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IDENTIFICATION OF DISEASE GENES IN RARE NEUROLOGICAL CONDITIONS

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Identification of disease genes in rare neurological conditions
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Martin Engvall

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In memory of Lennart, Turid, Daphne, Björn, Bertil, Johan and Natalie.

POPULAR SCIENCE SUMMARY OF THE THESIS

The goal of all medical research should be to advance knowledge in order to be able to offer effective treatment or preventive measures for diseases. In neurology there has, until recently, been treatment available for very few disorders. Examples of recent advances are better drugs to minimize the symptoms in multiple sclerosis and trombolytic therapy for cardiovascular diseases such as stroke.

This thesis focusses on genetic diseases caused by defects in single genes, monogenic disorders. Also for this category of conditions, novel treatments are emerging. For spinal muscular atrophy, the number one genetic killer in children, intense research has led to treatments that are very promising. Reports are also coming of a future treatment for Huntington disease, a devastating neurodegenerative disease caused by a toxic protein, where the defective gene is silenced using advanced molecular therapy.

A prerequisite for developing treatment for a disease is that the exact cause and mechanisms underlying the development of symptoms are known. This makes a correct diagnosis of utmost importance, but diagnosing rare diseases is a challenging task. In recent years diagnostic possibilities have improved at an increasing pace due to rapid developments in several areas. Most importantly, the possibilities of genetic diagnostics have improved immensely. Just ten years ago one had to guess what disease the patient had on clinical grounds and then look at the genetic sequence of the corresponding gene, if known, a process that could take months. But many disorders are not possible to pinpoint in that way, some disorders are caused by defects in one of several genes, and other diseases have unspecific symptoms. Recent advances have made it possible to, instead of sequencing individual genes, sequence all the genes in the human genome simultaneously. The resulting data, often several tens of gigabytes in size, is impossible to inspect manually. Instead the data undergoes a bioinformatic pipeline of a series of computer algorithms that is aimed at “finding the needle in the haystack”. An important part of this process is to apply a predefined filter of genes that could fit with the disease under investigation, this filter can consist of any number of genes.

Other diagnostic advances in recent years are improved imaging capabilities with magnetic resonance tomography and positron emission tomography, and improved possibilities for advanced biochemical analysis to look for aberrations in metabolites and proteins.

In this thesis three new very rare monogenic disorders are described. One disease affects the muscles and leads to severe loss of function. Another disease, spinocerebellar ataxia, leads to impaired balance and coordination and failure to control several body functions, including instable blood pressure. The third disease is a rare defect in an enzyme affecting important biochemical processes in the body, adenosine kinase deficiency. Persons affected by this disorder become both intellectually and physically impaired at an early age. For two of these disorders the genetic defect is now known, and light has been shed on some of the mechanisms involved in generating symptoms. It is my sincere hope that the findings described here will contribute to the eventual development of treatments for the disorders.

ABSTRACT

This thesis is about improving diagnosis and treatment to persons affected by rare diseases. Diagnosis before treatment is a principle often told to medical students. But what if a diagnosis can't be made with the resources and knowledge at hand? The number of disorders where the genetic background and the molecular mechanisms are known is increasing rapidly with the advent of massive parallel sequencing, but there are still many disorders awaiting genetic and biological characterization. In my PhD project I have tried to contribute to characterization of new rare diseases, and in two cases this was successful all the way to finding the causative mutations. The success owes a great deal to two things, the access to massive parallel sequencing platforms and to the extensive resources available through my workplace, Centre for Inherited Metabolic Disorders, CMMS.

Paper I is the detailed description of a rare muscle disease, Sarcoplasmic body myopathy. The first description of a family from Sweden affected by this condition was published by our group 40 years ago and paper II provides comprehensive clinical investigations of nine individuals.

Paper II is about the genetic and molecular characterization of the disorder, leading to the finding of the responsible gene, *MB* encoding myoglobin, and description of an additional five families. In paper II we also claim that the damage to muscle cells is caused by oxidative damage.

Paper III provides a detailed clinical description of the first two Swedish families affected by spinocerebellar ataxia type 4, SCA4. Besides ataxia and polyneuropathy striking autonomic dysfunction was found, expanding the phenotype significantly. By linkage analysis, custom capture and sequencing we could narrow down the genomic region of interest. Further studies are ongoing in order to identify the causative gene

Paper IV involves the clinical, biochemical and genetic characterization of a novel inborn error of metabolism, adenosine kinase deficiency, ADK. The two Swedish siblings affected by this disorder had an unusual biochemical abnormality, elevated methionine. After excluding all known causes of hypermethioninemia we could show that the siblings, as well as four additional patients from two unrelated families, were suffering from a previously unknown metabolic defect in the methionine cycle.

Paper V constitutes a proof-of-concept study on how rapid diagnosis can be established in acutely ill infants in the neonatal intensive care unit, using customized, rapid whole genome sequencing. Babies with metabolic disorders often respond to treatment provided that diagnosis can be made before permanent damage to the central nervous system and other organs. In this study we provide evidence that a genetic diagnosis can be made in 15-18 hours.

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INNEHÅLL

LIST OF ABBREVIATIONS

ADCA	Autosomal dominant cerebellar ataxia
ADK	Adenosine kinase gene
AdoHcy	Adenosylhomocysteine
AdoMet	Adenosylmethionine
ADP	Adenosine diphosphate
AMP	Adenosyl monophosphate
AO	Age of onset
ATP	Adenosine triphosphate
CK	Creatine kinase
CMMS	Centre for inherited metabolic diseases
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computerized tomography
DD	Disease duration
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EMG	Electromyography
ENeg	Electroneurography
FKRP	Fukutin related protein
FRDA	Friedreich ataxia
GSD	Glycogen storage disorder
HIBM	Hereditary inclusion body myositis
HMERF	Hereditary myopathy with early respiratory failure
IEM	Inborn error of metabolism
LGMD	Limb girdle myopathy
LOD score	Logarithm of odds
LOPD	Late onset Pompe disease
MB	Myoglobin gene
MFM	Myofibrillar myopathies
MMT	Manual muscle testing
MPS	Massive parallel sequencing
MRI	Magnetic resonance imaging
NanoSIMS	Nanoscale Secondary Ion mass spectrometry
NBT	Nitro-blue-tetrazolium
NGS	Next generation sequencing
NICU	Neonatal intensive care unit
OMIM	Online mendelian inheritance in man
PA	Propionic acidemia
PAS	Periodic acid-Shiff, to stain glycogen
PCR	Polymerase chain reaction
PDHD	Pyruvate dehydrogenase deficiency
PET	Positron emission tomography
PGD	Preimplantation genetic diagnosis
PGT	Preimplantation genetic testing
Q10	Co-enzyme Q10
QST	Quantitative sensibility testing
RBM	Reducing body myopathy
RNA	Ribonucleic acid
SBM	Sarcoplasmic body myopathy
SCA	Spinocerebellar ataxia
sIBM	Sporadic inclusion body myositis
SSR	Skin sudomotor response
WES	Whole exome sequencing
WGS	Whole genome sequencing
μFTIR	Fourier transform infrared microscopy

1 BACKGROUND

Monogenic diseases are individually rare or ultra-rare, but the number of disorders is very high, and their collective prevalence is therefore considerable. In Europe a disease is considered rare if less than 1 in 2000 people are affected and in total there is an estimated number of 6000 to 7000 rare diseases (1). Many, but not all, rare diseases have a genetic cause. The symptoms often start during childhood, but over 50% of rare diseases first appear in adult life (1). For 6545 disorders there is a known genetic defect (2). In 70% of rare diseases neurologic symptoms are present (3).

Neurology has traditionally been a specialty characterized by and largely limited to detailed observation of the disease course with careful documentation of symptoms and clinical signs. A diagnosis was established by defining the constellation of symptoms and clinical signs together with the specifics of the disease course. During my PhD project, which has been ongoing for ten years, the advancements of the methodologies in genetics and molecular biology have been spectacular. From painstakingly slow Sanger sequencing of individual genes, or fragments of genes, whole exome or genome sequencing (WES or WGS) is now a part of routine diagnostics.

The purpose of this PhD project has been to help patients with undetermined neurological diseases to get a specific diagnosis. The motivation is that patients without a diagnosis tend to suffer more than patients who have specific diagnoses, due to the burden of uncertainty. A specific diagnosis may also result in opportunities for treatment. Inherited neurological disorders comprise a wide spectrum of conditions with involvement of the nervous system as the common denominator. Traditionally neuromuscular disorders involving striated skeletal muscles, the neuromuscular junction and the peripheral nerves, are also considered neurological diseases.

The PhD project consists of four parts. The first includes the characterization of sarcoplasmic body myopathy (SBM), a dominantly inherited late-onset muscular dystrophy. The second project involves the detailed clinical phenotyping and linkage analysis of a family with late-onset spinocerebellar ataxia. The third project includes description of the clinical and biochemical phenotype and subsequent elucidation of the molecular mechanism behind adenosine kinase deficiency. Finally, the fourth project is aimed at facilitating rapid genetic diagnosis for acutely ill neonates and infants with potentially treatable neurological conditions in the neonatal intensive care unit, using whole genome sequencing.

All studies have been performed in a collaborative, multidisciplinary environment. My role has been the clinician-scientist's, with responsibility for patient selection and management, clinical work-up, selection of biochemical and genetic investigations, overall coordination, interpretation and compilation of results. The team also includes experts in e.g., genetics, biochemistry, molecular biology and bioinformatics.

1.1 INHERITED MYOPATHIES

Inherited myopathies can be caused by various genetic defects. When unspecific structural abnormalities are seen in either light- or electron microscopy the disorder is referred to as a muscle *dystrophy*, and when the morphology is normal the term *myopathy* is used. Inherited myopathies have been classified in different ways over the years. The first classifications date back to the late 19th century when Erb coined the term "dystrophia muscularis progressive". Modern classifications often take several aspects of the disease groups into account including genetic defect, symptom distribution, age at debut and morphology.

Congenital muscle dystrophies comprise over 20 different, mostly recessively inherited, disorders and affects collectively roughly 1/100 000 individuals (4). The distinguishing features in this group are early onset hypotonia, dystrophic muscle and increased creatinine kinase (CK). There is often involvement of other organs e.g. in muscle eye brain disease (5).

Congenital myopathies share the early debut, and the often recessive inheritance with the congenital dystrophies, but are characterized by lack of dystrophic features in muscle biopsy. Around 1/25 000 are affected by congenital myopathies (6). There are, however, often other distinguishing morphological findings, e.g. cores in central core myopathy. Patients with these disorders generally have normal CK. The group includes more than 20 separate disorders and are, in general, non-progressive.

The *dystrophinopathies* Duchenne and Becker muscular dystrophy are more common, affecting 15-20/100 000 boys age 5-9 years (7, 8). Symptoms start at age 4-7 with muscle weakness most pronounced in the lower extremities. Ambulation is lost in early teens and later respiratory failure and cardiomyopathy follow. The disorder is caused by almost complete lack (Duchenne) or reduced amounts (Becker) of functioning dystrophin, a protein linking the contractile elements to other structural proteins. The biopsy in dystrophinopathies shows general dystrophic features and absent or reduced staining for dystrophin. CK is commonly very high.

Limb-girdle myopathies, LGMD, is a large group of disorders containing both autosomal dominant and recessive disease. The former classification distinguished the inheritance pattern naming the dominant disorders LGMD1 followed by a letter and the recessive as LGMD2 followed by a letter, e.g. LGMD2I for the recessively inherited muscle dystrophy with defect in fukutin-related protein (FKRP). The disease group had to be re-classified recently when the disorder LGMD2Z was characterized and the letters of the alphabet were exhausted. The current system uses the term LGMD followed by the letter D (dominant) or R (recessive) followed by the defective gene or protein, e.g. LGMD2I is now named LGMD R9 FKRP-related (9). Symptoms in these disorders typically start in proximal muscles in legs, hips and shoulders. Debut is often in the teens, but there is a great variability. Muscle biopsy shows unspecific dystrophic changes but sometimes the defective protein can be identified, e.g. by reduced staining in Western blot for dysferlin in LGMD R2 dysferlin-related (formerly

LGMD2B). CK is generally elevated in these disorders, ranging from mild elevation to very high values. The prevalence of LGMD varies in different populations and the collective prevalence is not known, but has been estimated to be somewhere between 1-7/100 000 (4, 10).

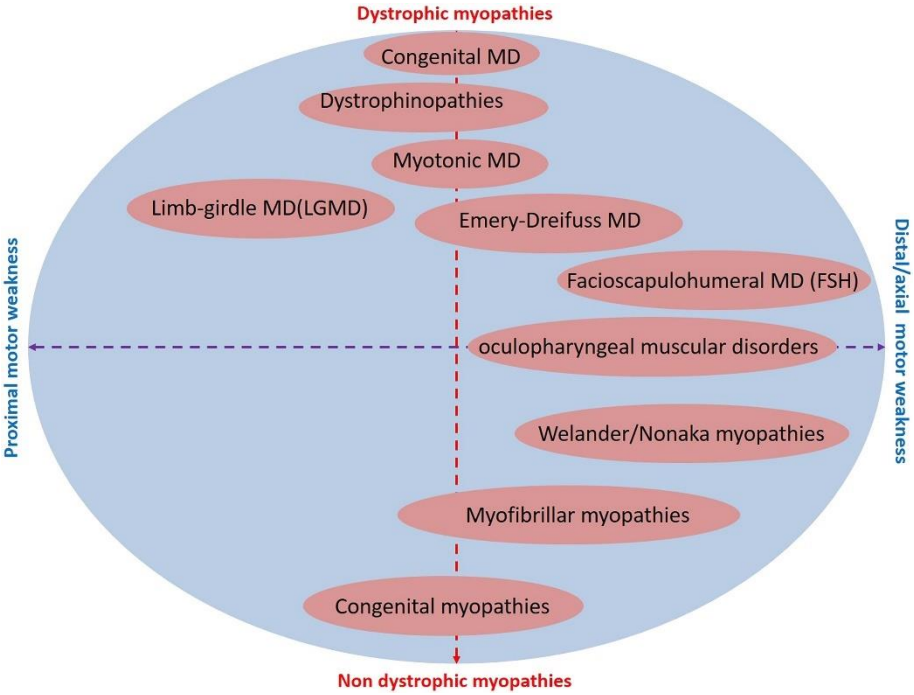


Figure 1. Illustration of the degree of dystrophic features and muscle symptoms of some of the major disease groups in inherited myopathies. On the horizontal axis the proximal to distal involvement is shown and the vertical axis reflects the degree of dystrophy seen in muscle biopsies. Reproduced with permission (11).

1.1.2 Myopathies with inclusions

Below are some examples of myopathies where inclusions are seen in muscle biopsies. The list is far from complete but serves as a comparison to distinguish from the unique inclusions seen in sarcoplasmic body myopathy.

Disorder	**Weakness distribution at debut**	**Age of onset, years**	**CK**	**Inclusion body characteristics**	**Gene/ OMIM**
Welander	Distal upper extremity	40-60	N-3 x normal	Rimmed vacuoles, autophagic bodies	*TIA1*/ 604454
HIBM (sIBM)	Distal lower extremity	10-25	N-5 x normal	Rimmed vacuoles, β-amyloid, filamentous inclusions	*GNE*/ 605820
Myofibrillar myopathies	Variable, lower extremities	40-50	N-8 x normal	Variable	Several
Reducing body myopathy	Proximal	0-40	N-10 x normal	Cytoplasmic reducing bodies immunoreactive to FHL1	*FHL1*/ 300718
Vitamin E deficiency	Normal strength	Childhood	? High	Autofluorescent, basophilic, electron dense	-
Pompe disease	Infantile: General	0-70	N – 10X Normal	Glycogen, lipofuscin, autophagic bodies	*GAA*/ 232300
Sarcoplasmic body myopathy	Proximal or distal	40-50	2-5 x normal	Autofluorescent. Oxidized lipids and proteins, rimmed vacuoles	See results section/ No OMIM

Table 1. Summary of features of a selection of myopathies with inclusions. Each condition is described below.

