the patients' association (NASPA)
4. A detailed family history was taken in every index patient. We investigated as many affected members as possible in each family

In the prevalence study the STROBE-statement checklist (Supplement 1) was used in order to get as reliable data as possible and to report our results in an informative way (83).

Inclusion and examination of the patients

All adult patients included were examined by Jeanette Koht and /or Chantal Tallaksen and Anne Kjersti Erichsen according to two standard clinical charts established by the SPATAX network (Supplement 2 and 3) (84). All clinical data were systematically registered in a database. Symptoms and signs were described in details after clinical investigations for each patient and all supplementary investigations were stored in the same database and in binders. Supplementary investigations including cerebral MRI were performed in as many as possible in each family. The hereditary ataxia diagnosis was in some families easy to make due to family history and typical symptoms. In other patients painstaking supplementary work was done. All index patients were investigated following updated diagnostic criteria in order to exclude wrong diagnosis and include correct patients (5;6;8;9;11-15;17-21;23;51;57). Patients were diagnosed with hereditary ataxia if they fulfilled the inclusion and exclusion criteria in Table 6.

Registration of the patients and controls

Each index subject was registered in the research database using the Excel 2003 software. All investigators filled in clinical data continuously into this database. The database included the standardized clinical sheets of the patient, geographical origin, consanguinity, the pedigree and additional clinical information such as results from supplementary investigations (MRI, nerve conduction examinations and blood samples). All index subjects were categorized into one of the four groups; autosomal recessive, dominant, X-linked trace or sporadic forms. The latter subjects were not included in this work. Whenever it was necessary to do statistical calculations the data was transferred to the SPSS 16.0 software.

The prevalence date was set to February 1, 2008 and the database was at that time frozen for calculations for the prevalence study, but the inclusion of patients was at the same time ongoing the whole study period and therefore more ADCA families were included in Paper 3.

Table 6. *Diagnostic criteria for hereditary ataxia in our study*

Inclusion criteria (1,2,3 or 1, 2 or 1, 3):	Exclusion criteria:
1. Progressive cerebellar ataxia	1. Secondary ataxias
2. Other family members with HA	2. Sporadic ataxias
3. Verified molecular diagnose	a) no family members affected
	b) no verified molecular diagnose

Collection of DNA

A blood sample was collected from each index subject and all available and informative subjects in the families included for further linkage studies. DNA was extracted from leucocytes from a blood sample using standard procedure at the Department of Medical Genetics, Ullevål.

Selection of families

Families were included for further linkage studies if the theoretically LOD score was >3. All index subjects with dominant trace were enrolled in the study for diagnosing newly known genotypes (Paper 3).

Molecular methods

To give patients exact genetic diagnoses and find new genes/seldom genotypes different strategies were used;

1) Sequencing already known genes

2) Sequencing already known, but seldom genes using a combination of candidate gene approach and linkage study with microsatellites flanking the region(s) containing the gene/genes
3) Whole genome linkage study in order to find new genes/locus

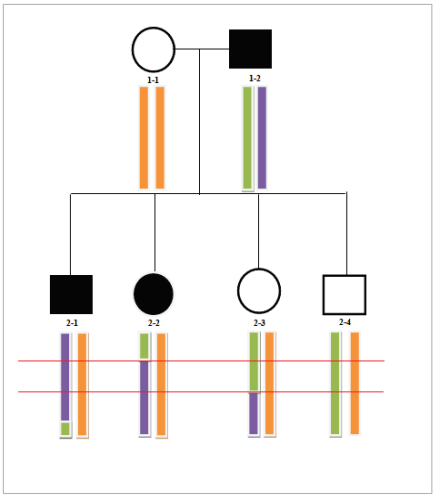
After Paper 1 we had included 171 subjects/87 families with HA, of these 111 subjects/48 families with ADCA. Many of these families were small, consisting of only 1-2 affected subjects alive.

Families with more than 6 affected available for investigations and blood samples were considered as candidates for linkage analyses. Power analyses with the simulation program fastS-LINK of the EASY linkage software program were used (85). After these analyses we choose three ADCA families with different phenotypes for further linkage studies.

Linkage analysis is based on identifying chromosomal regions co-segregating with the disease in the family and the identified region is expected to harbor the disease causing mutation (86). The further apart two loci are on the same chromosome the more likely it is that recombination has happened. Chromosomal regions are broken up during the meiosis and recombination information is utilized in linkage studies. A linkage analysis of the whole genome can identify loci that show evidence of containing the disease causing gene. The principle of linkage study with recombination/crossover events is shown in Figure 5 for one chromosome in a small autosomal dominant family, as seen in the figure a part of the chromosome (often referred to as the shared haplotype) are inherited together as a block. Genetic markers are used to genotype individuals (making a chromosome map) and utilized when performing linkage studies. Microsatellites and SNP (single nucleotide polymorphism) are today commonly used to genotype individuals and were used in Paper 3 and 4. A microsatellite marker (simple tandem repeat polymorphism) is made of repeats of two, three or four base pairs and varies in length between individuals. On one chromosome, there might be six repeats (CACACACACACA), while on the other chromosome there might be ten (CA_{10}). Microsatellite markers are easy to genotype, have multiple of alleles and they occur almost everywhere across the chromosomes. The principle of genotyping microsatellites is based on analyzing the size of the fragments amplified with primers flanking the microsatellites. The size of the amplification product varies depending on the number of repetitions in each specific allele. The analysis in this work was carried out on a sequencer

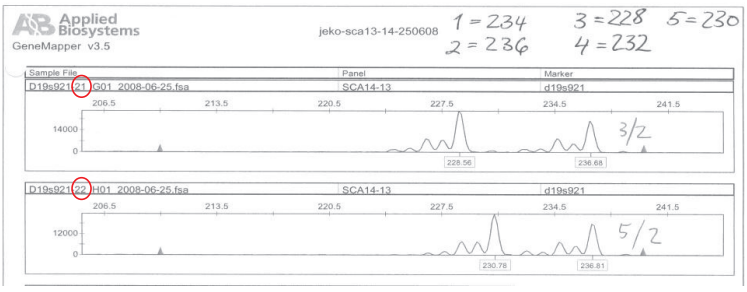
ABI PRISM 3730 Genetic Analyzer (Applied Biosystem) which measured the migration of the DNA fragments in a capillary loaded polymer and then analyzed by the software Gene-Mapper 4.0 (Applied Biosystem). The results are presented as peaks, with the base pairs (bp) numbers at the x-axis and the fluorescence intensity in the y-axis (Figure 6).

Figure 5. *An example of cross-over events (recombinations) in an autosomal dominant family*



Square=male, circle=female. Filled=affected, Clear=unaffected individuals. In the pedigree the affected siblings share the violet haplotype. The green haplotype has to be healthy due to the healthy brother 2-4. Sibling 2-3 has a part of the violet haplotype and a recombination has occurred. This means that the mutated gene is more likely located between the two red lines marked.

Figure 6. *Example from the genotyping with microsatellites. D19s921was one of many genotyped markers used in Paper 3.*



Subject 21 is heterozygous for the alleles with the sizes 228 bp and 236bp, translated to 3/2 which makes it easier read. Subject 22 is heterozygous for the alleles with the sizes 230 bp and 236 bp, translated to 5/2.

The SNP markers have their variations at a single base on the chromosome, i.e. there can be only three different combinations, for instant; AA, AT, TT. Enough SNP's have been identified to allow a very dense coverage of the entire genome and SNP's genotyping was therefore preferred in the whole genome genotyping in our families.
LOD score is used to prove evidence for linkage. LOD score means the "logarithm of the odds" and is a statistical estimate of the likelihood of a linkage between two loci. The evidence of linkage in dominant disorders is normally set to LOD score >3 as we did in our families. A LOD score of 3 means that the odds are a thousand to one in favor of genetic linkage. If however the region is broken up during meiosis in many of the affected it less likely that the disease causing gene is in this region. Gene linkage is excluded if the LOD score is less than -2.
Before selecting a family we performed a thorough examination of all participating members to find as many affected as possible in the families, in order to increase the chances to find the linked region and the gene.

Paper 1

The mutation analyses for SCA1, 2, 3, 6 and FRDA for the prevalence study were done as a standard diagnostic test at OUS, Ullevål and Haukeland Hospital, Medical Genetic department. The SCA8 analysis performed in selected ADCA families was done by Jørgen E. Nielsen, Department of Medical Genetics, Panum Institute, København as part of a collaborative work.

Paper 2

The mutation analysis was done at the Department of Human Genetics, Nijmegen Medical Centre in The Netherlands as a standard diagnostic test.

Paper 3

Genetic linkage analysis with candidate gene approach was a good method to use before starting the time consuming sequencing step. Linkage to the chromosome 19q13.4 region was therefore tested in dominant families who had tested negative for mutations in the SCA1, 2,3,6,7 and 8 genes and who were informative for linkage studies. The families were investigated with microsatellites flanking the SCA13 and 14 genes and haplotypes were then manually drawn. Selected exons in the SCA14 gene were sequenced in two families and all index cases in our ADCA cohort at that time.

Microsatellites primers were designed with Primer3 software program (87). Exons 1, 2, 3, 4, 5 10 and 18 were amplified by PCR in an Abi-Veriti apparatus using the primers and conditions mentioned in Table 7. The standard set-up for the PCR reaction used in these studies is shown in Figure 7. PCR amplified fragments were purified with exonuclease 1 and phosphatase and sequenced in an ABI automated sequencer in the forward and reverse direction with the PCR primers and with a BigDye kit (Applied Biosystem) (88). Segregation of the mutation was verified by direct sequencing in all family members were DNA were available. The mutation was supported by LOD scores.

In addition 576 healthy control chromosomes from Norway, France and North Africa were screened for the same mutation. The identified missense mutation was also checked in a bioinformatic program (Polyphen).

Paper 4

Two families with a theoretically measured LOD score>3 was chosen for SNP microarray genotyping followed by linkage analyses. One of these families is described in Paper 4, the other family is not yet published. In this paper ten family members were genotyped with 6090 SNP markers using the Illumina Gene Chip Human Mapping array at the platform situated at the Hospital Salpêtrière. The markers in this array are spanning all chromosomes with an approximately average genetically distance at 0.58 cM and an average physical distance at 441 kb. Standard protocol supplied by the manufacturer was followed. The procedure was done by Wassila Carpentier at P3S plateforme, Pitié-Salpêtrière Hôpital, Paris.