1.1.2.1 Welander distal myopathy

Welander distal myopathy (OMIM 604454) is common in Sweden and Finland. In Sweden it is sometimes referred to as "Hedesundasjukan" due to the high prevalence in the small village Hedesunda. In mid-Sweden and Finland the overall gene frequency is roughly 1/10 000 but in the surroundings of Hedesunda it was estimated to be as high as 1/10 (12). The disorder is of late onset, usually between 40 and 60 years of age and starts with weakness of the long extensors of the hand affecting fine motor skills. Later in the disease course weakness of foot extensors ensues. The histopathological findings include, apart from unspecific myopathic changes, inclusions of rimmed vacuoles and autophagic vacuoles in both degenerating and normal fibers. The genetic origin remained elusive until 2013 when a Finnish group found the causative genetic defect, a c.1150G>A, p.E384K transition in the *TIA1* gene (13). A Swedish group, working in parallel, found the same genetic defect through exome sequencing and proceeded to study splicing effects. They found increased *SMN2* exon 7 skipping and hypothesized that, although increased skipping of exon 7 in *SMN2* probably did not play a pathogenetic role, the finding could reflect a more widespread splicing dysfunction in muscle. The group also calculated that the ancient founder mutation causing the disorder had appeared first around 1050 years ago (14).

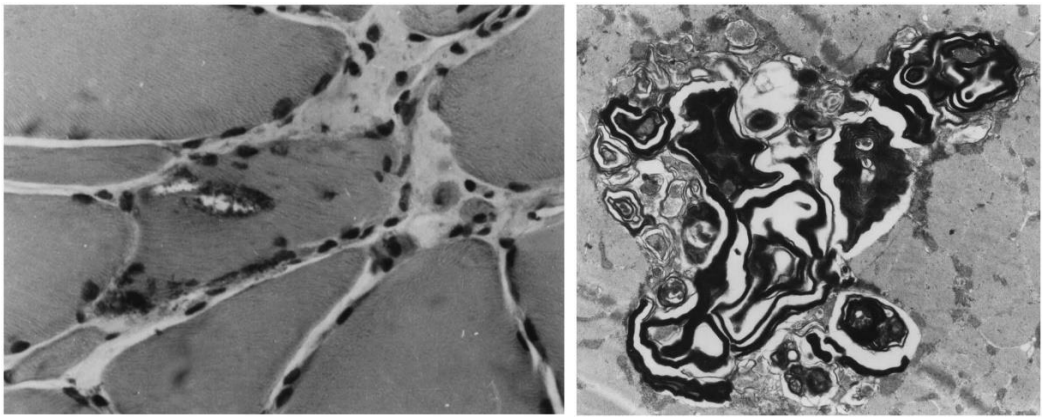
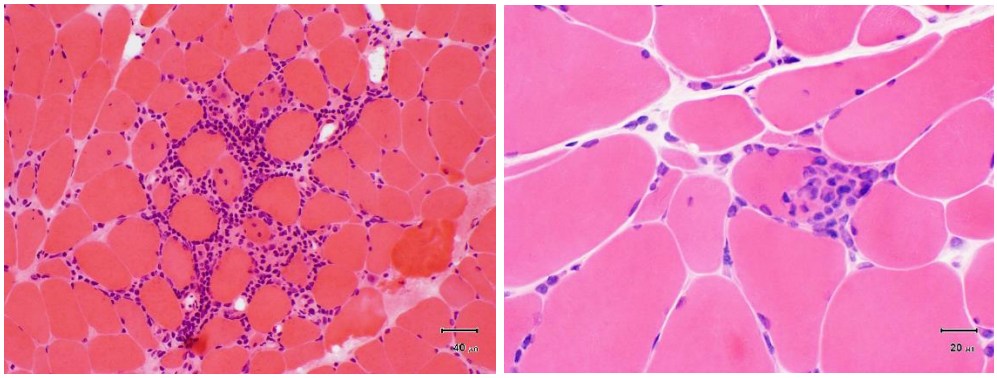


Figure 2. Morphology of muscle in Welander distal myopathy. **A**. Rimmed vacuole in a slightly atrophic muscle fiber (haematoxylin and eosin stain). **B**. Electron micrograph of anterior tibial muscle showing an autophagic vacuole containing myelin bodies surrounded by normal myofilaments. Magnification 7000×. Reprinted with permission (15).

1.1.2.2 Sporadic inclusion body myositis, sIBM

This is typically a late onset disorder starting after age 50 in 80% of patients. There is also a male preponderance which is more pronounced in higher ages of onset. Women with the disorder tend to have an earlier age of debut. Symptoms typically start with knee extensor weakness; other common symptoms are flexor weakness in the distal upper extremities and swallowing difficulties. The disorder usually progresses slowly and can be considered an intermediate between an inflammatory myopathy and a degenerative muscle disease. Muscle biopsy in IBM shows inflammation with mononuclear cell infiltration, degenerative changes and inclusions. The infiltrated cells are mainly $CD8^+$ cytotoxic T-cells (16). The inclusions

found in this disorder are rimmed vacuoles and protein inclusions including amyloid-β as in Alzheimer disease (17).



A B

Figure 3. Typical morphological findings in sIBM. **A**. Endomysial inflammatory infiltrates (haematoxylin and eosin stain). **B.** Invasion/compression of a non-necrotic fiber by inflammatory cells (haematoxylin and eosin stain). Reprinted with permission (18).

1.1.2.3 Hereditary inclusion body myositis, HIBM

HIBM, often referred to as GNE-myopathy (OMIM 605820), is a recessive disorder where most cases have mutations in the *GNE* gene. The highest prevalence is in the Jewish Persian population in the middle east (HIBM) and in Japan (previously Nonaka distal myopathy, now considered HIBM), with common founder mutations in the *GNE* gene present in the respective populations (19, 20). The onset of symptoms is earlier than in sIBM, usually in the late teens. In contrast to sIBM, the quadriceps muscle is often spared, while other muscles of the lower extremity display both weakness and atrophy. The histopathological findings are similar to what is found in sIBM, including rimmed vacuoles and accumulation of amyloid-β (21).

The gene *GNE* encodes UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase, a bifunctional enzyme involved in sialic acid synthesis and possibly leading to hyposialylation of proteins. It has therefore been hypothesized that substitution with oral sialic acid may slow disease progression. Prophylactic treatment in a mouse model of HIBM showed that development of a a myopathic phenotype could be prevented by administration of sialic acid (22). However, a double blind placebo controlled phase 3 study failed to show effect of treatment after 48 weeks (23).

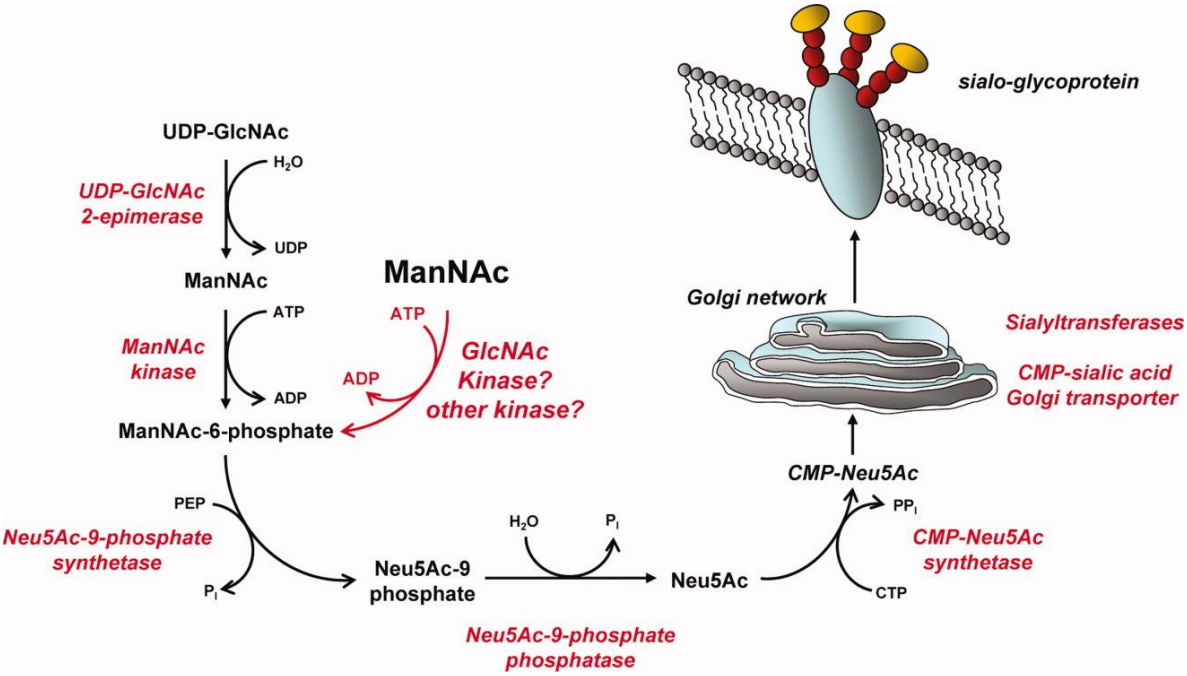


Figure 4. Overview of sialic acid metabolism. The metabolic defect is in the bifunctional enzyme UDP–GlcNAc-2-epimerase/N-acetylmannosamine kinase, that catalyzes the first two and rate-limiting steps of the sialic acid biosynthetic pathway. N-Acetyl-D-mannosamine (ManNAc) can enter the sialic acid pathway immediately downstream from the metabolic block after phosphorylation. Reproduced with permission (24).

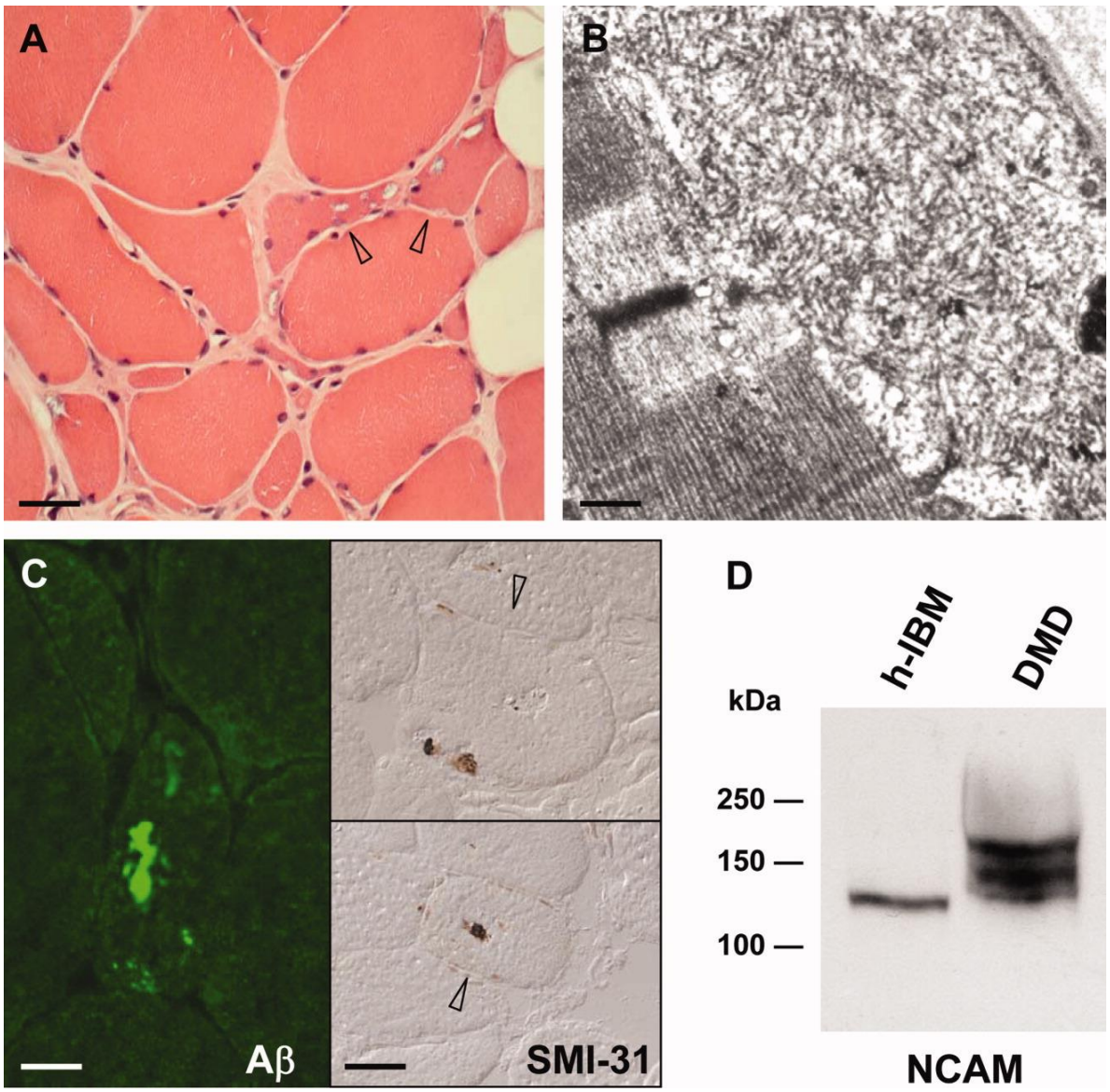


Figure 5. **A.** Hematoxylin and eosin stain of a representative HIBM muscle biopsy. There is increased variation in fiber size, angulated atrophic fibers, and fibers bearing cytoplasmic vacuoles (arrowheads). Scale bar = 20 μm. **B.** Electron micrograph showing a subsarcolemmal collection of typical 15–21 nm filaments. Scale bar = 300 nm. **C.** Left, confocal microscope image showing an HIBM abnormal muscle fiber with a cytoplasmic inclusion immunoreactive with anti-amyloid β (Aβ) antibody. Scale bar = 10 μm. Right, two abnormal muscle fibers (arrowhead) with cytoplasmic inclusions immunopositive with the SMI-31 antibody recognizing hyperphosphorylated tau protein. Scale bar = 20 μm. **D.** By Western blot analysis, in HIBM muscle, NCAM migrates as a discrete band of ≈130 kDa, whereas in control myopathies (Duchenne muscular dystrophy in this case) NCAM migrates as a broad band of ≈150–200 kDa. This evidence suggests abnormal sialylation of NCAM in HIBM muscle. Reproduced with permission (24).

1.1.2.4 Myofibrillar myopathies

Myofibrillar myopathies (MFM) is a group of rare mostly dominantly inherited disorders. They share some microscopic similarities consisting of inclusions of aggregates of abnormal sarcomeric proteins, e.g. actin, myosin, desmin, myotilin, filamin C and Z-band proteins beginning at the Z-disk. Most patients with MFM experience symptoms late in life, typically in the fourth or fifth decade.