PedCheck program was used to detect the pedigree file for Mendelian inconsistencies in the pedigree data (89). Linkage analyses were performed using the Merlin 1.0 Software package (90) and the Allegro package (91). For the disease allele the penetrance was set at 0.8 and with a gene frequency at 0.00005 in the general population.

To confirm linkage to the regions with positive LOD scores additional heterozygous microsatellite markers were used and primers were designed with Primer3 software program (87) covering the possible linked regions, Table 8. Haplotypes were drawn manually for all loci.

The INPP5gene, (coding regions, UTRs and splice site junctions) was sequenced by Genoscreen in Lille in France in an automatic ABI3730 sequencer (Applied Biosystems).

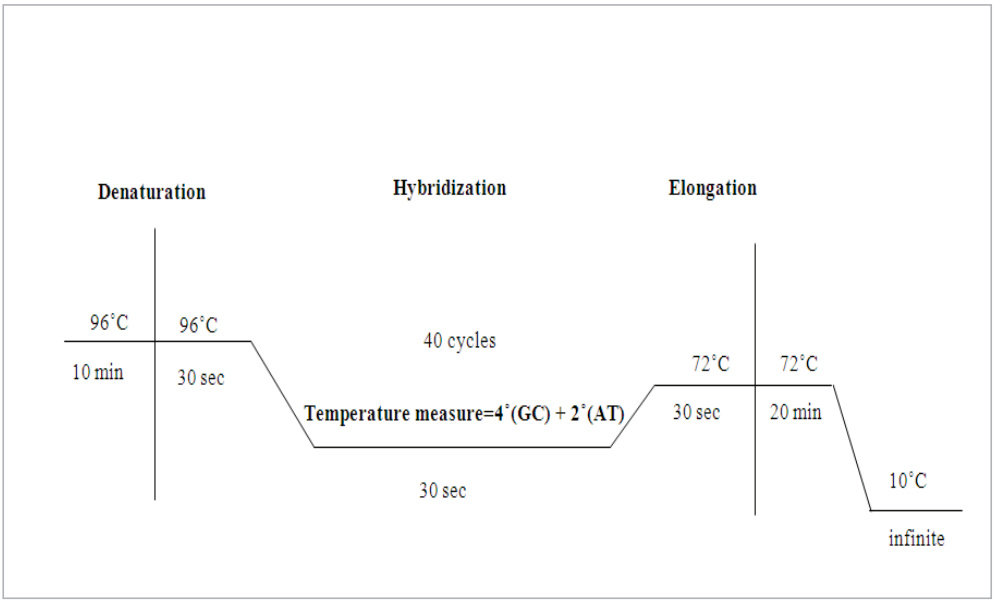
Table 7. *Primer pairs and annealing temperatures used for amplification and direct sequencing selected exons in the PRKCG gene (Paper 3).*

Exon number	Annealing temperature	Primer forward	Primer reverse
EXON 1	72-62°C	CGCCCTCTCGGTCGTCCTG	AGCCCCCTCCTTCTTCCTTC
EXON 2	65-55°C	GGGTCTGAAGGAGGAAGAAA	CAAATATCTGTCCCCACCTG
EXON 3	58°C	CGCTCTCTCTTTCCAATTTT	GAGGAGGAGAACCAGGTGT
EXON 4	62°C	GAGAGCAAGGCAGGAGGAAA	CCCCAAGCCAAGACTCCA
EXON 5	60°C	GCATGAAATGCTCCTGTGAC	GGCGTGACCATAGAAAGAGG
EXON 10	60°C	GTGGCCATTTTCCTCTGTCT	ACCTCTCCTCTCCAGGAACC
EXON 18	58°C	GTGCAGACACCATGAAGCAT	GGGAGGCACAGAACTACCAA

Table 8. *Primer pairs, annealing temperatures and positions for the microsatellites used to confirm linkage to chromosome 10 in Paper 4*

Marker	Heterozygousity	Position	cM	Temp.°C	Primer forward	Primer reverse
D10S1676	0.67	129896120	159.78	55	CCACCACTCAGAGGTAAGG	AATTGTATGATCCCAACTTTG
D10S1248	0.76	130982451	166.09	55	TCTCTTTTTCCCTTGTCTTG	TCTCTTTTTCCCTTGTCTTG
VNTR14GT	Unknown	132200000	?	55	GCTTGTTGTGAGGCAGATGA	TCTTGGGTGTCCATGGTAGG
D10S169	0.71	132411477	169.48	55	AAGGTATAACGAGGCAGTCC	GACAGGGATCACTTTTCAGA
D10S1770	0.88	132575634	170.04	55	CACTATCCCTTCATCCATCC	ATCCATCAGCTCTCCAGTCG
D101675	0,71	133428283	172.22	55	GAAGGAGCTACCACTGTGAG	GTGCTGTTTGTGCATGTTAT
D10S590	0.59	133497512	172.22	55	GTGGTCTCTGCCACACCTA	GCACAGTCTGCTGCATCTC
D10S212	0.66	134299489	174.19	55	GAAGTAAAGCAAGTTCTATCCACG	TCTGTGTACGTTGAAAATCCC
D10S1700	0.72	135026673	175.02	55	TGTGTTGGAGTAGGACGTGA	ACAGATGCGTGTACAGATGC

Figure 7. *The principle for Polymerase chain reaction (PCR) for the DNA amplification*



Statistical methods

In Paper 1 the Fleiss's method was used to calculate age, sex and county-specific rates (92) and the Poisson model (93) was used to estimate the prevalence in the different counties. Before starting the study we performed a power analysis in order to capture the real prevalence of these disorders.

In addition descriptive statistics were performed in all papers.

Results

For the prevalence study (Paper 1) 171 patients with hereditary ataxia were included, 365 patients all together (including HSP), the prevalence day was set at 1.February 2008. All later papers were based on this study and the families found in the prevalence study were then further explored for the genetic studies (Paper 2, 3 and 4).

Paper 1

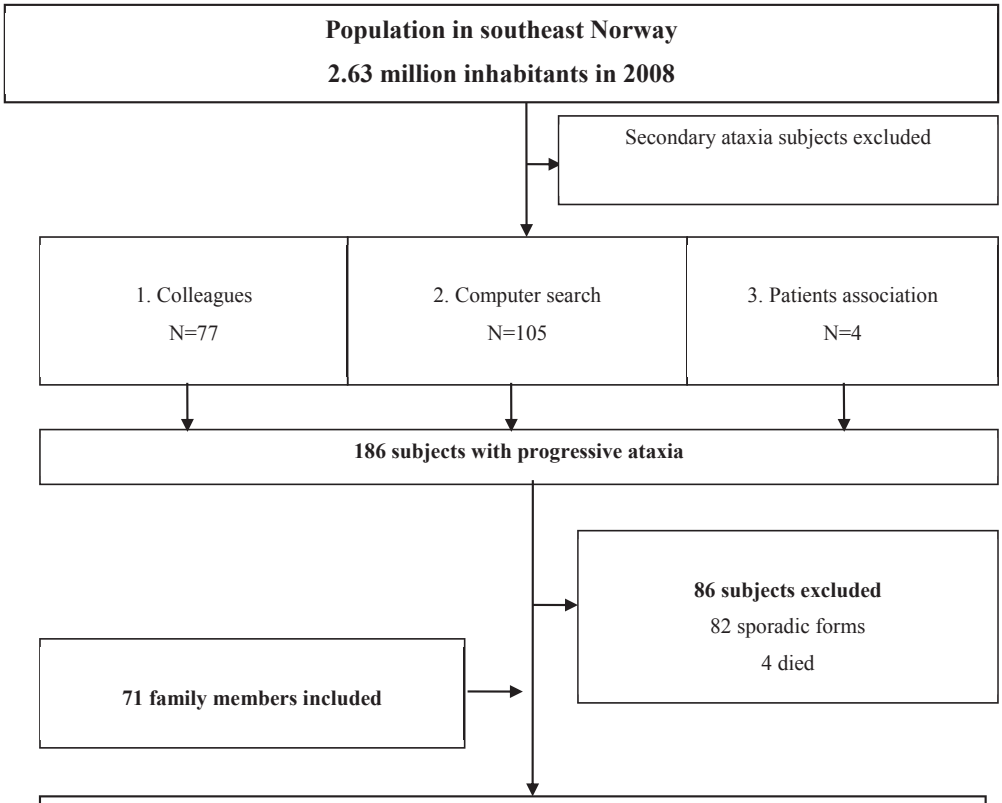
All collected patients are shown in Figure 8 with prevalence estimations for ARCA and ADCA and distribution of patients according to ages. The prevalence increased with age, with the highest prevalence in the age group over 60 years. Clinical data is given in Table 9. Thirteen small dominant families (21 subjects) had a pure phenotype with late disease onset (>40 years). Twenty-four of the 111 subjects were of non-Norwegian origin. Very few families with the well-known SCA1, 2, 3, 6 and 7 were found. Only 8% of the families in the ADCA group were given a genetic diagnosis and 46% among recessive families (Figure 9). SCA3 was the only dominant genetic disorder among dominant Norwegian families. A-T was the most common recessive ataxia form and FRDA was found in surprisingly few recessive subjects. Both AOA2 (74) and AVED (Paper 2) were diagnosed among Norwegian ARCA patients.

Table 9. *Clinical data at prevalence day, 1.February 2008.*

	ADCA	ARCA
Mean age at disease onset (range), years	32 (1-72)	9 (1-55)
Mean age at prevalence day (range), years	57 (13-94)	32 (4-71)
Percentages of the patients using wheelchair	6.3	40.7

ADCA-autosomal dominant cerebellar ataxia, ARCA-autosomal recessive cerebellar ataxia

Figure 8. *Flowchart of all included and excluded ataxia subjects and distribution of ADCA and ARCA subjects by ages.*



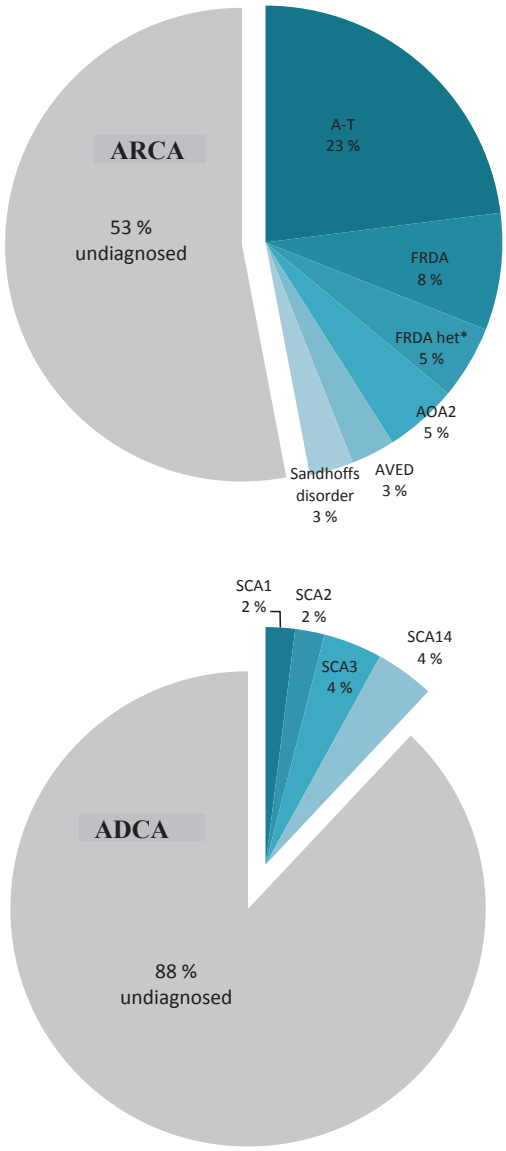
171 subjects with HA included

ADCA prevalence 4.27/100 000 (111 subjects/48 families)

ARCA prevalence 2.27/100 000 (60 subjects/39 families)

	ADCA subjects	ARCA subjects
0-19 years	3	25
20-39 years	15	11
40-59 years	36	16
> 60 years	57	8
Total	**111**	**60**

Figure 9. *ARCA and ADCA occurrences in our HA cohort in 2008. Percentage of the families with the different genetic diagnosis*



FRDA het*- patients with heterozygous mutation in one allele of the Frataxin gene, but with a FRDA-like phenotype. ADCA-autosomal dominant cerebellar ataxia, ARCA-autosomal recessive cerebellar ataxia

Paper 2

In Paper 2 we reported the first genetically confirmed subject in Norway due to mutations in the α-tocopherol transport protein gene resulting in vitamin E deficiency. Through the systematic population-based study of hereditary ataxia in southeast Norway one patient with ataxia with vitamin E deficiency (AVED) was identified among the 39 recessive families and 82 sporadic subjects included.

The subject was a 45 years old woman with progressive ataxia from preschool age. She was given a diagnosis of FRDA in the early eighties after clinical examination before genetic tests were available. At the age of 45 re-evaluation and re-examination were performed as part of the project and vitamin E analysis and genetic analysis were done. Vitamin E in serum was undetectable and genetic analysis detected a compound heterozygous mutation, A120T and R134X, in the α-tocopherol transport protein gene on chromosome 8q13. Clinically the patient had a FRDA phenotype with truncal and extremities ataxia, pes cavus, inverted plantar response, loss of proprioceptive and vibration sense and a severe sensory neuropathy. In addition she had titubation of the head typically seen in AVED. SARA score was 30 out of 40 points.

Optimal treatment with vitamin E supplementation was started.

Paper 3

In this paper we reported a new mutation in the PRKCG gene and the first two Scandinavian families with SCA14. This new mutation causes a mild, slowly progressive ataxia with pyramidal signs in the affected family members.

Genetic studies demonstrated linkage to the markers covering the SCA14 gene with a maximal bi-point LOD score of 3.21 at theta=0 for marker *D19S921* in family 1 and not to the SCA13 gene in one family (Figure 10). Haplotype analysis identified shared alleles at 3 microsatellite markers for these two families in the chromosomal region surrounding the SCA14 gene, suggesting a shared ancestral chromosome. These families were from the same small geographically region in Norway. Direct sequencing revealed a novel C to A missense mutation at position 417 in exon 5 in the PRKCG gene (Figure 11). The wild type amino-acid was in a highly conserved region in the gene and was perfectly conserved in all mammals and in other protein kinases and was predicted with in silico analysis to be damaging to the protein stability (Polyphen). The mutation co-segregated with the affected family members. The mutation was absent in 576 control chromosomes. One healthy family member (53 years)

carried the mutation, indicating incomplete penetrance at this age. Mutations in the PRKCG gene explain only 3.5% of the families in our ADCA cohort.

Figure 10. *SCA14 family linked to the SCA14 gene. The shared haplotype is marked with orange colour.*

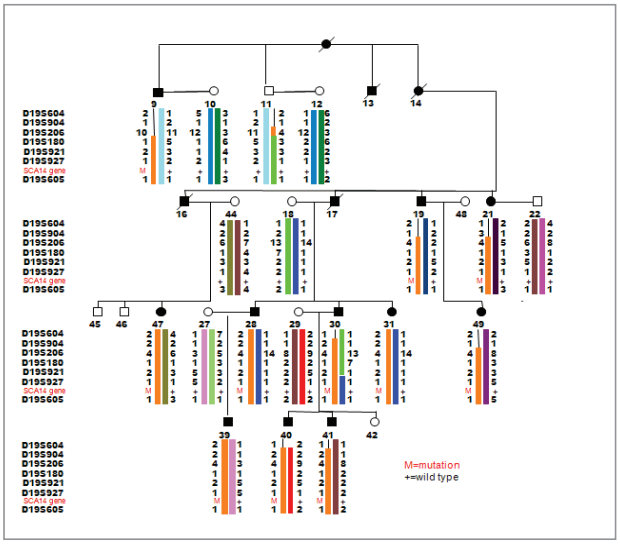
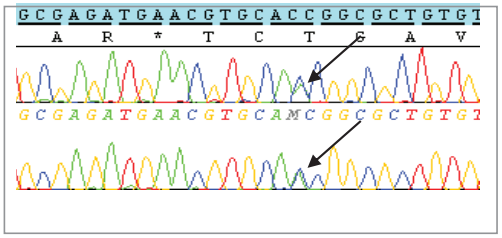


Figure 11. *Sequence chromatograms for portions of the PRKCG exon 5 (forward and reverse), showing the heterozygous mutation in one affected family member, changing a C to an A*



Paper 4

In Paper 4 we reported a very late onset autosomal dominant ataxia linked to a new ataxia locus on chromosome 10q. Twenty-four family members were included. Mean age of onset in the seven affected subjects was 62 years (range 60-68). All affected had mild to severe gait

unsteadiness and limb incoordination that progressed slowly over years with periodic dizziness. All had saccadic pursuit observed at eye examination. The whole genome scan identified one chromosome region with a multipoint LOD score > 2. There were no linkages to already known autosomal dominant ataxia loci. The most suggestive evidence of linkage was obtained to markers on chromosome 10 with a maximal multipoint LOD score (Merlin) of 2.67 between SNP markers rs570967 and rs880340 at position 131311320-134903113bp. Genotyping additional microsatellite markers for all family members available verified linkage and generated a maximal two-point LOD score of 3.1 to this region.

General Discussion

In our work we 1) report the prevalence of HA and occurrences of known genotypes 2) describe new genotypes in Norway and 3) present a new ADCA linked to a new locus.

Epidemiology

Håvard Skre did the first epidemiological study on hereditary ataxias in the seventies. With new genetic knowledge and improved epidemiologic methods we wished to contribute to new and reliable data on these disorders. Southeast Norway was well suited to do a cross-sectional population-based study because; 1) the population size was large enough 2) the population was stable and had a long average life expectancy 3) in the region we had good cooperation with the clinicians and 4) few private clinics and few genetic laboratories served genetic diseases. These factors made the sampling manageable and reliable.

In accordance to the power analysis performed in our study, a population size of at least 1 394 805 was required to estimate a precise prevalence. Many previous studies (Table 4) were conducted on small populations. Estimating the prevalence in a small population can bias the results. The prevalence estimation in the different counties in our study illustrates this well, because the prevalence ranged from 3.7 to 16.2/100 000.

One limitation of the prevalence study was the possibility to miss hereditary forms hiding among the sporadic forms which we excluded. Patients with ARCA are therefore likely missed if no biomarkers or diagnostic tests were available and if only one subject in a family was affected. Also mild phenotypes were possible to miss, because patients may not be aware of the family disorder or they may not be followed up by a neurologist. In order to try to find all subjects with hereditary ataxia in southeast Norway we searched back to 1992 in the journals archives and had a tight collaboration with clinicians in our region and investigated as many family members as possible in each family. We were very strict with the inclusion criteria and if there was doubt about the patient's diagnosis he/she was not included. There are many different disorders giving ataxia (Table 2). Sometimes it was difficult to differentiate ataxia from other neurodegenerative diseases, especially among children, and they were therefore not included. By choosing strict inclusion criteria and employing molecular testing

and biomarkers, we believed that we have avoided the inclusion of subjects with other neurodegenerative diseases in our study.
Our prevalence estimation at 6.5/100 000 is therefore likely an underestimation rather than an overestimation of the true prevalence in the region.

There are studies with higher prevalence estimation compared to our study. This is mainly due to different inclusion criteria. The study conducted in Japan (39) estimated a prevalence as high as 17.8/1000, because both HSP and sporadic subjects were included. If we include sporadic subjects (82 subjects) in our estimated prevalence study, our prevalence rises to 9.6/100 000 if we also add HSP and sporadic spastic paraparesis the prevalence estimation rises to 17/100 000 and this is almost the same as the highest prevalence reported for these disorders. These examples also show that it is difficult to compare the different studies done in different areas and countries.
In Paper 1 we showed a surprisingly high prevalence of ADCA, a high proportion of mild disease, a high prevalence of A-T and a high proportion of patients with undefined genetic diagnosis. Compared to other European countries where over 50% of the dominant ataxias are diagnosed after testing for SCA1,2,3,6 and 7 (6) and ~1/3 of the recessive ataxias is diagnosed with FRDA (81) we had a population with a high percentage of genetically undiagnosed subjects and a different occurrence of SCA1, 2, 3, 6 and 7 compared to other European countries. If we include the families explored in Paper 3 we still did not have exact genetic diagnosis to more than ~10% of our ADCA families (Figures 2 and 9).
As expected the prevalence increased with age and was highest among those over 60 years of age and in subjects with ADCA. Many small families with late onset and pure forms were without a genetic diagnose, this phenotype was explored in Paper 4. We reported also a lower prevalence of ARCA in older age groups, as expected because of the shorter life expectancy, mostly due to the high proportion of A-T among our ARCA families (8;94).