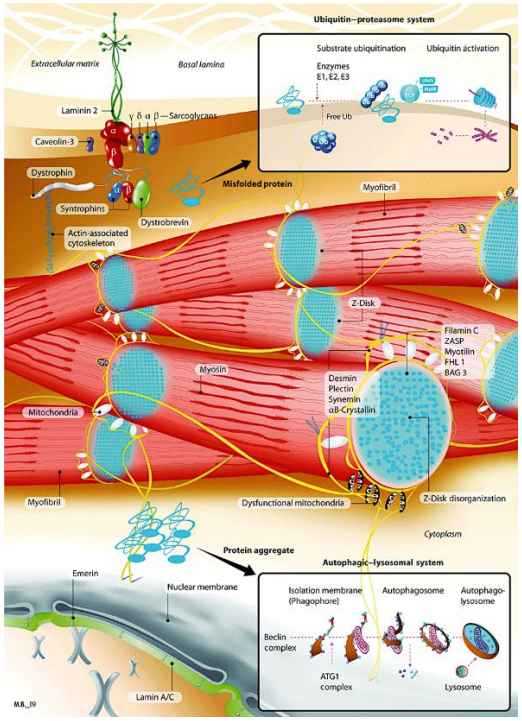


Figure 6. Cartoon highlighting the proteins involved in myofibrillar myopathies. The myofibrillar proteins constituting the myofibrils (filamin C, myotilin, Z-band alternatively spliced PDZ-containing protein, four-and-a-half LIM domain 1, Bcl-2-associated athanogene-3) are scaffolded by the extramyofibrillar proteins (desmin, aB-crystallin, plectin) that also link the myofibrils to nuclei, sarcolemma and mitochondria. Also included in the cartoon is the linkage to the structural proteins required to transmit the contractile force to the extracellular matrix, and the ubiquitin–proteasome and the autophagic–lysosomal systems essential for breakdown of abnormal proteins. Reproduced with permission (25).

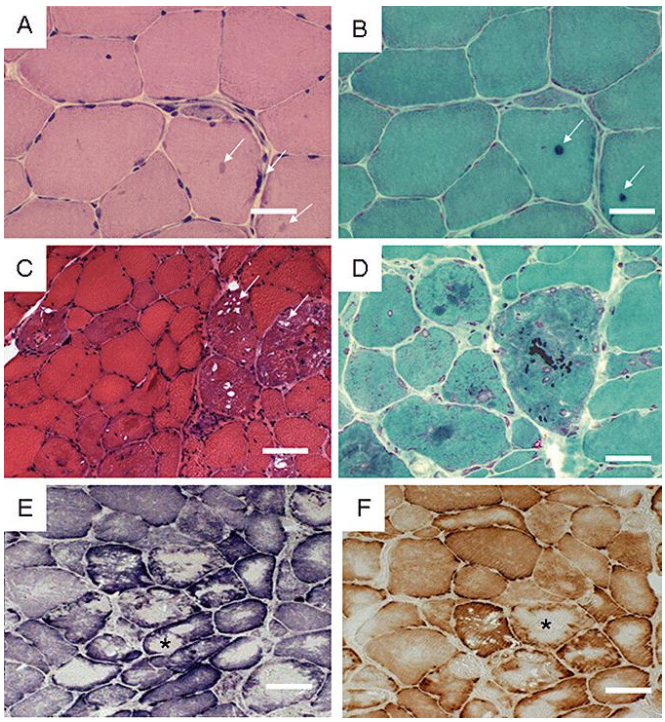


Figure 7. Histopathological findings in myofibrillar myopathies (MFMs). **A.** Hematoxylin & eosin (H&E) and **B.** Gomori trichrome (G-Tri) (B) staining in desminopathy. Arrows indicate the presence of isolated sarcoplasmic and subsarcolemmal protein aggregates (bars = 50 mm). **C.** H&E and **D.** G-Tri staining in myotilinopathy and in a patient with MFM of unknown aetiology, respectively. Note the vacuolar changes (arrows) in myotilinopathy (bar = 75 mm) and the polymorphic protein aggregates in MFM of unknown aetiology (bar = 50 mm). **E.** Succinic dehydrogenase and **F.** cytochrome-C oxidase (F) staining in desminopathy. Reproduced with permission (25).

A typical example of a myofibrillar myopathy is MFM 9, Hereditary myopathy with early respiratory failure (HMERF, OMIM 603689), a disorder sometimes referred to as Edström myopathy since it was first described in Sweden in 16 individuals from 7 families (26). This disorder is inherited as an autosomal dominant trait and is characterized by late onset slowly progressive muscle weakness also involving the diaphragm, causing respiratory failure. Respiratory insufficiency may be the presenting symptom in some cases (27). Since the original report in 1990 several other groups have reported more families from UK (28, 29),

Finland, France, Germany and Argentina (27). The muscle pathology typically shows unspecific myopathy, rimmed vacuoles and cytoplasmic bodies positive for anti-myotilin and anti-alpha B-crystallin. Nicolao et al found linkage to 2q24-q31 (30) and considered titin (TTN) to be a strong candidate gene. Lange et al (31), found a heterozygous missense mutation in TTN. Later Palmio et al (27) investigated several unrelated families and found different missense mutations affecting the FN3 119 domain in A-band of titin. It remains to elucidate the functional effects of the mutations, but abnormal autophagy and dysregulation of protein turnover is hypothesized (27).

Gene	**Protein**	**Disorder**	**OMIM**
DES	Desmin	MFM1	601419
CRYAB	αB-crystallin	MFM2	613869
MYOT	Myotilin	MFM3	609200
LDB3	LIM domain-binding protein C3 (ZASP)	MFM4	609452
FLNC	filamin C	MFM5	609524
BAG3	Bag3	MFM6	612954
PLEC1	Plectin	epidermolysis bullosa simplex with muscle dystrophy	226670
TTN	Titin	MFM 9 Hereditary myopathy with early respiratory failure (HMERF)	603689

Table 2. Examples of myofibrillar myopathies and their corresponding genes and proteins.

1.1.2.5 Reducing body myopathy

Reducing body myopathy (RBM, OMIM 300718) was first described in 1972 by Brooke and Neville (32). It is a rare myopathy belonging to the group of myofibrillar myopathies (see above) but with some distinguishing features, both clinically and genetically. RBM has a wide clinical spectrum ranging from early onset fatal disease to a mild disorder with onset in adulthood (33). Apart from muscle weakness and atrophy contractures and rigid spine also occurs (34). The hallmark of this disease is intracytoplasmic aggregates displaying strong reducing activity when stained with nitro-blue-tetrazolium (NBT), presumed to be due to reduction by sulphydryl groups. All reported cases were later shown to be caused by mutations in the four and a half LIM domain gene *FHL1* on the X chromosome. The inheritance can be *de novo*, X-linked dominant and X-linked recessive. In inherited cases, due to the X-chromosomal location of the gene, males are often more severely affected than their

mothers or grandmothers (33). In contrast to the allelic, and somewhat phenotypically overlapping, disorders Emery-Dreifuss muscular dystrophy 6, myopathy with postural muscle atrophy and scapuloperoneal myopathy, patients with reducing body myopathy usually have mutations affecting the second LIM domain in the FHL1 protein encoded by exon 3 of the *FHL1* gene. The allelic disorders have mutations affecting other domains, e.g. exon 5 to 8 in Emery Dreifuss muscular dystrophy type 6 (35). RBM due to *FHL1* mutations should also be considered as a differential diagnosis in rigid spine syndrome (36).

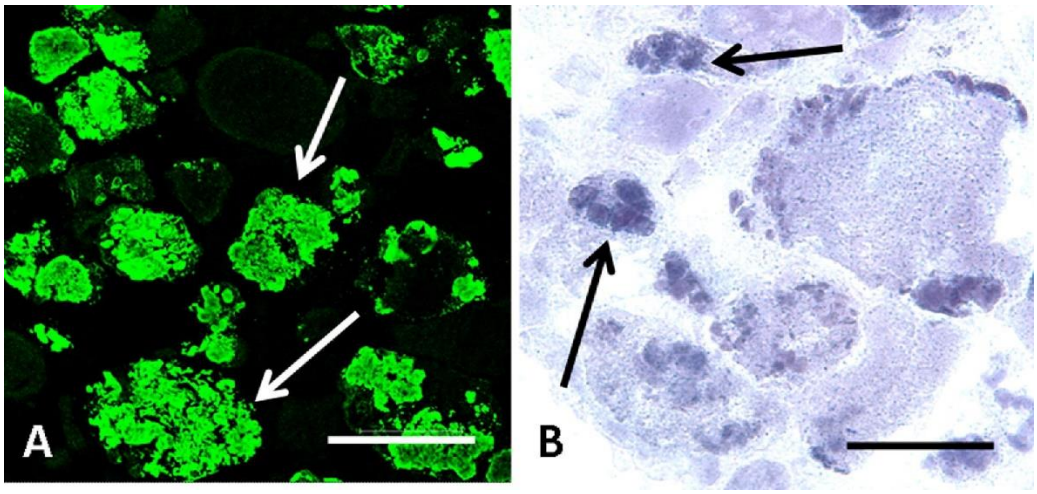
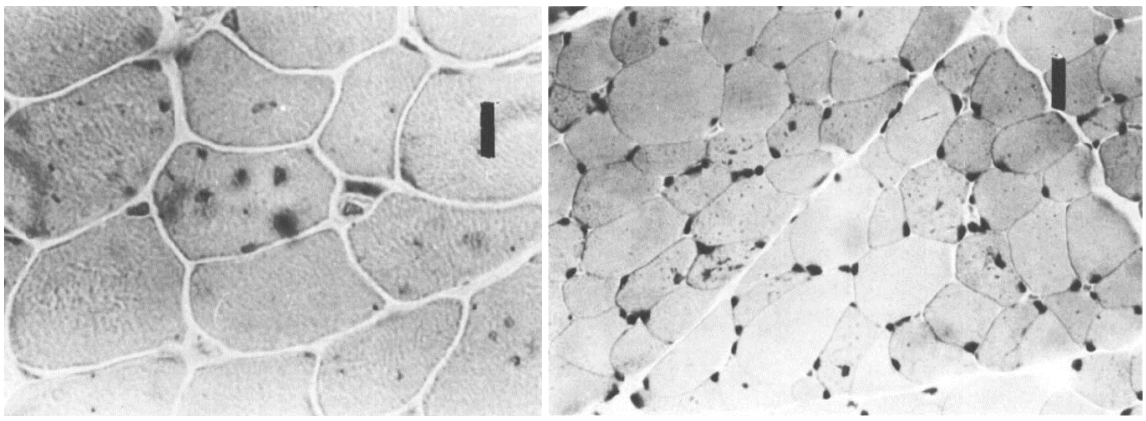


Figure 8. **A.** Immunostaining for FHL1-positive inclusions. **B.** Stain with nitro-blue-tetrazolium (NBT) showing strong reducing reactivity. Scale bars 100 µm. Reprinted with permission (37).

1.1.2.6 Myopathy with vitamin E deficiency

Deficiency of vitamin E is today a very uncommon cause of myopathy in the developed world. It is sometimes occurs in association with cholestasis and reduced uptake of vitamin E (38). Other reported symptoms of chronic deficiency of vitamin E are ataxia (39) and cardiomyopathy (40). Vitamin E deficiency does not cause isolated myopathy and the muscular symptoms are overshadowed by other neurological features. The histological features in vitamin E deficient induced myopathy include unspecific myopathic and neurogenic changes, but also accumulation of autofluorescent lipofuscin granules, like the autofluorescent inclusions seen in sarcoplasmic body myopathy (see below). There are some differences compared to the inclusions seen in sarcoplasmic body myopathy: the inclusions in vitamin E deficiency are PAS-positive, often localized just beneath the sarcolemma and emit yellow autofluorescence (38) while the inclusions in sarcoplasmic body myopathy are PAS-negative, localized to the sarcoplasm and emit orange autofluorescence (41).



A **B**

Figure 9. Muscle biopsy from unspecified muscle in patient with cholestasis and deficiency of vitamin E. **A.** Abundant basophilic inclusion (Htx stain), bar= 25 µM. **B.** Staining with acid phosphatase shows strong reaction, bar=50 µM. Reproduced with permission from (38).

1.1.2.7 Pompe disease

Pompe disease (OMIM 232300) is a recessively inherited disease belonging to the glycogen storage disorders (GSD2). It is caused by deficiency of the lysosomal enzyme acid alpha-glucosidase, encoded by the *GAA* gene, that accounts for the small percentage of glycogen that is broken down in the lysosomes. In Sweden Pompe disease is very rare, 0.25/100 000 persons (42), but in other countries the disorder is more common, e.g. in The Netherlands where the frequency is ten times higher, 2.5/100 000. This is most likely due to founder mutations in the population, 60% of patients carry either IVS1(-13T>G), 525delT or delexon18 (43). The disorder presents as a continuum of phenotypes ranging from debut in infancy to late onset cases. Infants presents at, or soon after, birth with severe hypotonia, hypertrophic cardiomyopathy, myopathy, respiratory insufficiency and macroglossia (44). In adult cases, late onset Pompe disease (LOPD), the symptoms resemble limb girdle muscle dystrophy with weakness predominantly in proximal muscles. Adult patients do not have cardiac involvement but usually pronounced involvement of respiratory muscles (45). Diagnosis is made by measurement of alfa-glucosidase in lymphocytes, fibroblasts, muscle tissue or in dry blood spots. Muscle biopsies are seldom performed nowadays but show general dystrophic features, round vacuoles and PAS-positive accumulation of glycogen. Since the morphological abnormalities are often very discreet in adult patients and the diagnosis can be missed if only biopsy is performed, measurement of enzyme activity is considered the gold standard for correct diagnosis. One frequent, but less known, finding in biopsies is a high prevalence of autophagic inclusions containing lipofuscin, this finding is more pronounced in severe cases (46). Treatment with recombinant enzyme is available for both infantile and adult onset patients. Enzyme replacement in infant patients seems to be more beneficial if started early (47), which has led to inclusion of enzyme testing as part of newborn screening in several countries. Taiwan was first (48) but the disease in now included in the Recommended uniform screening panel in the USA (49). The treatment effect is

excellent when survival is studied, but myopathy with atrophy, signal changes on muscle MRI and weakness of proximal muscles develops in spite of treatment (48).

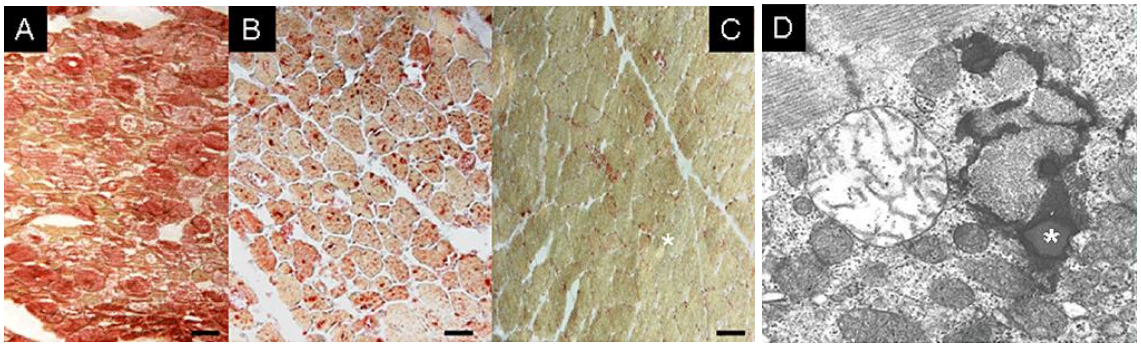


Figure 10. **A-C** show PAS staining for glycogen accumulation from **A.** infantile, **B.** juvenile and **C.** adult onset Pompe disease with intense staining in the infant case but a very discrete glycogen accumulation in the adult case. **D.** An ultrastructurally swollen mitochondrion and lipofuscin accumulation, Asterix shows a lysosome with lipid accumulation. Reprinted with permission (46).

1.1.2.8 Sarcoplasmic body myopathy

Sarcoplasmic body myopathy (SBM) is an autosomal dominant myopathy first described in Sweden in the late 1970ies by Professor Edström and colleagues (41). They were studying microscopy sections from a muscle biopsy from a man with distal muscle weakness and saw black stains in the muscle, but thought it was caused by dirt on the microscopy lens. The inclusions, however, remained after cleaning the lens. The muscle tissue also had signs of myopathy with increased fibrous tissue, abnormal variation in fiber size and centrally located nuclei. The inclusions could be seen on every available immunohistochemical stain but did not absorb any staining themselves (Fig 11). Professor Edström went on to study the muscle specimen by electron microscopy (Fig 12) and concluded that the inclusions looked like, but were not identical to, lipofuscin granules (50). Lipofuscin is an insoluble yellow-brown pigment composed of oxidized lipids and proteins (51). It has been postulated that lipofuscin forms secondary to oxidative stress by radical oxygen species (ROS) generated by mitochondria (52). Lipofuscin inclusions in muscle can also be an unspecific sign of ageing thought to be the result of deficient autophagy and mitophagy rather than ROS-driven oxidative injury as mitochondrial ROS-production is similar in the elderly and the young (53). As mentioned above lipofuscin is also seen in other disease states affecting muscle tissue (46, 54, 55). In SBM there is also some resemblance to the inclusions seen in vitamin E deficiency (38) and the inclusions sometimes seen in Pompe disease (46) but to conclude the inclusions seen in sarcoplasmic body myopathy are distinctive from almost all inclusions seen in other myopathies. Three more patients, siblings of the index case, were located. The initial four patients were described in some detail in 1980 (41). The sarcoplasmic inclusions were then further characterized in 1981 (56). Professor Edström early on realized that the unique findings indicated that this was a new not previously described muscle dystrophy. Clinical and genetic characterization of this novel disease forms the first part of my thesis.

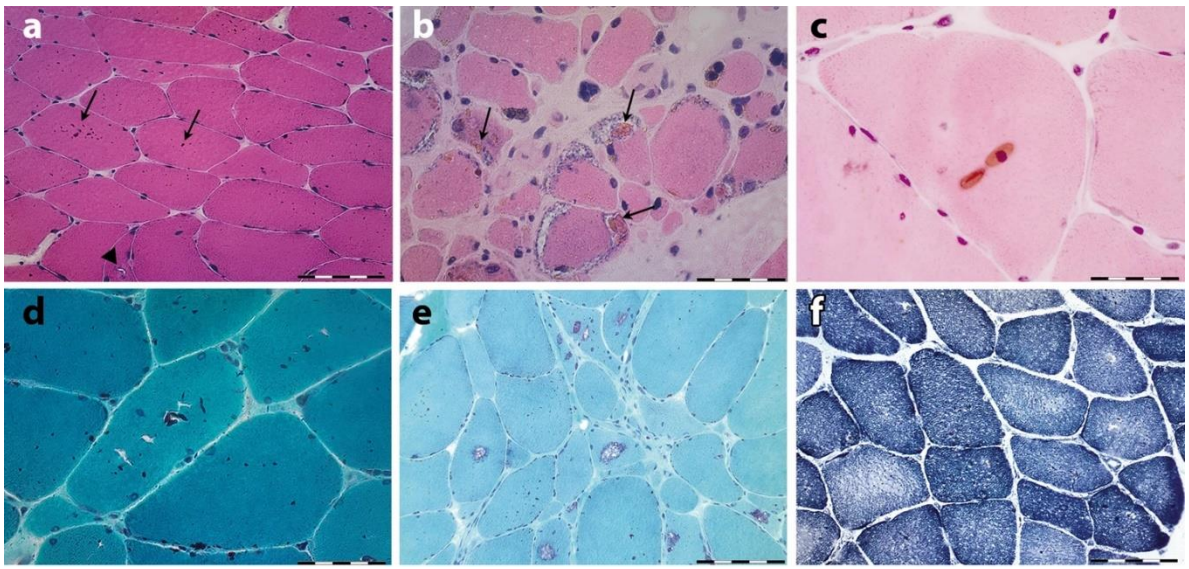
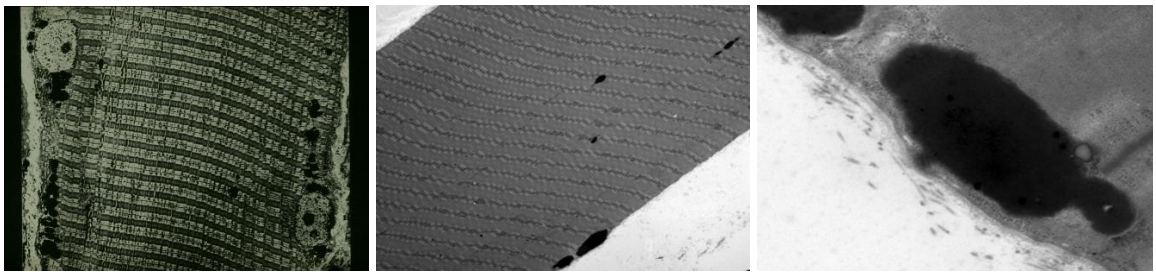


Figure 11. Histochemical features of sarcoplasmic body myopathy. **a.** Anterior tibial muscle biopsy from an individual 10 years prior to the onset of symptoms, stained with hematoxylin and eosin, showing several rounded brown inclusions (arrows) (sarcoplasmic bodies) in the majority of myofibers and very small vacuoles in some myofibers (arrowhead in a). **b**, **c.** Biceps brachii from a Spanish patient, 15 years after disease onset. Note the presence of collections of sarcoplasmic bodies within the rimmed vacuoles. **d**, **e**. Sarcoplasmic bodies appear red on modified Gomori trichrome stain. **e.** In muscle biopsies with more advanced pathological lesions, large numbers of rimmed vacuoles are observed. **f.** No major architectural changes are seen on NADH reaction, apart from lack of oxidative activity at the site of vacuoles. Scale bar in a and f = 50 μm; scale bar in b, c and d = 20 μm. Adapted from (57) with permission



A B C

Figure 12. Electron micrographs of SBM muscle. In **A.** accumulation of sarcoplasmic bodies close to nuclei is shown. **B.** shows inclusions interspersed between myofibrils and along the sarcoplasma. **C.** is a high magnification of a sarcoplasmic body displaying its electron density and irregular shape. Unpublished images from personal collection.

1.2 INHERITED ATAXIAS

The term *ataxia* is derived from the Greek word for *without order*, *disorder* and illustrates the main ataxia symptoms; disturbance of gait, speech, eye movements and fine motor skills. Ataxia is a common symptom in neurological practice and ataxia of non-genetic origin can be secondary to e.g. alcohol abuse, trauma, multiple sclerosis, paramalignant causes or coeliac disease (58). These will not be further discussed here.

When investigating a patient with ataxia, one must bear in mind that some ataxias, not only acquired ones but also some of genetic origin, are treatable. The initial steps in the investigation should therefore aim at identifying those conditions.

Primary coenzyme Q10 deficiency syndromes are caused by deficiencies in the co-enzyme Q10 (ubiquinone) biosynthetic pathway. The most common symptom of primary coenzyme Q10 deficiency is ataxia, but several other symptoms are often present. This group of syndromes are due to biallelic pathogenic mutations in the genes encoding the enzymes for coenzyme Q10 synthesis*; PDSS1, PDSS2, COQ2, COQ4, COQ6, COQ7, COQ8A* (*ADCK3*), *COQ8B* (*ADCK4*), and *COQ9*. Primary coenzyme Q10 deficiency is important to identify since many of the conditions respond to high doses of ubiquinone (59). Other treatment options may eventually be available, i.e. with drugs bypassing the enzymatic defect (60).

Other treatable causes of ataxia are cerebrotendinous xanthomatosis, Refsum disease, Niemann Pick type C, Wilson disease and ataxia with vitamin E deficiency.

The heterogenous group of hereditary ataxias are usually associated with cerebellar atrophy and can be inherited dominantly, recessively, X-linked or as a maternally inherited mitochondrial syndrome.

Recessive ataxia syndromes most frequently have debut in childhood. Two examples are described briefly below.

The most common recessive ataxia is *Friedreich ataxia* (FRDA) with disease onset between 5-15 years of age and a frequency of 2/100 000. FRDA is almost exclusively caused by a homozygous GAA expansion in the *FXN* gene leading to a reduced expression of the gene product *frataxin*. Deficiency of frataxin leads to a progressive neurodegenerative disease which affects the cerebellum, spinal cord and the myelin sheaths of the peripheral nerves. The heart is involved in most cases causing hypertrophy, dilation and cardiac arrythmias. Frataxin has been shown to be involved in iron-sulphur cluster biogenesis and cause mitochondrial dysfunction, but the exact biochemical processes are poorly understood (61).

Ataxia Telangiectasia presents in childhood with progressive cerebellar ataxia. Later a systemic disease with conjunctival telangiectasias, immune deficiency and malignancies develop. The disorder is caused by mutations in the *ATM* gene and the gene product, a phosphatidyl-3-kinase, is involved in phosphorylation of substrates involved in DNA repair and cell cycle control (62).

1.2.1 Autosomal dominant cerebellar ataxia

Autosomal dominant cerebellar ataxias (ADCAs) generally have later age of onset than recessive ataxias but overlap regarding debut age exists. ADCAs can in turn be categorized as spinocerebellar ataxias (SCA), currently numbered 1-48 (OMIM 2020-11-10), four different episodic ataxias (EA) and one spastic ataxia (SPAX1) (63). This background will focus on SCAs.

The most common and earliest identified SCA syndromes (SCAs), e.g. SCA1, SCA2, SCA3, SCA6, SCA7, SCA12, SCA17, and DRPLA, are all caused by heterozygous CAG trinucleotide expansions within the protein coding regions of the respective genes. The trinucleotide CAG encodes glutamine and thereby introduces a polyglutamine stretch that affects function of the respective gene products. The CAG repeat length is unstable and tends to increase in successive generations, a phenomenon known as anticipation. This often results in earlier age of onset and more severe disease in subsequent generations of the family. Molecular testing for the different SCAs is both sensitive and specific once the expansion is known. Genetic counselling is, however, not straightforward since disease prognosis cannot be concluded from repeat length only. There are, for instance reports of both mosaicism and the opposite of anticipation, contraction of the repeat length, that complicate the picture (64, 65). Not all SCAs are caused by CAG expansions, SCA8 is caused by another trinucleotide repeat, CTG, and SCA10 is caused by the pentanucleotide repeat ATTCT. Several SCAs are caused by missense mutations, examples are SCA5 caused by mutations in the *SPTBN2* gene encoding an isoform of β-spectrin with high expression in the cerebellum and SCA14 caused by mutations in *PRKCG* encoding protein kinase Cγ with highest expression in cerebral cortex and the spinal cord. SCAs caused by missense mutations are rarely associated with anticipation, with the possible exception of SCA5 (66).

There are still several SCAs awaiting molecular characterization, for example SCA18 linked to 7q22-q32 and SCA20 linked to 11q12. SCA4 linked to 16q22.1 is another example of a unique SCA where the chromosomal region has been identified by linkage analysis, but the molecular cause is unknown. The first description of SCA4 was from a kindred of Scandinavian origin residing in Utah investigated by Flanigan et al in 1996 (67). The disorder was characterized by ataxia of late debut at a median age of 39.3 years. Apart from ataxia somatosensory axonal neuropathy was seen in all affected subjects. Linkage analysis was performed and resulted in a maximum LOD-score of 5.93 in a region of 6cM on chromosome 16.

The second description of SCA4 was of a five generation German pedigree published by Hellenbroich et al in 2003 (68). The affected individuals in this family had ataxia with debut at a median age of 38.3 years and all affected had axonal sensorimotor neuropathy. Linkage analysis showed, as in the Utah family, linkage to 16q22.1. Due to recombination events the group could narrow the chromosomal region to 3.69 cM. The group also screened the region for CAG/CTG repeats and found no expanded alleles and concluded that the disorder must be caused by another sort of expansion or by a different kind of mutation.

Both the group from Utah and the German group found a tendency to anticipation with earlier disease onset and worsening of symptoms in successive generations, but this suspicion of anticipation could not be statistically proven due to small sample sizes. Characterization of a large Swedish family with apparent spinocerebellar ataxia type 4 forms the second part of my PhD project.

1.3 INHERITED METABOLIC DISORDERS

The term inborn errors of metabolism was coined by Archibald Garrod in 1908, and later in a publication describing alkaptonuria and several other disorders (69). Inborn errors of metabolism comprise a huge number of diseases almost exclusively caused by defects in enzymes or transporters. Each individual disease is rare but since the number of disorders is so great the number of affected individuals is significant. Metabolic disorders can affect virtually every organ or tissue and impact on the nervous system is very common.

Figure 13. Sir Archibald Garrod

Metabolism can be divided in different pathways, e.g. the synthesis or breakdown of a substance or molecule. In metabolic disorders pathways become disrupted, leading to either accumulation of substrate, toxic metabolites, deficiency of product or impaired energy production.

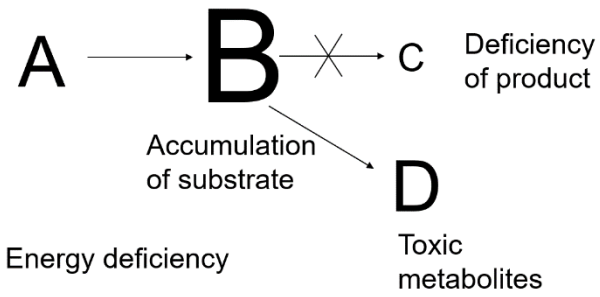


Figure 14. Illustration of a metabolic block

1.3.1 The methionine cycle

One very important biochemical pathway is the one carbon metabolism in the folate and methionine cycles (Fig 15). A correct function of these cycles is essential to provide methyl groups to a great number of methyl transferases, to produce cysteine and several other essential processes. There are many known defects of this pathway, both genetic and non-genetic. A common and well-known non-genetic cause of defective cycle function is deficiency of vitamin B12. This deficiency causes elevated homocysteine since methionine synthase requires B12 as a cofactor when a methyl group is donated from N-methyl tetrahydrofolate (N5-MTHF) to homocysteine to form methionine. Among the genetic causes the perhaps best known is homocystinuria, which is caused by biallelic mutations in the *CBS* gene encoding cystathionine β-synthetase. In this disorder both homocystein and methionine become elevated and cysteine synthesis is decreased. Five other genetic causes of elevated methionine are known; Mat I/III deficiency, Glycine-n-methyltransferase deficiency, S-adenosylhomocysteine hydrolase deficiency, citrin deficiency and tyrosinemia type 1 (70). In the latter disorder the defective enzyme is not a part of the methionine or folate cycle.