Genotypes and phenotypes in the Norwegian cohort

The database with spinocerebellar degenerative disorders consists in June 2011 of 564 patients (346 families) of which 287 patients have sporadic and hereditary ataxia, 40 families with ARCA and 60 families with ADCA.
Locus heterogeneity among the hereditary ataxias is a huge challenge when diagnosing patients. It was therefore difficult after the clinical examination to say which genotype the

family had. We started therefore to screen the population for the well-known genotypes. Linkage analyses were performed in selected families, with markers flanking known genes of interest or the whole genome in order to find the genotype or to find new genes.

Dominant families

Figure 12 shows all genotypes and phenotypes we have found in the Norwegian ADCA cohort until June 2011.

Phenotypes

Mild pure forms

We have 18 families with a mild phenotype in the cohort and the new locus on chromosome 10 (Paper 4) can explain one of these families. Seventeen of these families were uninformative for linkage studies, without mutations in the well-known genes and therefore leaving them without and exact genetic diagnosis. These families are not tested for mutations in all known SCA genes and it could have been of interest to test them for SCA11, 15, 28 and 31 which all are described with relatively pure phenotypes. There are occurrence studies published on 11, 15 and 28 (67;95;96), but these forms does not explain more than a few percentages of the families in these cohorts. SCA30 (gene not found) and 31 are described as pure forms and published in very few families as is with many of the SCA due to conventional mutations (6). Due to the high expenses and the time required to sequence these genes it was not possible to perform molecular analyses for the SCA11, 15, 28 and 31genes in the scope of this work. In addition it is not considered as part of a routine testing (9).

Also SCA26 is described with a pure phenotype and of particularly interest, because the family linked to this locus was originally from Norway (97). The gene is not yet found. DNA from selected families from this study has been sent to the group in USA, but no matches have yet been found.

17 families (30% of the families in the cohort), not informative for linkage analyses seem to have a very similar phenotype as the family explored in Paper 4. It will therefore be of interest to find the gene in the linked family and further explore if we have a founder mutation in Norway giving this new phenotype.

Episodic forms

Ten families reported episodic symptoms and were classified into this group. Two families had an EA2 phenotype, but tested negative for mutations in the CACNA1A gene. There are today many genes causing episodic ataxia and also SCA6, which is allelic to EA2, can give rise to episodic symptoms in the beginning of the disease. Vertigo previous to the development of the ataxia is often seen (98). We have not found any families with expansions in the SCA6 gene in our ADCA cohort. Many of these families have benefit from medication (Diamox). In our cohort many family members had symptoms in many years before they were diagnosed. We believe the number of episodic ataxia is underestimated in our study due to the diffuse symptoms, especially in the beginning of the disease.

Ataxia with tremor

Six families with autosomal dominant inheritance had cerebellar symptoms and a disabling intension and action tremor. One family with early onset and tremor/ataxia with pyramidal signs was included for further linkage studies. This is a new phenotype not linked to any known ADCA genes, but linked to a new locus on chromosome 3 (unpublished data). Tremor is reported also as an additional feature in families with SCA12, but most of the families reported are from Asia (99) and SCA12 seems to be a rare cause of hereditary ataxia. The families uninformative for linkage are not tested for mutations in this gene and we may have some SCA12, even though this seems to be a rare cause.

Fragile X tremor/ataxia syndrome with an expanded trinucleotide repeat in the FMR1 gene (55-200 CCG expansions) can give rise to tremor and ataxia symptoms, but this disorder is X-linked, not autosomal dominant as seen in our families, with a high degree of anticipation (giving rise to Fragile X syndrome), men are more often affected than woman, typical MRI changes can be seen as also Parkinson-like features and peripheral polyneuropathy (100). None of the families had this phenotype and this genotype may not explain the tremor/ ataxia symptoms among these families. We performed genetic testing in most families, but they tested all negative for mutations in the FMR1 gene. We have seen patients with Fragile X pre-mutations during our work, but they appear to be classified among tremor patients rather than ataxia in Norway. No Fragile X pre-mutations were found during our prevalence study. We might have found a few more families using tremor diagnoses when searching in journal archives in hospitals.

Other ataxia phenotypes

There are families with miscellaneous phenotypes, but all families were uninformative for linkage studies. This group was therefore not further explored in this thesis, interestingly the family with SCA4 (101), is originally from Scandinavia. The phenotype seems similar to some of the families in this cohort.

Genotypes

In monogenic diseases one mutation in a single gene alone is sufficient to cause the disease and the main goal will be to find as many as possible of the genes explaining these disorders. Linkage analysis still has a role in finding new genes, even though faster, parallel methods for sequencing are taking over for SNP genotyping (62).

The linkage analysis showed evidence for linkage to markers on chromosome 10 in Paper 4. We found genetically confirmed SCA families in the Norwegian ADCA cohort (Figure 9), even though the number was small, two families with SCA14 with the same mutation, both families from the same region in Norway with a probably common ancestor were reported in Paper 3. Also two SCA3 families from Norway were investigated, but one of these has its ancestor from Portugal. The other SCA3 family did not know about any ancestors from outside Norway. The other SCA families were all from outside Norway. In Paper 3 only selected exons were sequenced and therefore still some families in our cohort may have undiagnosed SCA14. According to earlier papers on SCA14 (102;103) we decided not to sequence all exons in this gene. It is time-consuming to sequence exon by exon and also expensive and other tasks were therefore prioritized for the time I was working in the laboratory. The occurrence of SCA14 in our cohort was very close to what have been published earlier. We therefore believe the missed subjects are few. A drawback in our work is of course that we did not sequence all genes causing hereditary ataxia. The growing number of SCA makes it even more difficult to know where to start. We have through our work searched among the most frequent SCAs (SCA1,2,3,6,7 and 8) and SCA14, but still after this work ~ 90% of the ADCA families are without an exact diagnosis.

We did a huge effort to find the gene in Paper 4, but even though we sequenced a very good candidate gene in this paper and performed RNA analysis (not published) on the INPP5A gene, 29 more genes in these regions are not explored. The naturally next step is to perform faster parallel sequencing methods and hopefully find the disease causing mutation in this family.

Recessive families

Figure 13 shows all genotypes and phenotypes we have found in the Norwegian ARCA cohort until June 2011.

Phenotypes

Among the families in the recessive group 54% of the families are left without an exact diagnose. Spastic ataxia seems to be a homozygous group among the Norwegian families. Ten families had spastic ataxia with onset before 25 years. We have during our work tested selected families for ARSACS, but all families were negative for mutations in this huge gene (unpublished data, genetic work done by S.Vermeer in Nijmegen Medical Centre, the Netherlands, personal communication). No other genes are reported with this genotype to our knowledge and these families can therefore represent a new phenotype. Two of the 41 Norwegian recessive families reported consanguinity, among these, one small family with spastic ataxia (ongoing project). Homozygous mapping is an excellent way to find new recessive genes or to find the exact diagnosis (104), the method is often used on consanguinity families or isolated populations.

Genotypes

Among the recessive ataxias we gave 46% of the families a genetic diagnoses (Paper 1), with A-T as the most prevalent diagnose due to the known Rendalen founder mutation. All sporadic and recessive subjects were screened for vitamin E deficiency (Paper 2) and only one AVED was only one AVED was found. Early screening for vitamin E deficiency in all ataxia subjects is strongly recommended even though it is a rare cause of neurodegenerative ataxia also in Norway. Two families with heterozygous mutations in the Frataxin gene were found, with a FRDA-like phenotype. We had very few FRDA patients in our Norwegian population and we hypothesized that the carrier frequency is low due to the prevalence estimation. It is therefore unlikely that the families with a FRDA-like phenotype and expansions in one allele have another ARCA in addition to this finding. These families are now further investigated by our group. Two families from our cohort were diagnosed with AOA2 (SCAR1) and included in a paper with 90 patients from a European cohort and part of the SPATAX network cooperation (74).

Figure 12. *Numbers of families with the different genotypes and phenotypes per June 2011 in the Norwegian ADCA cohort*

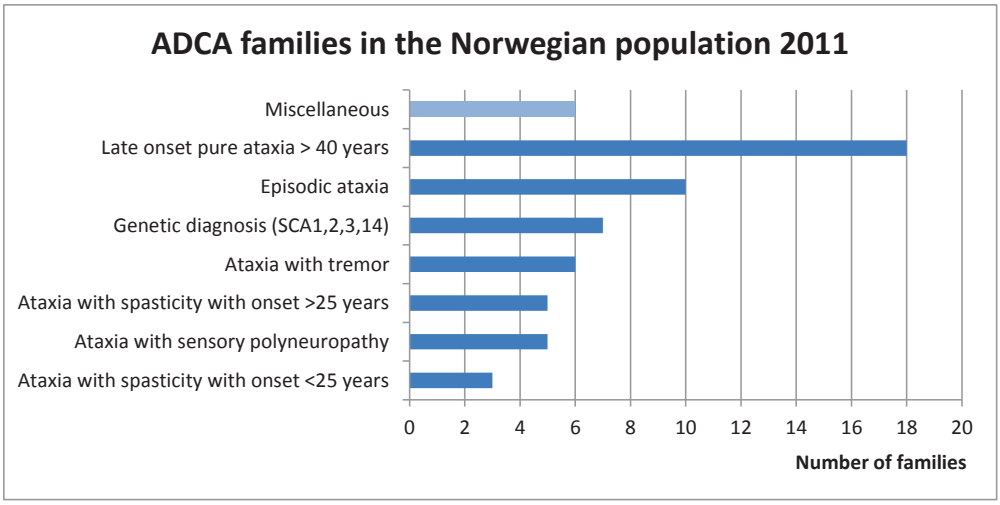
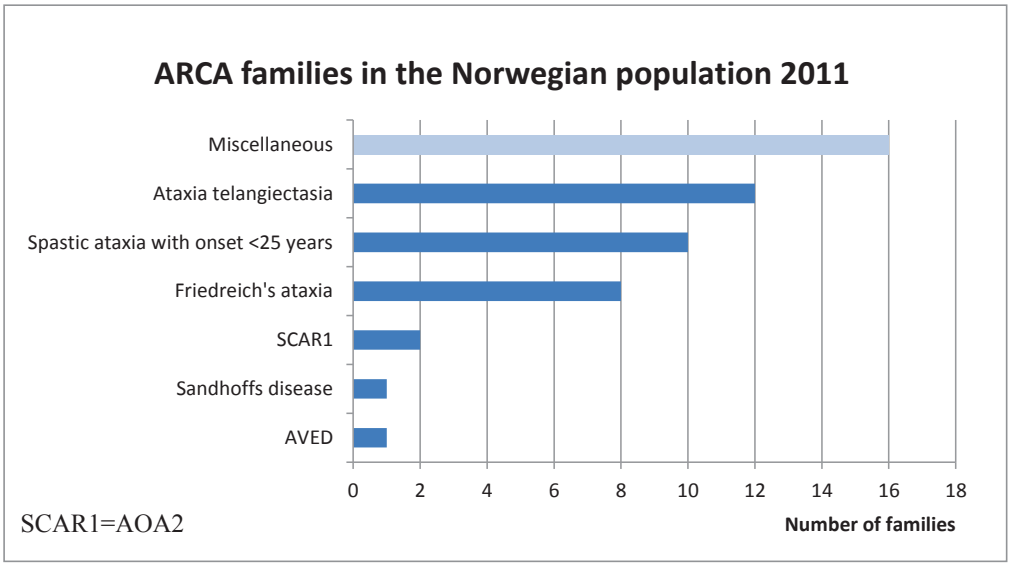


Figure 13. *Numbers of families with the different genotypes and phenotypes per June 2011 in the Norwegian ARCA cohort*



Why find exact genetic diagnoses and new genes?

Since few ADCA and ARCA, except AVED, FRDA and EA (10;11;57;105;106), have therapeutic options which prevent the degeneration (in greater or lesser extent) or decrease the episodic attacks it is always a cost-benefit question when to perform painstaking diagnostic work and expensive molecular analyses. There are a growing numbers of ADCA and ARCA, and the phenotype-genotype correlation is not always present and establishment of clinical criteria for genetic subtypes seems difficult (6;9). In addition testing for many HA is on research basis only. Many of these disorders are rare, SCA5, 11,15,23,27,28,31,35 and 36 are reported in few families. Screening projects have been performed in SCA11, 14, 15 and 28, but explained only a few percentages of the families in these ADCA populations. Except for the most common forms (SCA, 1, 2,3,6,7 and FRDA), the time consumed, the occurrences and the expenses can therefore be arguments against genetic testing for these disorders.

On the other hand through our work we have seen the importance getting an exact diagnosis. For many patients it is important to know whether a disease can be prevented or treated. Even though few medication options exist for hereditary ataxias the test results might help a person to realize that they have done what is possible to treat the disease. To get a genetic diagnosis is important in making life decisions, such as career choice and family planning. Patients can get more reliable information about the disorder and prognosis and they get more exact answers to their problems. Family members with variable expressivity can be tested if they want, misdiagnoses can be corrected, genetic counselling can be performed and finally these subjects can probably in the future be included into medication trials.

Research on monogenic diseases has contributed greatly to our understanding of pathogenic mutations, gene regulation and genetic mechanisms. Until today more than 2000 monogenetic disorders are found, but still more than over 1500 monogenetic disorders are only described with phenotypes in the OMIM (Online Mendelian disorders in Man) database (63). This means that more monogenetic disorders need to be explored. These future genotypes can give us valuable insight into the human biology.

The impact of finding a new gene is therefore great and this was therefore also the ultimate aim of this thesis. We faced many difficulties in Paper 4 as for instant; difficulties obtaining sufficient numbers of DNA samples, difficulties in finding other families with power enough for linkage analyses and lack of funding because these disorders are rare.

However, studies of monogenic diseases have contributed a great deal to the knowledge of complex diseases, such as Parkinson and Alzheimer. There are therefore good arguments for

why Mendelian disorders deserve more attention (107) and why we still search for new genes among the hereditary ataxia families.

Clinical recommendations based on our work

During our work we have tried to give patients diagnoses using different methods such as linkage studies, screening projects and routine diagnostic tests. Our work revealed difficulties and challenges in diagnosing hereditary ataxias and based on our experiences we suggest an approach to these patients (Figure 14).

An accurate family history can be of valuable help. Sometimes it is important to take the family history from different family members to find the phenotype of importance. To exclude sporadic ataxia supplementary examination can be of great help (Table 2). Brain MRI can be used to differentiate between pure and more complex forms (Figure 4). Atrophy of the brainstem is seen more often in coding triplet disorders. Also among ARCA atrophy of the cerebellum is seen in variable degrees (6;81). In recessive or isolated cases it is helpful to use the biomarkers which are cheap and easy to perform. It is of valuable help also to reconsider the diagnosis after a while and sometimes do some of the investigations again, because clinical signs and symptoms can be more prominent in later disease stages.

In all ARCA and sporadic progressive ataxia we recommend taking biomarkers, MRI of the cerebrum/medulla and neurography as the first diagnostic workup. If the biomarkers are negative it is today recommended to test for expansions in the FRDA gene first. Worldwide it is recommended to test for SCA1,2,3,6 and 7 among ADCA subjects and in addition perform supplementary investigations in order to exclude secondary causes (6;9). Since few of the well-known ADCA genotypes are found in Norway it can be argued not to test for these if no brainstem symptoms are found, if the brainstem has normal appearance on brain MRI and if no family members affected are from outside Norway. On the other hand these tests are easy to perform, cheap compared with other genetic tests and may give a few families an exact diagnosis which is important for follow up, prognosis and probably for therapy in the future.

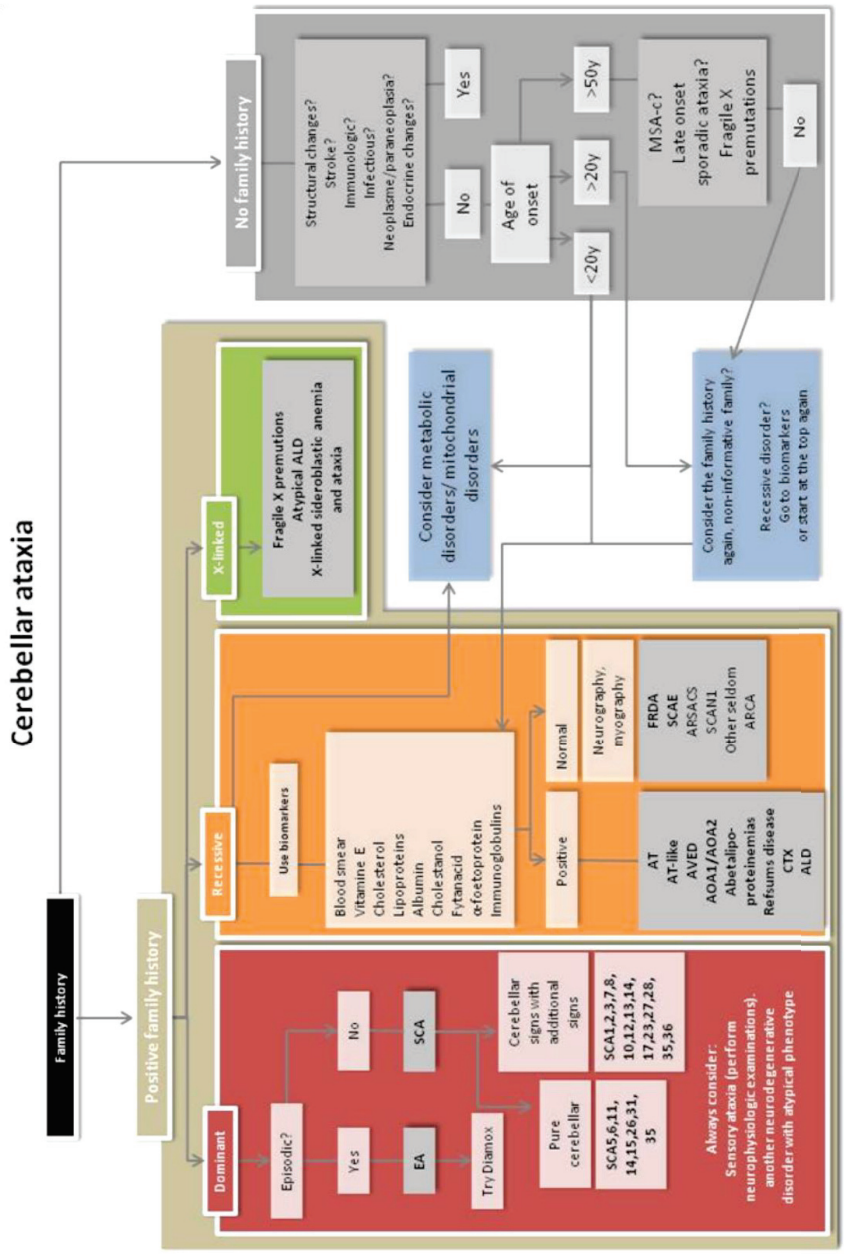
Many different medication and treatment options have been tried in order to reduce symptoms and to stop the neurodegeneration, but with little or no effect (11;105;106;108).

The main goal for designing treatment is to target the mechanism underlying the disorder. Despite the increased knowledge in genetics and the pathophysiology we still have too little knowledge about the pathways and why cells start to degenerate. The only medications shown

to have effect are vitamin E in AVED due to mutations in a vitamin E transport protein (53;57) and mild dose-dependent improvement in neurological function and reduction in cardiomyopathy in children with Friedreich's ataxia using Idebenone (105;109-111). We started vitamin E supplementation in the patient published in Paper 2, but she was diagnosed very late and little improvement is seen after medication was started. It is of great importance to start as early as possible in AVED subjects and therefore patients with progressive sensory and/or cerebellar ataxia should to be screened for vitamin E deficiency. Antiepileptic drugs are recommended in episodic ataxia (Azetolamide as the first choice), but medications only reduce attacks not the neurodegeneration (10). The treatment of hereditary ataxias today is therefore primarily supportive. Physical methods such as training (112), different walk-assistants (cane, walker, adding weights to the wrists) and education can in some degree compensate for the incoordination. Speech therapists are also key partners, because of the frequent problems with dysphagia and dysarthria. Last a good collaboration with rehabilitation institutions is important for follow up of these patients.