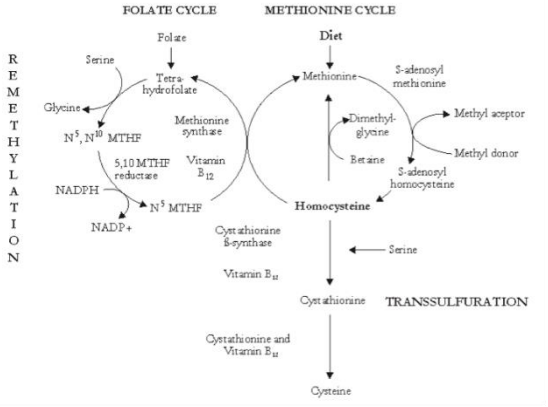


Figure 15. Folate and methionine cycles

Instead the defective enzyme fumarylacetoacetate hydrolase is involved in tyrosine metabolism and the defect causes accumulation of the toxic metabolite fumarylacetoacetate, which is a strong inhibitor of the MAT I/III enzyme.

Paper IV is about the biochemical, genetic and clinical characterization of a seventh disorder causing hypermethioninemia.

1.4 GENETIC DIAGNOSIS IN ACUTELY ILL INFANTS

Inherited metabolic disorders with debut in early infancy are often treatable but difficult to diagnose (71). The proportion of infants admitted to neonatal intensive care unit (NICU) due to metabolic disorders vary in different studies but tend to be under 1% (72) and the proportion with any genetic defect is unknown but thought to be high (73). Newborn screening is conducted in most Western countries to make a presymptomatic diagnosis but identifies only a subset of disorders. In Sweden, for example, 25 disorders (23 metabolic) of the over 1000 known metabolic disorders are included in the screening program (74), which is centralized to the Centre for Inherited Metabolic Diseases (CMMS) at Karolinska University Hospital. CMMS also performs biochemical and genetic follow-up of positive screening results. Occasionally a metabolic disorder presents before the results from the newborn screening is available, or even before the sample is taken (75). In metabolic disorders the typical symptoms are metabolic acidosis, lactic acidemia, hyperammonemia or severe hypotonia/floppy infant. In these cases, analysis of standard parameters as lactic acid, ammonia, blood gases, liver enzymes and CK can often give clues to the disease category but this is rarely enough for a specific diagnosis. More specialized methods, including urinary organic acids, plasma amino acids and plasma acyl carnitines, can sometimes lead to diagnosis if the baby is affected by an intoxication type metabolic disorder, but unspecific metabolite alterations due to feeding, medication, vitamin deficiencies and insufficient sample volumes often make diagnosis challenging (71). Specialized metabolic testing is time-consuming and diagnostic delay can worsen patient outcomes, making an alternative or complementary approach desirable.

2 AIMS

- To perform a detailed clinical characterization of sarcoplasmic body myopathy including neurophysiologic assement, muscle morphology and imaging
- To molecularly define sarcoplasmic body myopathy by linkage analysis, sequencing of candidate genes and next generation sequencing after custom capture of the linked chromosomal region. To elucidate the pathogenetic process whereby the muscle phenotype in Sarcoplasmic body myopathy evolves
- To perform a detailed clinical phenotyping in a large Swedish family with apparent SCA4 and to perform genetic investigations and study pathogenetic processes leading to damage in the cerebellum and periferal nerves
- To perform a detailed biochemical and clinical assesment in a family with hypermethioninemia and to find the underlying cause of disease through massive parallell sequencing
- To perform a proof-of-concept study to illustrate the value of adding rapid WGS to the diagnostic work-up in acutely presenting neonates with suspected IEM

3 METHODS

A broad range of diagnostic methods have been used in the different projects.

Clinical investigations	**Used in paper**
Rating scales; Hospital Anxiety and Depression Scale (HADS) Scale for the Assessment and Rating of Ataxia (SARA) Inventory of non-ataxia Symptoms (INAS)	III
Biochemical analysis	
CSF analysis of markers of neurodegeneration, monoamine metabolites, AdoMet and AdoHcy	III and IV
Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS)	II
Specialized metabolite analysis (amino acids, acyl carnitines, very long chain fatty acids etc.)	III, IV and V
Enzyme activity measurement of SAHH activity in fibroblasts	IV
Cloning, expression and determination of activity in mutant ADK enzyme.	IV
Imaging	
MRI of muscle, heart or brain	I, II, III and IV
Peripheral nerve magnetization transfer ratio and peripheral nerve diffusion tensor MRI	III
[11C]Flumazenil-PET and [18F]FluoroDeoxyGlucose (FDG)-PET	III
Pathology	
Neuropathological studies and immunohistochemistry of CNS	III
Muscle biopsy, morphological investigations with light- and electron microscopy	I, II, III and IV
Fourier transform infrared microscopy (μFTIR)	II
Neurophysiology and Physiology	
Electroneurography (ENeG), electromyography (EMG)	I, II, III and IV
Quantitative sensory test (QST), variability of RR-interval, skin sudomotor response (SSR) and ambulatory polysomnography with Embletta	III
Spirometry	I
Cardiac ultrasound	I and II
Electrocardiogram (ECG)	I and III
Genetic investigations	
Polymerase chain reaction (PCR) and Sanger sequencing	II, III and IV
Whole exome/genome sequencing (WES/WGS)	IV and V
Custom capture followed by massive parallel sequencing	II and III
Multipoint linkage analysis and haplotype analysis with microsatellite markers	II and III

4 ETHICAL CONSIDERATIONS

Below is a discussion of the ethical considerations that have been raised in the studies constituting this thesis. The ethical implications have been extensively discussed within the research group. In sarcoplasmic body myopathy, paper I and II, I alone have had most contacts with the research subjects. For autosomal dominant ataxia, paper III, we have been two investigators leading the project and had many discussions among ourselves and with our supervisors on how to conduct the research in an ethically correct manner. For the studies involving minors, paper IV and V, the situation is a bit different. These studies all involved children with severe recessive disorders and communication has exclusively been with the children's guardians. All projects have been approved by the regional ethics committee.

4.1 GENERAL REMARKS

When performing genetic studies or testing in humans it is of paramount importance to adhere to consensus ethical guidelines (http://www.eurogentest.org/index.php?id=645, https://www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/Practice-Guidelines.aspx).

4.2 SARCOPLASMIC BODY MYOPATHY

All patients and unaffected carriers entering the studies reported in paper I and paper II have received written and oral information before signing the informed consent document. Early on we realized that inclusion bodies could be detected in muscle biopsies decades before the onset of clinical symptoms. This finding was evident long before linkage analysis enabled us to make the diagnosis with haplotype analysis or, even later, by direct sequencing of the mutated gene.

When discussing the possible inclusion of a new individual in the studies we gave extensive information of what was known about the disease, it's inheritance and it's predicted course. We also offered the right for each person to remain unknowing of their disease status, meaning that we refrained from communicating the results from various investigations such as muscle biopsy, linkage analysis and genetic investigations. Minors, who are all asymptomatic, were told they had to wait until they reach legal age (18 years) before entering the study, parents were not allowed to enter their children.

Several of the patients opted for not knowing their disease status. These individuals were kept uninformed, either until they developed symptoms or until they changed their minds and wanted to be informed if they were affected or not. In case a study subject wished to be informed of his or her disease status we used an approach similar to what is done in genetic counseling for Huntington disease (76) and other severe late onset dominant diseases.

For pre-symptomatic persons wishing to either know the results of previous investigations or to have a presymptomatic test, a personal visit was scheduled. On this visit, which was almost exclusively hosted by me, extensive disease information and the current status of the research project were conveyed. We then proceeded to discuss why the individual wanted testing and

whether knowledge of disease status would do any good at all in their current life situation. In several instances the testing or conveying of previously known information was wanted because the individuals were planning to have children and did not wish to propagate the disease to following generations. After exploring the strength of motivation for testing a description of the testing procedure was given. The visit then continued with a discussion what the result would mean to the individual as both a positive and a negative result could have impact of the person's well-being. According to my experiences a positive result may lead to a life crisis and a negative result may elicit a reaction similar to "survivors' guilt". After leaving room for questions the visit then ended without making any decision to test or not but instead the individual was told to think the decision through for a minimum of one week's time.

The follow up was usually made by a phone call. If the individual still wanted testing/knowledge of results a new visit was booked to communicate the results. No results have been communicated by phone and the persons receiving the results of testing were encouraged to be accompanied by someone to support them. When the visit for result communication took place it always started with me asking if they still wanted to be informed of the result, as this is something that can never be undone.

The results were then revealed as plainly as possible. Strong emotional reactions were the rule, whether the result was positive or negative. Often renewed disease information was necessary. For persons with especially strong reactions counselors connected to the neuromuscular team were available and some of the patients underwent crisis therapy. Regardless of the result follow-up questions from the tested person were common and the persons therefore received both my email address and my telephone number and were told not to hesitate to take contact.

Prenatal diagnosis has been performed in several instances. One couple where the disease status was known in one of the parents underwent preimplantation genetic testing (PGT), sometimes referred to as preimplantatory genetic diagnosis (PGD). By this procedure the first step is to take a semen specimen from the to-be father and to collect oocytes from the to-be mother after ovarian stimulation. An in-vitro fertilization is then made, and embryos are investigated for the disease-causing mutation followed by implantation of an unaffected embryo. In other instances, where the person at risk did not want to know the disease status, we chose a different approach through a naturally induced pregnancy followed by a chorion villus biopsy. That biopsy was then used for grand parental exclusion test using the alleles from the subject's grandparent to exclude that the disease allele had been inherited to the fetus without revealing disease status to the future parent. The same procedure can be used in PGT if the couple wishes to have that procedure.

4.3 SPINOCEREBELLAR ATAXIA TYPE 4

In this project all subjects receive extensive oral and written information regarding the disease we study, including the different investigations we plan to perform and the genetic

investigations that are underway. Study subjects are then invited to enter the study and sign the consent forms. For a long time, we lacked pre-symptomatic testing for this disorder and didn't have access to pre-symptomatic biomarkers. A possibility may be to use pre-symptomatic signs of autonomic disturbances. Now the situation is different when we have been able to establish a haplotype with strong linkage to the disease locus enabling pre-symptomatic testing. This has not yet been requested from any of the family members, but there will most likely arise such requests. We will, when the issue comes up, adopt the same strategy as when testing for sarcoplasmic body myopathy.

4.4 ADENOSINE KINASE DEFICIENCY

The two Swedish children included in this study were both severely ill with an intellectual involvement that made direct communication with them impossible. One sibling was deceased. Instead the parents were informed about the ongoing research from their pediatrician and from myself and my supervisor. When whole exome sequencing became available, we travelled to Dalarna North of Stockholm to meet the surviving older male sibling, the parents and the responsible pediatrician. This made a more comprehensive discussion possible. I also had an appointment for genetic counseling with the healthy brother and his spouse.

4.5 RAPID PULSED WHOLE GENOME SEQUENCING

In this project the parents of the investigated infants were informed about the project before signing informed consent. After investigations they were also informed about the results. In the case of the child included prospectively, in whom we could not find a genetic cause of the condition, a thorough metabolic workup was performed that did not reveal any indication of a metabolic disorder. This child had a lactic acidosis of unknown cause that subsided spontaneously for unknown reasons after exclusion of primary and secondary causes.

5 RESULTS

5.1 SARCOPLASMIC BODY MYOPATHY, SBM

I became involved in this project 1999 when I had recently started my training to become a neurology specialist at Karolinska University Hospital. The early work in the SBM family of professor Edström et al had been followed up by a linkage analysis that showed linkage to chromosome 22 (unpublished).

I started extended investigations of additional members of the family in year 2000, most of them descendants of the initial affected members from the publications of Edström et al (41, 56).

5.1.1 Paper I

When I started out to further define the phenotype of sarcoplasmic body myopathy (SBM) we realized that the highly unusual inclusions in muscle tissue were pathognomonic of the disease leading to the hypothesis that they might be present presymptomatically. I then contacted the different members of the family and offered all at-risk persons inclusion in the study. In this process it became evident that individual II:2 and his descendants were related to the rest of the family due to a common ancestor (I:2) interlinking the two branches, see pedigree (Fig 16). All newly identified family members were offered to take part in the study, without being informed of the results if they wished. Nine persons accepted to be included. Some declined, two since they did not want to undergo the investigations, a third thought the research was futile and the rest lived in faraway locations and declined for logistical reasons.

The research protocol included a detailed history and manual muscle testing (MMT) (77) of all relevant muscle groups. Also included in the protocol were laboratory testing for CK, alpha-tocopherol, muscle biopsies of the anterior tibial muscle or vastus lateralis and neurophysiology testing including electromyography (EMG), electroneurography (ENeG) and quantitative sensory testing (QST). To visualize the distribution of muscle affection muscle MRI was performed in three individuals, two symptomatic and one pre-symptomatic. Spirometry was included to investigate whether affected respiration could be detected at early disease stages. At the time no patients had complained of or died from heart affection. In order to address this issue electrocardiogram (ECG) and cardiac ultrasound were included in the protocol.

In this study I did all clinical investigations myself and performed the neurophysiology examinations (under supervision from an experienced neurophysiologist). I also handled all communication with the family, constructed the pedigree and wrote the first draft of paper I. Spirometry, MRI and cardiac investigations were performed in the respective hospital laboratories.

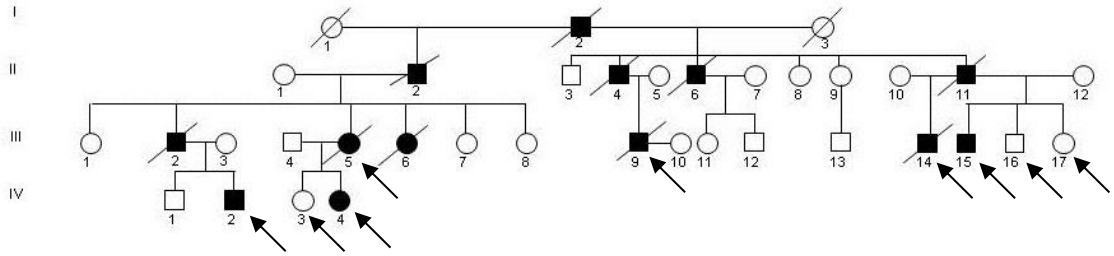
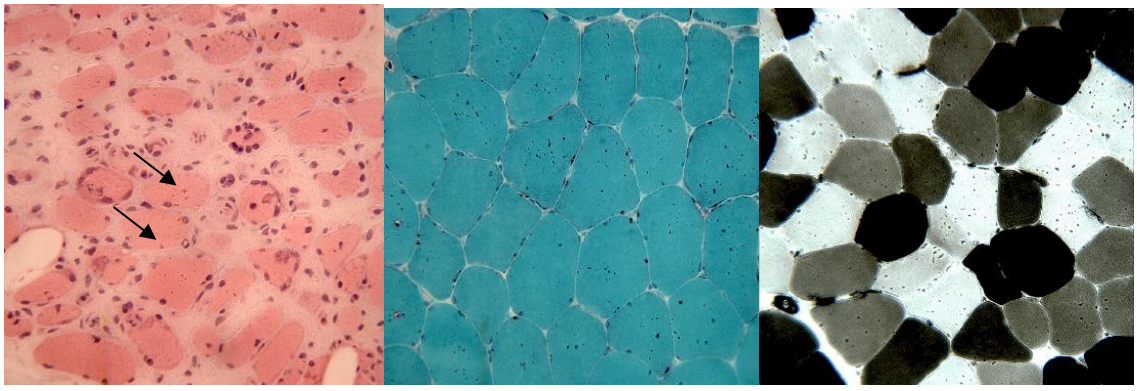


Figure 16. Pedigree of Swedish family with sarcoplasmic body myopathy showing a common ancestor in individual I:2. Individuals included in paper I are marked with arrows. Affected individuals are defined as those who have both presence of inclusions and elevated creatinine kinase (CK). Individuals not wishing to be informed of their disease status are excluded.

An early symptom noted in the previous studies was weakness of the thenar muscles between the thumb and the index finger. In paper I, I show that some of the individuals have proximal weakness with difficulty rising from chairs and walking stairs as initial complaint. An initial complaint can also be symptoms only at exertion, as one patient (IV:4) complained of muscle fatigue without associated weakness. From interviews and clinical investigations, I could conclude that, although two subjects had distal hand weakness (III:5 and III:9), proximal weakness seemed to be a far more common first complaint than distal weakness. The most affected muscle groups were the hip flexors.

Muscle biopsies were obtained from all nine individuals and showed inclusions in six (IV:2, IV:4, III:7, III:9, III:14 and III:15) (Fig 17, Table 3). It became evident that the sarcoplasmic inclusions could indeed be seen before overt clinical symptoms appeared, as seen in three patients (IV:2, IV:4 and III:15). In individuals with clear disease symptoms there were, in addition to inclusions, signs of unspecific myopathy with increased number of centralized nuclei, variation in fiber size and fibrosis. In the pre-symptomatic individuals muscle morphology was completely normal except for the sarcoplasmic inclusions.



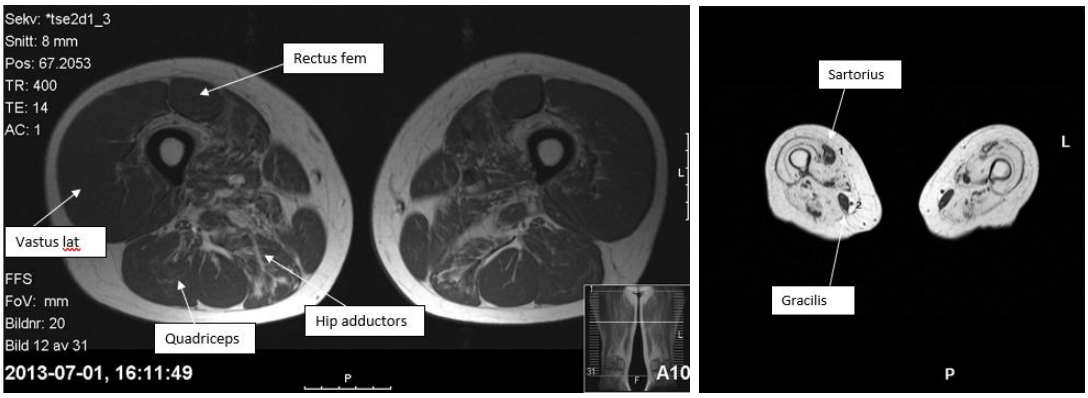
A B C

Figure 17. Muscle biopsy sections from patients affected by SBM. **A** Hematoxylin eosin staining from patient III:9 in pedigree (Fig 16), taken approximately 10 years after debut of symptoms. There are unspecific dystrophic features with increased centralized nuclei, variability in fiber dimension and increased connective tissue. Sarcoplasmic inclusions can be seen scattered in sarcoplasm (arrows). **B** Gomori trichrome stain from a biopsy taken pre-symptomatically from individual III:15 showing an abundance of sarcoplasmic inclusions in otherwise normal muscle. **C** ATP:ase staining showing type 1 fibers (white), type 2B fibers (grey) and type 2A fibers (black). Sarcoplasmic inclusions are seen in all fiber types.

In the laboratory investigations all affected individuals had slightly increased CK, varying between 1.5-4 times the normal level. One unaffected female individual, IV:3, had slightly increased CK of unknown reason. In order to try to exclude deficiency of vitamin E we measured alpha-tocopherol in blood, which was normal in all subjects.

Because of the presumed debut with distal muscle weakness neurophysiologic studies were performed that ruled out neuropathy as contributing to the distal weakness. From the neurophysiologic study we learned that myopathic signals could be detected pre-symptomatically only in the iliopsoas muscle in two subjects (IV:2 and IV:4). There were no consistent signs of neuropathy except in one individual who had concomitant hepatitis C.

MRI of both proximal and distal muscles of the upper and lower extremities were obtained from three individuals (Fig 18).



A B

Figure 18. **A** T1 image from the thigh of Swedish individual III:15 four years after onset of symptoms. Vastus lateralis and rectus femoris are not much affected but signs of degeneration can be seen in hip adductors and knee flexors. **B** T1 weighted image of individual III:5 19 years after onset. Note the relative sparing of gracilis and sartorius muscles in the otherwise generalized fatty atrophied musculature. Unpublished images from personal collection.

Cardiac ultrasound, ECG and spirometry showed no signs of early affection of either the heart or the respiratory systems.

Table 3 summarizes the clinical and laboratory findings of the nine individuals investigated for paper I.

Subject/ sex	Age at study (years)	Onset age (years)	Debut symptoms	Hip flexor strength (MMT 0–5)	Muscle atrophy	Biopsy findings	CK×UNL ukat/L	Neurophysiology (EMG, ENeG, QST)	Initial Spirometry
IV:2/M	43	–	Asymptomatic	4†	–	Inclusions, normal structure	1.5 × normal	Myopathy	Normal
IV:4/F	43	42	Muscle fatigue	4†	–	Inclusions, normal structure	2 × normal	Myopathy	Normal
IV:5/F	44	–	Asymptomatic	5	–	Normal	1.5 × normal	Normal	Normal
III:7/F	68	39	Hip flexor weakness	0–1	General weakness	Inclusions, myopathy	Normal	Myopathy*	impaired
III:9/M	51	43	Hip flexor weakness	0–1	General weakness	Inclusions, myopathy	2 × normal	Myopathy	Impaired
III:14/M	51	51	Hip flexor weakness	4	Slight thenar atrophy	Inclusions, normal structure	2.5 × normal	Myopathy and neuropathy	Normal
III:15/M	35	–	Asymptomatic	5†	–	Inclusions, normal structure	4 × normal	Normal	Not available
III:16/M	32	–	Asymptomatic	5	–	Normal	Normal	Normal	Normal
III:17/F	39	–	Asymptomatic	5	–	Normal	Not available	Normal	Normal

Table 3 with an overview of characteristics of the nine individuals included in paper I. Four of the affected individuals had complaints of muscle symptoms, two of the affected were pre-symptomatic and three were non-affected. In one of the presymptomatic cases EMG changes were detectable only in the iliopsoas muscle. All the affected and one of the unaffected had increased CK at the time of the study. The reason for the increased CK in the unaffected is unknown. The hallmark of the disease, the characteristic sarcoplasmic inclusions, could be seen in all affected subjects, presymptomatically in two subjects. MMT, manual muscle testing; CK, creatine kinase; UNL, upper normal limit; EMG, electromyography; ENeG, electroneurography; QST, quantitative sensibility testing. * Subject III:7 was investigated at another center. † In these subjects the biopsied muscles, tibialis anterior or vastus lateralis, were of normal strength and morphologically normal except for numerous sarcoplasmic inclusions. Numbers correspond to generation and subject number in pedigree in Fig 16.

5.1.2 Paper II

In parallel with the clinical studies and as more individuals in the family became affected or were otherwise located, an extended linkage analysis focusing on chromosome 22 was performed by our group. This resulted in the highest LOD-score, 5.8, in a region on chromosome 22 containing approximately 100 genes (Fig 19).

The myoglobin gene (*MB*) was in this region but was initially not considered a primary candidate gene since the mechanism of disease seemed to be oxidative damage. Instead we sequenced a few candidate genes thought to be involved in redox biology, e.g. *TXNRD2*, encoding mitochondrial thioreoxin reductase 2, without finding any disease-causing variants. We also found accumulation of intermediate filaments with an estimated weight of 55 kD seen in electron microscopy. An early hypothesis was that the filaments, and possibly the inclusions, were aggregated defective desmin filaments. Desmin filaments have a similar molecular weight (53 kD) as the identified filaments, but sequence analysis of the *DES* gene encoding desmin did not detect any pathogenic variants.

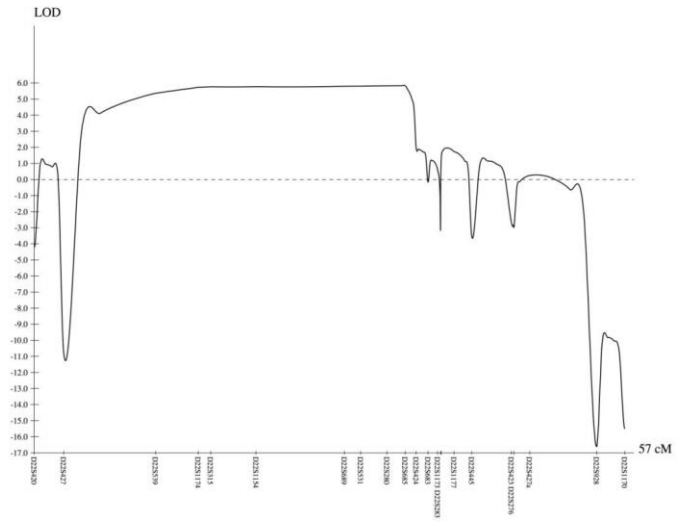


Figure 19. Multipoint LOD (logarithm of the odds) score plot of the linkage region identified on chromosome 22 for the Swedish SBM family. A LOD-score above 3 is usually considered significant and represents roughly a 1000 to one odds that the disease and the marker are linked. In this case the chance is $10^{5.8}$, translating into an odds of 600 000 to one that the disease gene and the linkage region are linked in the family.

The years went by and technology advanced. When next-generation sequencing became available we designed a custom capture of the entire linked region and flanking genomic sequences, in total comprising over 400 genes. This was performed in eight family members, 6 affected and 2 unaffected. After bioinformatic analysis the top candidate gene was *MB*, where the variant c.292C>T (p.His98Tyr) was found in all affected individuals but not in the two unaffected persons.

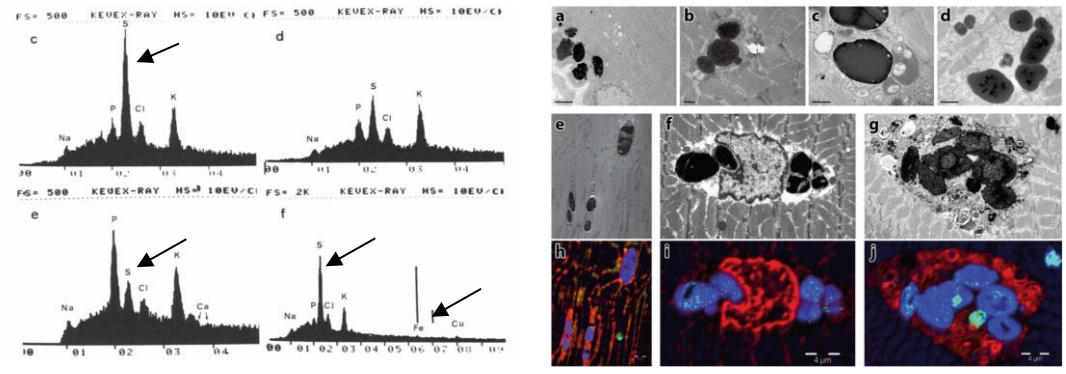
The *MB* gene product, myoglobin, was the first protein to have its 3D structure described by X-ray crystallography (78). Since mice where the equivalent gene was knocked out showed no obvious muscular phenotype (79), we realized that it would be a difficult task to prove that the mutation had functional effects. Attempts to use morpholino antisense oligonucleotides (80) in zebra fish were unsuccessful. The fish behaved normally, and no muscle inclusions developed, possibly because the effect of morpholino treatment was transient or the time was too short to develop muscle inclusions. However, our favored hypothesized mechanism was a slowly developing pathology due to a toxic gain rather than loss of function. We were in the process of trying to study the oxygen dissociation curve in homogenized muscle specimens when I got contacted by Professor Nigel Laing in Perth, Australia, who in turn collaborated with Professor Montse Olivé in Barcelona, Spain. They were investigating patients from two families, from Mallorca and mainland Spain, who had similar phenotypes and morphological findings. The Australian group performed genetic investigations with a different approach compared to us. Instead of linkage analysis followed by custom capture this group used whole exome sequencing from three affected members of the two families followed by filtering for genes encoding proteins with high expression in skeletal muscle from the FANTOM5 project (81). This strategy resulted in finding the same mutation in *MB* in both

families, c.292C>T (p.His98Tyr), which is identical to the mutation found in the Swedish family.

Together we decided to prepare a joint publication. My part in this work was to design the study in collaboration with the Spanish-Australian group. I also coordinated all contacts between the Swedish group and other collaborators. My close and long-standing contact with the family proved to be crucial since new muscle samples were needed for different functional studies and availability of patients from the other families was very limited. I also provided images from light and electron microscopy, MRI images, clinical and laboratory data, linkage analysis and data from custom capture and MPS. The Swedish group did the haplotype analysis and Sanger sequencing from the other families and my responsibility was to coordinate shipping and registration of samples and results. The biochemical and physical characterization of the *MB* mutation was performed in collaboration with groups in Italy, Spain and Austria. I also wrote drafts of the manuscript.

Later three more families, two from France and one from the Netherlands, were identified. The functional validation experiments were based on a hypothesis of a toxic gain of function as a result of the mutation. The rationale for this was that myoglobin knockout mice did not develop muscle weakness and survived into adulthood displaying several adaptations to the lack of myoglobin without developing any inclusions.

Nanoscale secondary ion mass spectrometry (NanoSIMS) analysis of the sarcoplasmic inclusions showed increased signals for sulphur and iron, signs of oxidative damage and myoglobin degradation with possible lysosomal iron accumulation (82, 83). The high sulphur and the somewhat increased iron content were known before. When Edström et al in (56) studied X-ray spectra combined with electron microscopy in scanning mode in different cellular compartments of muscle tissue they found increases of both sulphur and iron peaks (Fig 20).



A **B**

Figure 20. Comparison of findings from (56) published 1981 and paper II published 2019. **A** X-ray spectra from sarcoplasmic bodies (c and f) show high sulphur peaks (arrows). The sulphur peak is also demonstrated in the myofibrillar region containing sarcoplasmic bodies (d) and in cell nuclei (e). The spectrum from a sarcoplasmic body shows that, in addition to high Sulphur, there is also a small iron peak (f). **B** parts of fig 5 from paper II. Electron micrographs a-d show sarcoplasmic bodies of different appearance; under the sarcolemma (a), enclosed by a membrane (b), near vesicles (c), and in cardiac muscle (d). Micrographs e-g corresponds to the NanoSIMS images h-j. Blue indicates Sulphur (32S), red phosphorus (31P), and green (56Fe), respectively.