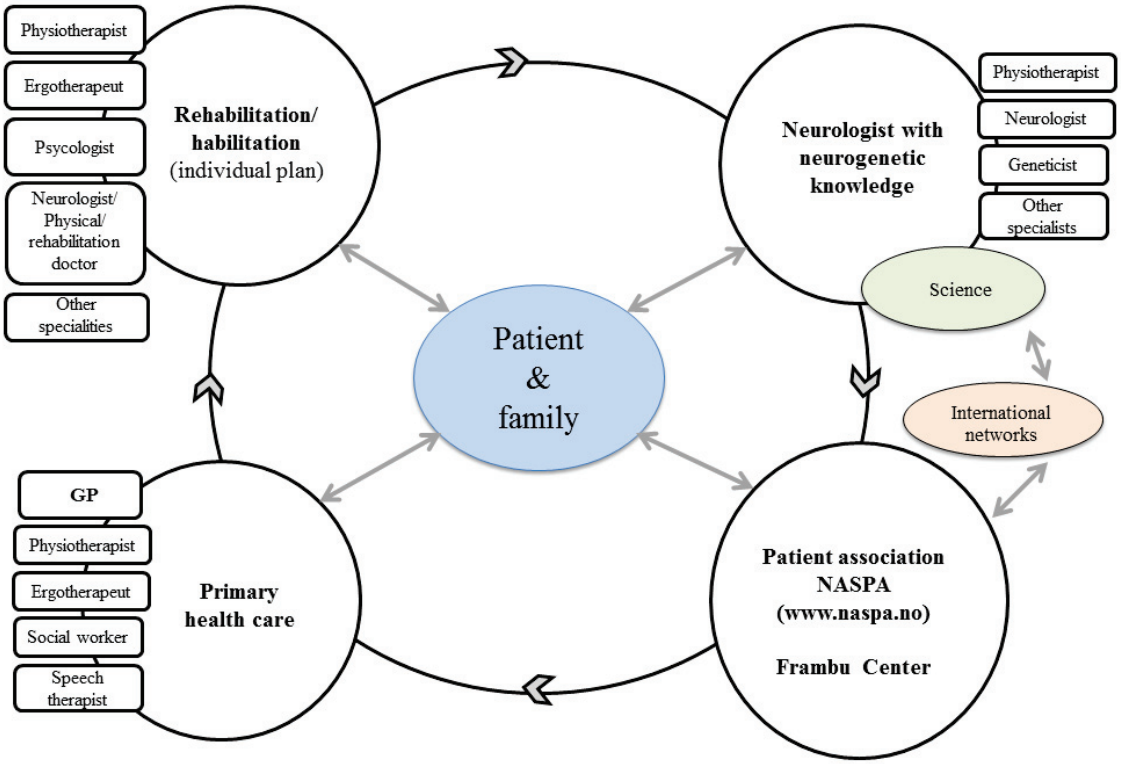
There is a need for strategies to handle referred patients with hereditary ataxia. Before we started our study there was no follow up strategies for these patients in Norway, there were no guidance for training, physiotherapy or how to cope with all the problems and symptoms. Therefore Figure 15 summarizes our advices based on our experiences with these disorders.

Figure 14. *Flowchart for approach to the patient with cerebellar ataxia*



ALD-Adrenoleukodystrophy, MSA-c-muliple system atrophy-cerebellum,CTX- Cerebrotendinous xanthomatosis, ARCA-autosomal recessive cerebellar ataxia, ADCA-autosomal dominant cerebellar ataxia,SCAE-spinocerebellar ataxia epilepsy, SCA-spinocerebellar ataxia, AVED-ataxia with vitamin E deficiency, ARSACS- autosomal recessive spastic ataxia of Charlevoix-Saguenay, AOA-ataxia with oculomotor apraxia, EA-episodic ataxia

Figure 15. *An illustration of how caretaking of hereditary ataxia patients can be practiced in Norway*



Modified after Anne Kjersti Erichsen, Hereditary spastic paraplegia in Norway, thesis 2009

Ethical aspects

The project has been approved by the Regional Ethical committee (Ethical agreement no 129/04011 Helse sørøst). As the project has been expanding during the past years, Chantal Tallaksen submitted a new protocol to REK 2010, including an enquiry for the establishment of a research biobank, which was accepted. At the Department of Medical Genetics, OUH they have a protocol for dealing with incidental findings of clinical relevance, including genetic counseling and an ethical review board. All patients have given informed written consent.

Conclusions

1. Prevalence of hereditary ataxias was estimated at 6.54/100 000, ADCA was 4.27/100 000 and ARCA at 2.27/100 000.
2. One subject in our material was diagnosed with AVED, having a Friedreich's ataxia phenotype.
3. A novel truly disease-causing mutation in the PRKCG gene, in exon 5, was discovered in the two first Norwegian SCA14 families with a slowly progressive ataxia.
4. A family with a late onset autosomal dominant ataxia was linked to a new locus on chromosome 10.

Further perspectives

Chantal Tallaksen and her research group have included families and patients in the database since 2002 and many families still not have exact diagnoses. It is likely to assume that we have new genotypes in the Norwegian population due to results from this thesis and further research on these families is needed. In Paper 4 we published a new locus and another family, not yet published is also linked to another new locus. Exom-sequencing is currently started in order to find the genes in these families.

The database has expanded every year with more families and patients. In the following years this database will be a research platform for exploring new phenotypes especially among subgroups such as; spastic ARCA, episodic ADCA and pure, late onset ADCA. The new technological developments with exom sequencing and Next-Generation-Sequencing have revolutionized genetic research the past years improving the possibilities to find the gene and reducing the often limiting step; time. In a short time the next limiting factor; money, will also be improved as costs are rapidly diminishing. We have now a great opportunity with selected phenotypes included in the database to find new genes with newer and faster methods.

This database will also help us to understand the clinical aspects of these disorders and hopefully in the future include patients in clinical medication trials. Monogenetic disorders are excellent models for understanding genes and their role in cellular and intracellular pathways. In collaboration with Joel Glover at the Stem Cell Center at Rikshospitalet we have started a project on SCA14. Induced pluripotent stem cells will be cultured from fibroblast cells from patients with SCA14 with the aim to generate human neurons. This provides us with exceptional opportunities for developing an in-vitro model for this disorder and trough this model provide better understanding of the ongoing neurodegenerative processes inside the Purkinje cells.

Collaboration

The European network for spinocerebellar degenerative disorders (SPATAX) was established in 2000 and includes clinicians, neurologists, basic scientists, geneticists, pathologists and patients' associations. A neurogenetic platform was recently established at Oslo University Hospital. This collaboration is an important network in order to discuss new methods, to find new phenotypes/genotypes, to start screening projects and to be kept updated in diagnostic and treatment options.

The laboratory work has been achieved in collaboration with European research centers, mostly INSERM at the Salpêtrière Hospital in Paris and Oslo University Hospital, Ullevål, Department of Medical Genetic.

Moreover, the European collaboration has been extended to the ASG (Ataxia Study Group) which comprises partners from most European countries.

Appendix 1

Autosomal dominant cerebellar ataxias

SCA1 (MIM ID #164400)
SCA1 was the first dominant genetic form discovered (1993). It is caused by CAG repeat expansions. Repeat numbers greater than 39 is mostly pathogenic. Symptoms can start in all ages, but most often in the mid 30's. Brain MRI reveals atrophy of the cerebellum and pons. The first symptoms are usually unsteadiness and coordination problems. Pyramidal signs are often seen and the phenotype can be ALS-like.

SCA2 (MIM ID #183090)
SCA2 was first described in families from Cuba and the gene was found in 1993. This form is also caused by a CAG repeat expansion. Repeat numbers greater than 35 is pathogenic. Common features are slowness and ophtalmoplegia of eye movements, neuropathy, reduced reflexes and memory loss in addition to the cerebellar ataxia. Brainstem atrophy with thinning of the pons is often seen on brain MRI.

SCA3 (MIM ID#109150)-
SCA3 is also known as Machado-Joseph disease (MJD). It is caused by a repeat expansion greater than 67 repetitions. Symptoms beside ataxia include tremor, rigidity, myokymias and neuropathy. SCA3 is the most common dominant ataxia in many parts of the world and especially in Portugal.

SCA5 (MIM ID#600223)
This form of ataxia was found in one branch of Abraham Lincoln's family, known as Lincoln's ataxia. It is caused by missense mutations in the *SPTBN2* gene. SCA5 has onset in all ages, but most often in young ages. The disease progress slowly. SCA5 is described as "pure".

SCA6 (MIM ID#183086)
SCA6 has mutations in the same gene as EA2 and hemiplegic migraine, but the mutation is caused by CAG repeats. SCA6 may initially present as an EA2-episodic disorder and many patients have migraine in addition to the cerebellar symptoms and signs. Acetazolamide (Diamox) can give symptom relief. It is often described as a relatively pure cerebellar disorder.

SCA7 (MIM ID#164500)
SCA7 is what Harding described as ADCAII, with retinopathy. This neurodegenerative disorder is caused by repeat expansions greater than 36. Anticipation is reported giving an aggressive juvenile form.

SCA8 (MIM ID#608768)
SCA8 gives poor coordination of the extremities, mostly the lower extremities, poor coordination of gait and speech. Spasticity is reported. Many patients with CAG/TAG expansion in one allele do not develop the disorder, due to factors not well understood. It is reduced penetrance in SCA8. An expansion larger than 71 can give SCA8.

SCA10 (MIM ID#603515)
SCA10 is a slowly progressive ataxia described in Mexico and Brazil. In addition to the cerebellar ataxia patients may develop epilepsy, weakness, and loss of sensation. SCA10 is caused by an ATTCT repeat expansion greater than 850 in the intron of the ataxin10 gene.

SCA11 (MIM ID#604432)
This is a seldom form, relatively pure late onset cerebellar ataxia caused by mutations in the TTBK2 gene. Some patients have pyramidal signs.

SCA12 (MIM ID#604326)
SCA12 is associated with tremor. Tremor can be the first symptom and later the ataxia is more prominent. In addition Parkinson-like feature may also occur and exaggerated tendon reflexes and loss of sensation can be seen. SCA12 is caused by a CAG repeat expansion. If one allele has 51-78 repeats the subjects will develop SCA12.

SCA13 (MIM ID#603259)
SCA13 is often early onset, progressive ataxia. Cognitive decline is often seen, especially among those with early onset. Pyramidal signs are also reported in some subjects. Missense mutations in a potassium channel gene.

SCA14 (MIM ID#605361)
SCA14 is found in the Norwegian ataxia population, but is rare. SCA14 is characterized by a slowly progressive ataxia with often additional pyramidal signs. Many patients are described with myoclonus and cognitive decline, but these findings are not seen among the Norwegian patients.

SCA15/16 606658)
This disorder was first described as two different SCA with loci close to each other. A huge deletion in the *ITPR1* gene in ataxic mice was found (66) and explained both the SCA15 and 16 families. Missense mutations in the gene do explain the genotype in some families, but most families published have huge deletions in the gene. This ataxia appears to be a slowly progressive form of ataxia from early childhood.

SCA17 (MIM ID#607136)
SCA17 is associated with chorea, dystonia, psychiatric symptoms, memory loss and ataxia. They may also have Parkinson-like features. This is a CAG repeat disorder. If one copy of the gene has 43-48 repeats, the individual might develop the disorder. If one copy of the gene has 49 or more repeats the individual will develop SCA17.

SCA23 (MIM ID#610245)
SCA23 has been described in Dutch families. Symptoms begin in the 40's and 50's. In addition to typical symptoms of ataxia, there are pronounced problems with eye movements. The *PDYN* gene for this SCA is recently found.

SCA27 (MIM ID#609307)
SCA27 was originally described in a Dutch family. SCA27 is caused by mutations in *FGF14* gene. This SCA is found in Norway (114). Mental impairment, cerebellar dysfunction, neuropathy, tremor exaggerated by physical exercise and stress and in some patients aggressive outbursts and orofacial dyskinesia are reported.

SCA28 (MIM ID#610246)
SCA28 has an early onset ataxia. It was first described in Italian and German families first. This is probably a seldom dominant ataxia due to an occurrence study newly performed. The phenotype is an early onset, slowly progressive relative pure ataxia, but with pyramidal signs in many of the reported patients. SCA28 is caused by sequence changes in *AFG3L2* gene.

SCA31 (MIM ID#117210)
This is a relatively pure cerebellar ataxia with late onset caused by an insertion of a penta-nucleotid repetition. In a few patients hearing loss is reported. The SCA4 locus is very close to this locus, but the phenotypes is described different. Few families are reported.

Episodic ataxias

EA1 (MIM ID #160120)
This type of ataxia is caused by sequence changes in the gene *KCNA1*. This gene is a potassium channel. Symptoms often begin in early life. Episodes of ataxia may last from seconds to minutes, but rarely hours. During the attacks patients have cerebellar symptoms with ataxia, double vision, nausea and difficulties in speaking, but also tightening of muscles/myotonia and headache are observed. In between episodes, patients may experience myokymia.

EA2 (MIM ID#108500)
This type of ataxia is caused by sequence changes in the gene *CACNA1A*. This is a Calcium channel gene. The symptoms during the attacks can be similar to EA1, but last longer, often for hours. Symptoms from the cerebellum with unsteady gait and nystagmus can be seen in between attacks and especially with longer disease duration. Episodes are often triggered by stress, caffeine, alcohol and physical exercise.

EA5 (MIM ID#613855)
This EA is caused by mutations in the *CACNB gene*, which also is an ion channel gene. This EA is described in a few families with ataxia and also in families with epilepsy.

EA6 (MIM ID#612656)
EA6 is caused by mutations in the *SLC1A* gene. This EA is seldom, only few families are published with associated migraine, hemiplegic features and with ataxia symptoms also between attacks.

Appendix 2

Autosomal recessive cerebellar ataxias

Friedreich's ataxia (MIM ID #229300)
This is a neurodegenerative disorder, mainly affecting the spinocerebellar tracts, dorsal columns and pyramidal tracts. Cerebellar atrophy is a late finding. Age of onset is in the first and second decades of life, but late age of onset is also reported. Weakness of the extremities and loss of sensation become more prominent as the disease progresses. Skeletal deformities, including scoliosis, heart problems and diabetes mellitus are commonly features. Most individuals with FRDA have repeat expansions in both alleles in the Frataxin gene, but 2% of the individuals are reported to have a combination of a repeat expansion in one gene and a missense mutation in the other allele. Lack of vibration sense and joint position/sensory ataxia is the main feature. Decreased reflexes are seen, but with positive Babinski sign. Skeletal deformities, heart problems and diabetes mellitus are also commonly seen.

Ataxia telangiectasia (MIN ID#208900)
Ataxia telangiectasia is an early onset recessive ataxia with a high prevalence in Norway due to the founder mutation in the county Hedmark, the mutation is called; Rendalen mutation. A-T is the second most frequent ARCA described worldwide and the most frequent form in Norway. The disorder is characterized by cerebellar ataxia, oculomotor apraxia, conjuctival telangiectasia, immune system defects and an increased risk for some types of cancers. Movement problems are commonly seen such as; choreatetosis and dystonia. The ataxia is often the first sign and telangiectasia is a later sign. Biomarkers in blood are; elevated α-foetoprotein and low levels of immunoglobulin. α -foetoprotein increases with age, due to mechanisms not well understood (50;51;115).

Ataxia with vitamin E deficiency (AVED) (MIM ID #277460)
Ataxia with vitamin E deficiency (AVED) manifests most often in childhood or early teens with poor balance when walking with loss of proprioception, cerebellar signs, dysarthria and areflexia. AVED may present as a FRDA like disorder. Some patients can have dystonia, head titubation (see article 2) retinopathy, cognitive symptoms and psychiatric symptoms. If not treated patients become wheelchair bound as a result of the neurodegeneration. A FRDA-like ataxia and very low serum vitamin E in the absence of fat malabsorbation are the diagnostic criteria. Genetic testing is available and recommended. Cardiomyopathy is not common in AVED. Treatment with vitamin E supplement at least 800 mg daily is recommended as early as possible in order to stop the neurodegeneration. It is recommended to screen all patients with recessive and sporadic ataxia for vitamin E deficiency, because this is the only ataxia with a treatment.

Spinocerebellar ataxia autosomal recessive 1/ Ataxia with oculomotor apraxia type 2 (AOA2)-(MIM ID #606002)
SCAR 1 is also called AOA2. This is an inherited ataxia with onset usually before 20 years. Patients often have problems to initiate eye movements, therefore given the descriptive name; oculomotor apraxia. α-foetoprotein in their blood is often elevated (not understood why) and can be used as a biomarker in this recessive disorder. Dystonia, chorea and neuropathy in addition to the ataxia are commonly seen. Two mutations in the Senetaxin gene are the genetic reason to this ataxia (74).

Ataxia with oculomotor apraxia type 1 (AOA1) (MIM ID #208920)
This form often have earlier onset than AOA2. The clinical feature can be similar to AOA2/SCAR1, but in addition often movement problems are seen (chorea and dystonia) and neuropathy is prominent. There are no good biomarkers for this disorder, but elevated levels of cholesterol and low levels of an albumin may be found.

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) (MIN ID #270550)
Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is characterized by early-onset spastic ataxia in addition to distal sensorimotor neuropathy and nystagmus. Hypermyelinated fibers which radiate from the edges of the optic fundus in the retina are seen in some families from Canada, but not in the French ARSACS families. This form is caused by mutations in the SACS gene.

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Supplements

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

DIAGNOSTIC FORM for SPINOCEREBELLAR DEGENERATION (ATAXIA AND/OR SPASTIC PARAPARESIS)

Date: _____ID-number: _____

Center : _____Examinator: _____

Proband: yes ❍ no ❍

Age at examination :_____Sex: M ❍ F❍ Geographical origin: _____

Initial examination yes ❍ no ❍ Follow-up number: _____

Age at death: _____Cause: _____

A. FAMILIAL HISTORY (add pedigree)

- Spastic paraparesis and or ataxia in the family yes ❍ no ❍
- Other familial disease yes ❍ no ❍ name: _____
- Consanguinity yes ❍ no ❍ precise: _____

B. AGE AT ONSET OF THE FIRST SIGN:_____

C. PRESENTING SIGNS

- Motor development normal ❍ delayed ❍ describe:
- Learning abilities normal ❍ delayed ❍ describe:

Signs at onset	Yes	No	At Age
- Unsteadiness			
- Dysarthria			
- Stiff legs			
- Cramps			
- Medical exam			
- Pain			
- Other (describe)			

D. PREDOMINANT SIGNS at present examination

	SPASTICITY				**CEREBELLAR ATAXIA**			
	None	Mild	Moderate	Severe	None	Mild	Moderate	Severe
Upper limbs	❍	❍	❍	❍	❍	❍	❍	❍
Lower limbs *	❍	❍	❍	❍	❍	❍	❍	❍
Gait	❍	❍	❍	❍	❍	❍	❍	❍
Dysarthria	❍	❍	❍	❍	❍	❍	❍	❍

*at rest for spasticity and knee-heel for cerebellar ataxia

E. DISABILITY STAGE

	At age
0: no functional handicap	
1: no functional handicap but signs at examination	
2: mild, able to run, walking unlimited	
3: moderate,unable to run, limited walking without aid	
4: severe,walking with one stick	
5. walking with two sticks	
6: unable to walk, requiring wheelchair	
7: confined to bed	

F. OTHER CLINICAL SIGNS

1. Reflexes	Normal	Enhanced	Very brisk	Diminished	Absent	Clonus
Jaw Jerk	❍	❍	❍			
Biceps	❍	❍	❍	❍	❍	❍
Finger flexor	❍	❍	❍	❍	❍	❍
Patellar	❍	❍	❍	❍	❍	❍
Adductor	❍	❍	❍	❍	❍	❍
Achilles	❍	❍	❍	❍	❍	❍

Hoffmann's sign absent ❍ present ❍

Plantar reflex flexor ❍ indifferent ❍ unilateral extensor ❍ bilateral extensor ❍

2. Motor deficit	None	Mild	Moderate	Severe
UL prox	❍	❍	❍	❍
UL distal	❍	❍	❍	❍
LL prox	❍	❍	❍	❍
LL distal	❍	❍	❍	❍
Facial palsy/atrophy	❍	❍	❍	❍

3. Muscle wasting	None	Mild	Moderate	Severe
UL prox	❍	❍	❍	❍
UL distal	❍	❍	❍	❍
LL prox	❍	❍	❍	❍
LL distal	❍	❍	❍	❍

4. Fasciculations yes ❍ no ❍ localisation:_____

5. Sensory deficit

- Vibration sense at ankles: abolished ❍ <5 seconds ❍ 5-10 sec ❍ >11 seconds (normal) ❍
- Superficial sensory loss yes ❍ no ❍ Underlign: Touch Prick Cold