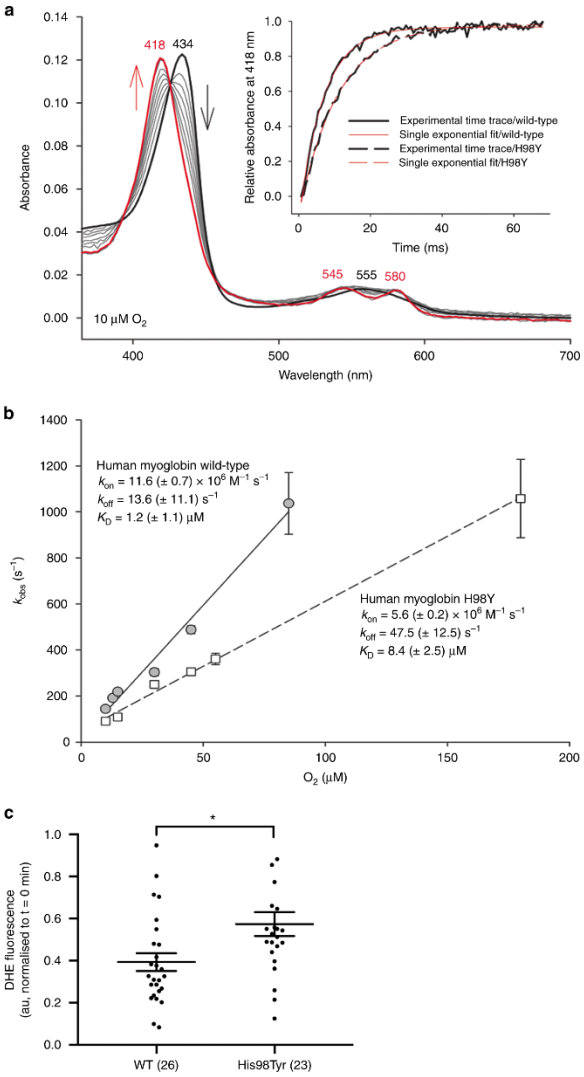
Further analysis with Fourier transform infrared microscopy (μFTIR) showed signs of lipid peroxidation, further strengthening the findings of oxidative damage.

Biochemical studies directed at the myoglobin binding of heme, the O2 -binding properties and the oxidative state of iron were also conducted. This showed that the dissociation constant for the heme prosthetic group in mutant myoglobin was significantly higher compared to wildtype myoglobin. The diffusion of heme to different cellular compartments may result in ROS-mediated oxidative damage (84). Next, we studied the oxygen-binding properties and found that mutated myoglobin had a significantly reduced affinity for oxygen compared to wildtype, which might affect the ability for mutant myoglobin to store oxygen. Finally, the oxidative state of iron was studied by measuring superoxide levels in HEK293FT cells expressing wild type (WT) and mutant myoglobin. The superoxide levels were slightly higher in cells expressing mutant myoglobin compared to WT. This could be an indication of increased ROS generation caused by free heme.

	$K_{D,\,O2}$ (μM)	k_{-H} at pH 7.0 (h^{-1}) [a]	k_{-H} at pH 5.0 (h^{-1}) [b]	$E^{\circ\prime}$ by spectroelectr. (V vs SHE) [c]	$E^{\circ\prime}$ by SWV (V vs SHE) [c]	k_{OX} at pH 7.0 (min^{-1})	Heme SASA (nm^2) [d]
WT	1.2 ± 1.1	0.22 ± 0.06	1.44 ± 0.09	+0.040 ± 0.005	−0.056 ± 0.015	1.96 ± 0.39	1.45 ± 0.04
His98Tyr	8.4 ± 2.5	1.19 ± 0.05	1.86 ± 0.05	+0.021 ± 0.005	−0.105 ± 0.015	1.97 ± 0.43	1.80 ± 0.05

Table 4. K_{-H}, the dissociation constant for heme at pH 7 and 5. Mutant myoglobin has 5 times higher K_{-H}, most likely caused by decreased interaction between heme propionate-7 and the mutant Tyr residue compared to the interaction between heme propionate-7 and the His residue in WT myoglobin. At pH 5 the K_{-H} is increased for both WT and mutant myoglobin but the difference in K_{-H} between WT and mutant myoglobin decreases, probably due to increased protonation of heme propionates resulting in reduced interaction with myoglobin. At pH 7 the increased tendency for heme to dissociate could cause heme mediated ROS activation resulting in oxidative damage to muscle cells. E°', the reduction potential of myoglobin, shows slightly lower values for mutant myoglobin that would thermodynamically favor auto-oxidation to MetMB even if k_{ox}, the autoxidation rate constant, is almost identical between WT and mutant myoglobin.

Figure 21. Kinetics of dioxygen binding to wild-type human myoglobin and the variant His98Tyr.



a) Spectral changes upon reaction of 1 µM ferrous wild type hMb (black line) with 10 µM O2. The final spectrum represents oxymyoglobin (red line, 68 ms after mixing). Gray lines represent spectra obtained at 0.68, 2.72, 4.08, 6.12, 8.84, 12.24, 34.00, and 51.00 ms after mixing. The inset depicts experimental time traces at 418 nm of wild-type hMb (solid black line) and p.His98Tyr MB (dashed black line) mixed with 10 µM O2 and corresponding single-exponential fits (solid red line, wild-type MB; dashed red line, His98Tyr MB).

b) Linear dependence of k_{obs} values from the O2 concentration for wild-type MB (gray circles, solid line) and p.His98Tyr MB (white squares, dashed line).

c) Basal intracellular superoxide levels in HEK293FT cells expressing WT or mutant MB-EGFP. Data presented as individual data points and the mean ± SEM, numbers in parenthesis represent n. *indicates p = 0.007 (Mann–Whitney test, two-tailed)

One interesting finding is that the same mutation was recurrent in all six families. To rule out that the families were distantly related to each other haplotype analysis on 3 Mbp adjacent to the MB gene was performed showing that the six families belonged to at least three different haplotypes (Table 5).

						Family 5 three sibs							
Microsatellite	Hg19 position	Family 1*	Family 2	Family 3	Family 4	1		2		3		Family 6	
D22685	34,595,593	302	302	310	306	306	306	306	306	306	306	314	314
D22S691	34,875,643	222	242/246	223	255	246	242	246	242	246	242	247	223
D22S1152	35,139,045	263	263	263	263	263	263	263	263	263	263	263	269
D22S1265	35,389,908	176	176	176	206	200	191	200	191	200	191	176	188
MB	36,002,811												
D22S277	36,271,500	162/166	162/166	156	150	160	160	160	160	160	160	166	158
D22S683	36,513,691	246	216	226	222	216	246	216	246	216	246	224	230
D22S692	37,125,545	156	160	160	152	160	160	160	160	160	160	160	156
IL2RB	37,536,285	245	251/255	257	245	259	255	259	255	259	255	255	245

Table 5. Microsatellite markers on 3 Mbp surrounding the *MB* gene in the six families. The Swedish family 3 differs in microsatellite marker from families 1-2 and 4-6 indicating no common ancestor and that the mutation has developed on different genetic backgrounds. In family 5 the three siblings, one affected and two unaffected, have identical microsatellite markers raising the possibility of a *de novo* mutation.

The finding of a possible *de novo* mutation in family 5 could imply that the mutation has arisen in a mutational hotspot and raises the possibility that more families with the disease are hitherto unrecognized. Unfortunately, the parents in family 5 were diseased and not available for genetic analysis, meaning that we could not prove that the mutation was *de novo*.

5.2 SPINOCEREBELLAR ATAXIA

In the year 1999 I first met a male patient born 1936. He was excluded from military service at age 20 due to areflexia, but debut of overt ataxia was at 42 years of age. At the time of investigations, he had both profound ataxia and symptoms of polyneuropathy. Apart from this one of the patient's main complaints was dizziness upon standing and attacks of profuse sweating. He also had erectile dysfunction since many years. A simple bed-side tilt test showed a blood pressure of 185/90 when lying down that dropped to 85/65 upon standing up.

The index case reported that several relatives, including his mother, had ataxia symptoms. The initial workup included MRI of the brain and spinal cord, neurophysiologic investigations including autonomic function testing and screening for common genetic causes of ataxia. In the workup analysis of very long chain fatty acids (VLCFA) to rule out adrenomyeloneuropathy, fat biopsy to investigate for amyloid accumulation and muscle biopsy was also included.

MRI of the brain in 1999 showed both supra- and infratentorial atrophy, most pronounced in the cerebellum where widened sulci were seen. MRI of the spinal cord was essentially normal except for slight degenerative changes.

Neurography (ENeG) showed a predominant axonal neuropathy with low amplitudes and only moderately reduced conduction velocities. EMG showed predominantly neurogenic abnormalities with high amplitude compound motor unit potentials. Quantitative sensibility testing (QST) showed complete absence of thresholds for all modalities, cold, heat and vibration, in the feet and elevated thresholds for vibration in the hands. Autonomic testing revealed profound affection of both the sympathetic and parasympathetic systems. Tilt test showed a significant drop in blood pressure when standing up. RR-variability, the change in heart rhythm in response to breathing, was only slightly decreased 1,017 (ref >1,098), but when the rate of change in heart rhythm was analyzed, the accelerations, the measurement became more significant. AI (acceleration index) was 1,2 (ref>4,5).

Genetic screening for common ataxia syndromes that included analysis for SCA1, 2, 3, 6 and 7, DPRLA, Friedreich ataxia and *CMT1A* was negative. Measurement of VLCFA was normal and a biopsy from abdominal fat showed no amyloid accumulation. Muscle biopsy showed neurogenic abnormalities with increased fiber type grouping and groups of atrophic fibers, but no signs of myopathy or mitochondrial dysfunction.

At this point I concluded that the patient must have a rare form of autosomal dominant ataxia and involved Martin Paucar, a colleague working subspecialized in ataxias. We hypothesized that the family was affected by SCA4.

5.2.1 Paper III

After designing the study and approval from the ethics committee we contacted relatives of the index case. Early on we met a second large family affected by ataxia, polyneuropathy and dysautonomia. This family was also included in the study.

In family 1 a total of seven affected persons were available for clinical investigations, for another six persons medical records were available, for an additional three subjects, history from relatives could confirm ataxia. In family 2 two individuals were available. The disease is inherited in an autosomal dominant fashion with equal affection of both genders. All affected subjects had ataxia, polyneuropathy and all, with the possible exception of one subject in family 2, had autonomic symptoms (Tables 6 and 7).

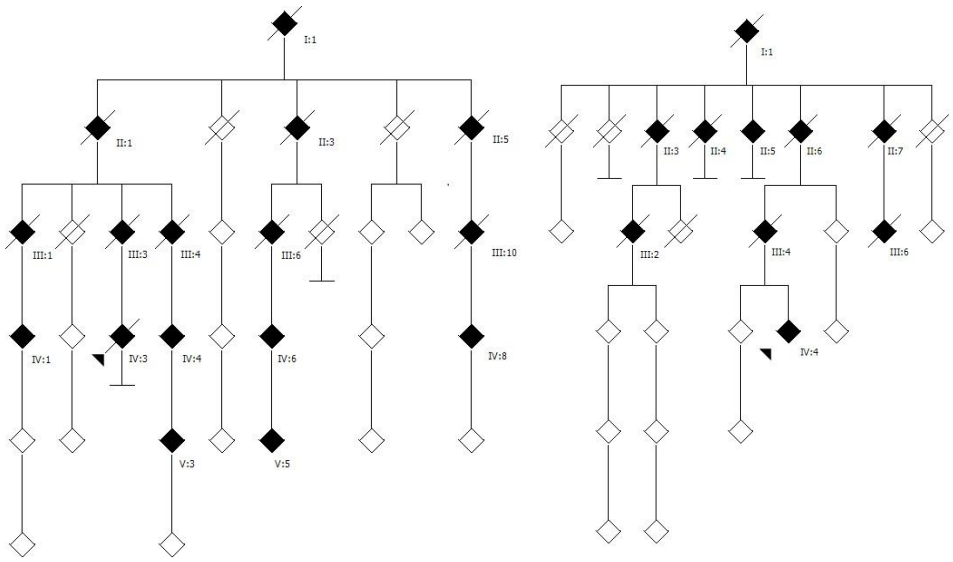


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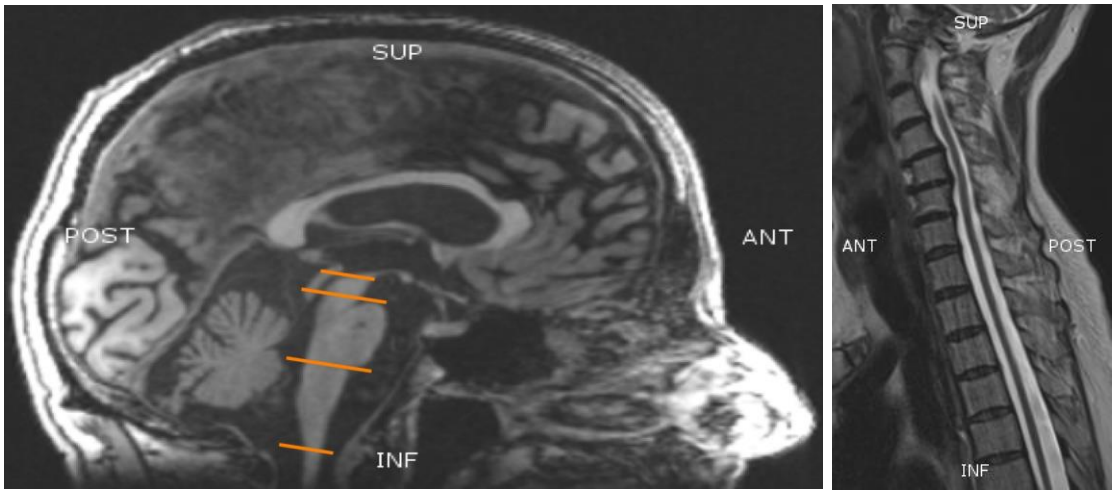
Figure 22. Pedigrees of Swedish family 1 and 2 with spinocerebellar ataxia. **A**. Swedish family 1. The index case in family 1 is number IV:3. This part of study includes 16 affected patients, 7 have been examined by us. Disease status of the remainder was corroborated by review of medical records and/or history. **B** Pedigree of family 2 with spinocerebellar ataxia. The index case is number IV:4, only this patient and the parent, III:4, have been available for investigation. The remaining subjects have been reviewed by medical records and/or by history.

Age of onset was 48.2 years in family 1 and 40 years in family 2. The clinical investigation indicated a combination of cerebellar and sensory ataxia in line with previous studies. Autonomic symptoms were almost universal in family 1 and present in the oldest patient in family 2 (III:4). Pedigrees of family 1 and 2 can be seen in Fig 22, clinical features are summarized in Table 6 and autonomic features are summarized in Table 7.

Neurophysiological investigations showed a predominantly axonal neuropathy in all investigated patients as shown in Table 6. All investigated patients also had small fiber impairment that was most prominent when heat thresholds were measured. Autonomic

dysfunction demonstrated in tilt test and pathological RR-variability was evident in all tested subjects. In Table 7 subject IV:4 is annotated as not having overt autonomic dysfunction since sympathetic skin response (SSR) was normal.

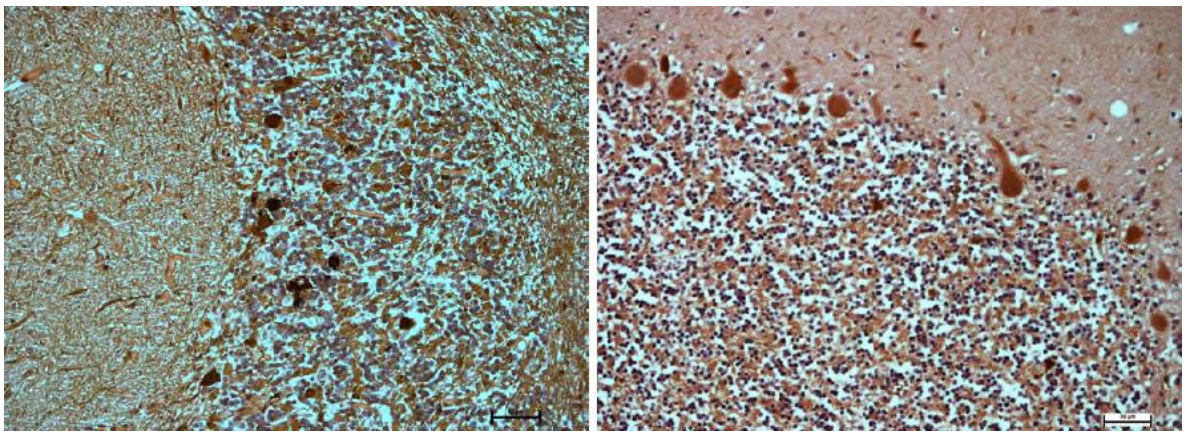
Four patients from family 1 who underwent MRI of the brain and spinal cord displayed significant atrophy of the vermis, pons, medulla and spinal cord. In family 2 subject IV:4 had atrophy of both vermis and pons, the spinal cord was not significantly atrophied. In individual III:2 atrophy of vermis was reported on CT-scan only. Figure 23 shows typical findings in brain and spinal cord MRI.