6. *Skeletal abnormalities*

	None	Mild	Moderate	Severe	Other:
- Scoliosis	❍	❍	❍	❍	
- Pes cavus	❍	❍	❍	❍	

7. Facial dysmorphia yes ❍ no ❍ describe:

8. Sphincter and sexual disturbances

	None	Mild	Moderate	Severe
Urinary urgency	❍	❍	❍	❍
Urinary incontinence	❍	❍	❍	❍
Urinary retention	❍	❍	❍	❍
Anal incontinence	❍	❍	❍	❍
Impaired sexual function	❍	❍	❍	❍

Early menopause ❍ yes ❍ no age: _____

9. Extra-pyramidal symptoms	None	Mild	Moderate	Severe	Site
- Rest/postural/action tremor	❍	❍	❍	❍	_____
- Chorea	❍	❍	❍	❍	_____
- Dystonia	❍	❍	❍	❍	_____
- Myoclonus	❍	❍	❍	❍	_____
- Hypokinesia	❍	❍	❍	❍	_____
- Rigidity	❍	❍	❍	❍	_____

10. Ophthalmological signs

*Observation	Yes	No	
- Diplopia	❍	❍	
- Ptosis	❍	❍	
- Eye lid retraction (bulging eyes)	❍	❍	
- Diminished visual acuity	❍	❍	age at onset: _____

**Movement:*

- Nystagmus	❍	❍	describe: _____
- Saccadic pursuit	❍	❍	describe: _____
- Slow saccades	❍	❍	
- Ocular motor apraxia	❍	❍	
- Supranuclear ophthalmoplegia	❍	❍	describe: _____
- Nuclear ophthalmoplegia	❍	❍	describe: _____

**Eye fundus*

- Abnormal fundii ❍ ❍
  - ❍ Optic atrophy ❍ Retinis Pigmentosa ❍ Other : _____

11. Mental status

- Intellectual deterioration	❍	❍	onset/type: _____
- Mental retardation	❍	❍	onset and degree: _____
- Psychiatric symptoms	❍	❍	describe: _____
- Depression	❍	❍	

12. Other signs

	Yes	No		
- Dysphagia	❍	❍	describe: _____	onset: _____
Graduation: Mild ❍		Moderate❍	Severe❍	
- Skin problems	❍	❍	describe:	
- Hearing impairment	❍	❍	describe:	
- Epilepsia	❍	❍	type and onset:	

13. Other medical complaints:

G. SCORES OF CLINICAL EVALUATIONS

Cerebellar gait score (Annex 1a): _____
Adiadocho-score (Annex 1b): _____
Dysarthria score (Annex 1c): _____
Ashworth score (Annex 2a): _____
Ambulatory score (Annex 2b): _____
PATA test (Annex 3): _____

H. DIAGNOSTIC CONCLUSION

Cerebellar ataxia ❍

- AR / AD / ISOLATED/ X-LINKED
- Pure / complex

1. ❍ Definitely affected
2. ❍Probably affected
(Only dysarthria)
3. ❍*Possibly affected*
(Only mild gait ataxia)

Spastic paraparesis ❍

- AR / AD / ISOLATED/ X-LINKED
- Pure / complex

1. ❍ *Definitely affected*
2. ❍ Probably affected
(enhanced or very brisk LL reflexes ± Babinski)
3. ❍ Possibly affected
(enhanced reflexes in LL)

I. MOLECULAR DIAGNOSIS:

J. INVESTIGATIONS

	not done	normal	abnormal	describe
1. VLCFA	❍	❍	❍	_____
2. α-foetoprotein	❍	❍	❍	_____
3. Cholesterol	❍	❍	❍	_____
4. Serum protein electrophoresis	❍	❍	❍	_____
5. Cerebral MRI	❍	❍	❍	_____
6. Medullar MRI	❍	❍	❍	_____
7. EMG + NCV UL	❍	❍	❍	_____
8. EMG + NCV LL	❍	❍	❍	_____
9. VEP	❍	❍	❍	_____
10. AEP	❍	❍	❍	_____
11. MEP	❍	❍	❍	_____
12. SEP	❍	❍	❍	_____
13. Muscle biopsy	❍	❍	❍	_____
14. Skin biopsy	❍	❍	❍	_____
15. ERG	❍	❍	❍	_____
16. Fundus examination	❍	❍	❍	_____
17. Neuropsychological exam	❍	❍	❍	_____
18. Urodynamics	❍	❍	❍	_____
19. Urine density	❍	❍	❍	_____
20. Vitamin E	❍	❍	❍	_____
21. Apolipoprotein A , B	❍	❍	❍	_____

K. STORED MATERIAL

	Yes	No
DNA	❍	❍
Immortalized cell lines	❍	❍
Muscle tissue	❍	❍
Skin biopsy	❍	❍
Nerve biopsy	❍	❍
Other:		

ANNEXES

ANNEX 1a: Cerebellar gait score (*Standing capacities, eyes open)*

0 Normal: able to stand on one foot more than 10 s.
1 Able to stand with feet together, but no longer able to stand on one foot more than 10 s.
2 Able to stand with feet together, but no longer able to stand with feet on tandem position.
3 No longer able to stand with feet together, but able to stand in natural position without support, with no or moderate sway.
4 Standing in natural position without support, with considerable sway and considerable corrections.
5 Standing in natural position with unilateral support
6 Standing in natural position with bilateral support
7 Unable to stand at all

ANNEX 1b: Adiadocho-score

In sitting position, count the number of alternate movements of the dominante hand. Count only dorsal touch during 10 seconds (normal: 15-20)

ANNEX 2a: Modified Ashworth Scale

0 No increase in muscle tone.
1 Slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end range of motion when the part is moved in flexion or extension/adduction, etc or followed by minimal resistance throughout the remainder (less than half) of the range of motion.
2 More marked increase in muscle tone through most of the range of motion, but the affected part is easily moved.
3 Considerable increase in muscle tone, passive movement is difficult.
4 Affected part is rigid in flexion or extension (abduction or adduction, etc.).

ANNEX 2b: ***Ambulatory Score (0 – 10) Add A+B***

Number of meters in 5 seconds

A) Without aid		**B) With aid**	
0	> 5 m	0	> 5 m
1	≥ 4 m	1	≥ 4 m
2	≥ 3 m	2	≥ 3 m
3	≥ 2 m	3	≥ 2 m
4	≥ 1 m	4	≥ 1 m
5	0 m	5	0 m

ANNEX 3: PATA test: dysarthria evaluation

Count the number of repetitions of the “Pa Ta” syllables pronounced during 10 seconds.

Rater: _____date: _____patient:_____

Scale for the assessment and rating of ataxia (SARA)

1) Gait

Proband is asked (1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and (2) to walk in tandem (heels to toes) without support.

0 Normal, no difficulties in walking, turning and walking tandem (up to one misstep allowed)
1 Slight difficulties, only visible when walking 10 consecutive steps in tandem
2 Clearly abnormal, tandem walking >10 steps not possible
3 Considerable staggering, difficulties in half-turn, but without support
4 Marked staggering, intermittent support of the wall required
5 Severe staggering, permanent support of one stick or light support by one arm required
6 Walking > 10 m only with strong support (two special sticks or stroller or accompanying person)
7 Walking < 10 m only with strong support (two special sticks or stroller or accompanying person)
8 Unable to walk, even supported

Score	

2) Stance

Proband is asked to stand (1) in natural position, (2) with feet together in parallel (big toes touching each other) and (3) in tandem (both feet on one line, no space between heel and toe). Proband does not wear shoes, eyes are open. For each condition, three trials are allowed. Best trial is rated.

0 Normal, able to stand in tandem for > 10 s
1 Able to stand with feet together without sway, but not in tandem for > 10s
2 Able to stand with feet together for > 10 s, but only with sway
3 Able to stand for > 10 s without support in natural position, but not with feet together
4 Able to stand for >10 s in natural position only with intermittent support
5 Able to stand >10 s in natural position only with constant support of one arm
6 Unable to stand for >10 s even with constant support of one arm

Score	

3) Sitting

Proband is asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front.

0 Normal, no difficulties sitting >10 sec
1 Slight difficulties, intermittent sway
2 Constant sway, but able to sit > 10 s without support
3 Able to sit for > 10 s only with intermittent support
4 Unable to sit for >10 s without continuous support

Score	

4) Speech disturbance

Speech is assessed during normal conversation.

0 Normal
1 Suggestion of speech disturbance
2 Impaired speech, but easy to understand
3 Occasional words difficult to understand
4 Many words difficult to understand
5 Only single words understandable
6 Speech unintelligible / anarthria

Score	

Rater: _____date: _____patient:_____

5) Finger chase

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50 % of proband´s reach. Movements have an amplitude of 30 cm and a frequency of 1 movement every 2 s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated.

- **0 No dysmetria**
- **1 Dysmetria, under/ overshooting target <5 cm**
- **2 Dysmetria, under/ overshooting target < 15 cm**
- **3 Dysmetria, under/ overshooting target > 15 cm**
- **4 Unable to perform 5 pointing movements**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

6) Nose-finger test

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90 % of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor.

- **0 No tremor**
- **1 Tremor with an amplitude < 2 cm**
- **2 Tremor with an amplitude < 5 cm**
- **3 Tremor with an amplitude > 5 cm**
- **4 Unable to perform 5 pointing movements**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

7) Fast alternating hand movements

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precise as possible. Movement is demonstrated by examiner at a speed of approx. 10 cycles within 7 s. Exact times for movement execution have to be taken.

- **0 Normal, no irregularities (performs <10s)**
- **1 Slightly irregular (performs <10s)**
- **2 Clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs <10s**
- **3 Very irregular, single movements difficult to distinguish or relevant interruptions, performs >10s**
- **4 Unable to complete 10 cycles**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

8) Heel-shin slide

Rated separately for each side
Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task is performed 3 times. Slide-down movements should be performed within 1 s. If proband slides down without contact to shin in all three trials, rate 4.

- **0 Normal**
- **1 Slightly abnormal, contact to shin maintained**
- **2 Clearly abnormal, goes off shin up to 3 times during 3 cycles**
- **3 Severely abnormal, goes off shin 4 or more times during 3 cycles**
- **4 Unable to perform the task**

Score	**R**ight	**L**eft
mean of both sides (R+L) / 2		

I

II

III

IV