A **B**

Figure 23. **A** sagittal T1-weighted brain image in patient IV:1 from family 1 showing significant atrophy of the vermis and pons. Segmentation of mesencephalon, pons and medulla oblongata is illustrated with orange lines. **B** sagittal T2-weighted image of the upper spinal cord in patient IV:6 in family 1. Spinal atrophy is evident. SUP: Superior; ANT: Anterior; POST: Posterior; INF: Inferior.

Neuropathology was performed in two individuals, IV:3 from family 1 and III:2 from family 2. Histopathological investigations showed thinning of the granular layer of cerebellum and loss of Purkinje cells in both subjects (Fig 24). Investigation of the spinal cord showed loss of motor neurons in the anterior horn, also in both subjects. Investigation of the sural nerve showed unspecific and moderate loss of myelinated small fibers in three patients, by nerve biopsy in subject IV:1 from family 1 and by post mortem examination in IV:3 from family 1 and III:4 from family 2.



A **B**

Figure 24. **A**. Patient III:3 from family 1. **B**. Healthy control. Reduced number of calretinin positive cells in the granular layer of the cerebellum in SCA4. Also evident is loss of Purkinje cells as compared to a healthy control.

Pedigree	Pedigree 1													Pedigree 2		
Parameter and case	II:1	II:5	III:1	III:3	III:6	III:10	IV:1	IV:3	IV:4	IV:6	IV:8	V:3	V:5	III:2	III:4	IV:4
AO (years)	55	48	60	60	NA	50	45	42	50	50	40	58	20	35	50	35
Current age/age at death	83	75	82	78	61	92	76	78	88	66	62	64	37	90	76	54
DD	28	27	22	18	NA	42	31	36	38	16	22	6	17	55	26	19
Latest SARA score (age)	NA	NA	NA	NA	NA	NA	28.5 (75)	25.5 (77)[a]	28.5 (87)	26.5 (65)	24 (61)	9.5 (63)	5 (36)	30.5 (88)	NA	16 (53)
Dysartrhia (Dysar) Dysphagia (Dysph)	Dysar (+++) Dysph NA	Dysar (+) Dysph NA	Dysar (+) Dysph (++)	NA	NA	Dysar (+) Dysph NA	Dysar (+++) Dysph (+)	Dysar (+) Dysph (+++)	Dysar (++) Dysph (++)	Dysar (+) Dysph (+)	Dysar (+++) Dysph (++)	Dysar (+) Dysph (N)	Dysar (+) Dysph (++)	Dysar (++) Dysph (+++)	Dysa (++) Dysph (++)	Dysar (++) Dysph (+)
Nystagmus	NA	N	N	NA	NA	N	Y	N	Y	Y	Y	N	N	N	NA	N
Opthalmoplegia	NA	N	N	NA	NA	N	Vertical palsy	Partial vertical palsy	Vertikal palsy	N	Y	N	N	N	NA	N
Hypometric or hypemetric saccades	NA	N	NA	NA	NA	N	Hypom.	Hypom	Hypom.	Both	Hypom	Both	N	Hypom	NA	Hypom
Slow saccadic eye movements	NA	NA	N	NA	NA	NA	Y	Y	Y	N	Y	N	N	N	NA	N
INAS score	NA	NA	NA	NA	NA	NA	4	5	4	3	5	3	1	3	NA	3
Areflexia	Y	Y	Y	NA	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Polyneuropathy on neurography	NA	NA	NA	NA	NA	NA	Y	Y	NA	Y	Y	Y	Y	Y	NA	Y
Small fiber neuropathy	NA	NA	NA	NA	NA	NA	Y	Y	NA	Y	NA	NA	NA	NA	NA	Y
Loss of vibration	NA	Y	N	NA	NA	NA	Y	Marked impairment	Y	Marked impairment	Y	Y	N	Y		Y
Dysautonomia	Y	Y	Y	NA	NA	Y	Y	Y	Y	N	Y	Y	Y	Y	N	N
Dystonia (D) and/or chorea (Ch)	Ch	N	N	NA	NA	N	N	N	N	N	N	D	N	A	D	D
Upper motor signs	Y	Y	Y	NA	NA	N	Y	Y	N	Y[c]	N	Y	N	Y	N[d]	N
Other abnormalities	Hearing loss Weakness Pes equinovarus	Hearing loss	Weakness	NA	NA	NA	Myok. Minipolym.	Myok. Minipolym. Weakness	Pain Fasciculat.	Hearing loss Mirroring Myok.	Mirroring Myok. Minipolym.	Myok.	N	Hearing loss Minipolym.	NA	Blepharosp. Myok. Minipolym Laryngosp.
Atrophy in cerebellum and/or pons	NA	NA	NA	NA	NA	NA	Cerb Pons	Cerb Pons	Cerb[b]	Cerb Pons	NA	Cerb	Cerb Pons	Cerb	Cerb[d]	Cerb[e]

Table 6. SCA4 pedigree 1 included 16 affected patients, 7 were examined and disease status for the remaining was attributed by medical records and/or history. Pedigree 2 consists of 10 affected patients, two subjects were available for examination, the remaining subjects were ascribed from medical journals and/or history. All examined patients had areflexia and in some cases muscle atrophy and contractures. N=No, Y=yes, NA=not assessed, AO=age of onset, DD= disease duration. [a]At age 76 this patient had an infarction in the right external capsule and *corona radiata*. At age 75 his SARA score was 20. [b]Mild atrophy but assessment was made with a CT-scan. [c]Babinski sign during last exam. [d]This atrophy was evident on a CT-scan. [e]Mild periventricular white matter abnormalities were also evident in this patient.

Pedigree and patient	Pedigree 1													Pedigree 2		
Parameter	II:1	II:5	III:1	III:3	III:6	III:10	IV:1	IV:3	IV:4	IV:6	IV:8	V:3	V:5	III:2	III:4	IV:4
Abnormal sweat	NA	NA	NA	NA	NA	NA	Y	Y	Y	N	N	N	N	N	NA	N
Hot flushes	NA	NA	NA	NA	NA	NA	Y	Y	Y	N	Y	N	N	N	NA	N
SSR	NA	NA	NA	NA	NA	NA	Absence of reaction	Absence of reaction	NA	Mild abnormality	N	NA	Normal	NA	NA	N
RR variation	NA	NA	NA	NA	NA	NA	Marked reduced	Marked reduced	NA	Reduced	Reduced	NA	Reduced	NA	NA	Reduced
Orthostatism	Suspected	NA	NA	NA	NA	NA	Y	Y	N	N	Y	N	N	NA	NA	N
Pattern of reaction on tilt test	NA	NA	NA	NA	NA	NA	Vasovagal syncope	Asympaticoton	NA	Asympaticoton	NA	NA	Asympaticoton	NA	NA	NA
Acrocyanosis	NA	NA	NA	NA	NA	NA	Y	Y	N	N	Y	N	N	N	NA	N
Urine incontinence (Y/N)	Y	Y	NA	NA	NA	NA	Y	Y	N	N	N	N	N	N	NA	N
ESS	NA	NA	NA	NA	NA	NA	2	10	NA	4	3	3	0	9	NA	3
Sleep registration	NA	NA	NA	NA	NA	NA	Central apnea	Central apnea	NA	N	Central apnea and OSA	NA	N	NA	NA	N
Obstipation	NA	Y	Y	NA	NA	Y	Y	Y	Y[a]	N	Y	N	N	Y	NA	Y
Weight loss	NA	Y	NA	NA	NA	Y	N	Y	Y	N	Y	Y	N	N	Y	Y

Table 7. Dysautonomic features in SCA4. ESS: Epsworth Sleepiness Scale; OSA: obstructive sleep apnea; RR interval variation on an electrocardiogram; SSR: sympathetic skin response. [a] This patient was diagnosed with severe irritable bowel syndrome early in life. N=No, Y=yes, NA=not assessed.

Multipoint linkage analysis was performed in family 1 using the markers D16S3031, D16S3019, D16S397 D16S3067, D16S3141, D16S496, D16S3085, D16S3107, D16S421, D16S3086, D16S3095, D16S3624, D16S3059, D16S512, D16S3018, D16S516and D16S402. This confirmed linkage to chromosome16q22.1 with a maximum LOD score of 3.7 (Fig 25). The linkage peak was located in a 3.69 cM region between markers D16S3019 and D16S512. The index case from family 2 shared the haplotype found in family 1 in the candidate region.

The whole linked region and surrounding genetic regions (chr16: 53633817-76593135, GRCh37) was custom captured and sequenced in six affected and four unaffected members from family1 by using a custom designed capture kit (NimbleGen SeqCap EZ Choice Library). No clear pathogenic variant could be identified that segregated with the disease. However, based on the sequence data, a refined haplotype could be established, reducing the linked region to 23 Mb (flanked by D16S415 and D16S515).

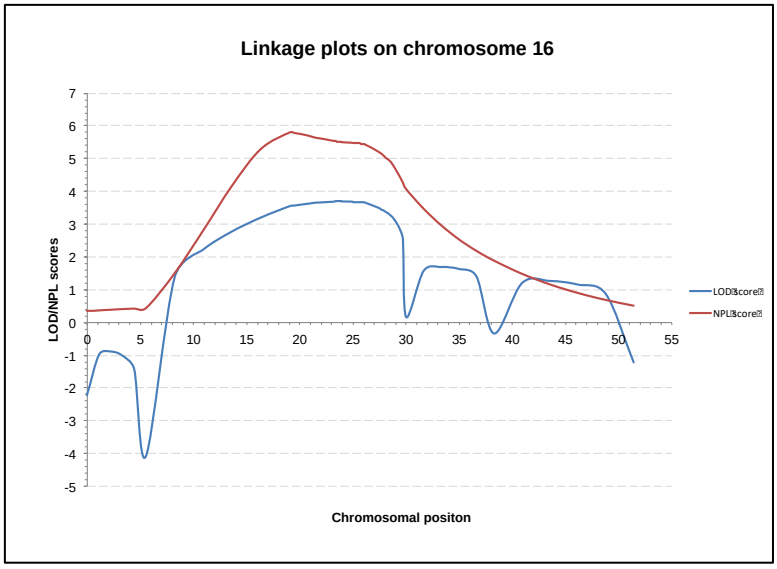


Figure 25. Multipoint linkage analysis confirmed linkage with a maximum LOD score of 3.7 on chromosome 16. Linkage peak was located in a 3.69 cM region between D16S3019 and D16S512.

5.3 ADENOSINE KINASE DEFICIENCY

5.3.1 PAPER IV

When we first started to use whole exome sequencing for identification of disease-causing genes, we turned to our old, unsolved cases. As the first family, we decided to study a pair of siblings who had increased levels of plasma methionine that could not be explained by known monogenic disorders.

The first affected child, a boy born 1987, exhibited failure to thrive, elevated liver enzymes and conjugated hyperbilirubinemia at 3-4 weeks of age. His sister, born 1996, had an almost identical disease course as her brother. The siblings come from a reportedly non-consanguineous family in Dalarna. In the family was also a healthy older brother born 1985.

At age 3-4 months there was an obvious delay in psychomotor development. At one year of age both siblings had macrocephalus with frontal bossing and debut of epilepsy with both generalized tonic-clonic and partial seizures before age 3. The first year of life metabolic screening with organic acids in urine and amino acids in plasma were unremarkable and the bilirubin levels normalized. The liver enzymes, however, remained elevated and there was also a slight increase in serum creatinine kinase (CK) of 10 μkat/L (ref <3.5). The combination of symptoms involving both liver, muscle and CNS raised the suspicion of a mitochondrial disorder, but mitochondrial biochemical and morphological investigations from a muscle biopsy were normal in one of the siblings, the boy. Liver biopsy was performed showing liver steatosis and fibrosis in porta zones. At eight years of age both siblings had severe intellectual disability with autistic features. They also developed reduced vision with atrophy of the optic nerves. The girl died at age 10 of a suspected epileptic seizure during sleep. The older brother had, at eleven years of age, a general muscle hypotrophy, scoliosis, kyphosis and shortened Achilles tendons. MRI of the brain showed atrophy of both white and grey matter in the brother but was essentially normal in the sister at age 14 months.

Both siblings had increased levels of plasma methionine and p-AdoMet (Table 8) pointing to a disrupted methionine cycle (Fig 26). The metabolite pattern suggested that the biochemical block was in the conversion of AdoHcy to homocysteine. Previously six genetic causes of elevated methionine had been described (70). The pattern of metabolite elevations in our patients could point to defective S-adenosyl-homocysteine hydrolase (SAHH) activity. After measurement of S-adenosyl-homocysteine hydrolase activity and sequencing of *AHCY*, the gene encoding this enzyme without finding any abnormalities, we concluded that this most likely was a novel disorder.

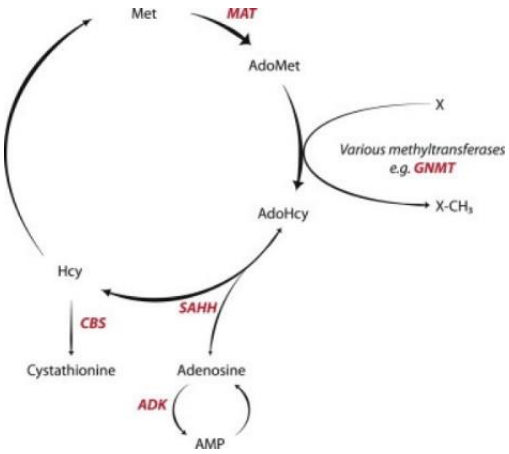


Figure 26. The methionine cycle

	Boy born 1987	**Girl born 1996**	**Reference values**
P-Methionine, µmol/L, highest values	455	886	15-35
P-homocysteine, µmol/L	9.7	15	5-15
P-AdoMet, nmol/L	677	496	55-116
P-AdoHcy, nmol/L	122	102	9-45
U-adenosine, µmol/mol creatinine	7.1	9.85	<1

Table 8. Biochemical investigations showed a pronounced elevation of methionine in plasma and indications of a functional block in the conversion of S-adenosyl-homocysteine (AdoHcy) to homocysteine (Hcy) in both siblings.

The family was selected for whole exome sequencing, making this the first Swedish family to undergo such an investigation.

After exome capture, sequencing and bioinformatic filtering a homozygous mutation, c.902C>A (p.Ala301Glu), was found in the *ADK* gene as described in paper I. This finding fit nicely with the biochemical findings.

	Sibling 1			Sibling 2			Siblings 1 and 2		
	Dom	**Rec**	**Hom**	**Dom**	**Rec**	**Hom**	**Dom**	**Rec**	**Hom**
NS/SS/I	4539	2789	2103	4452	2782	2111	3750	2270	1639
Not in dbSNP131	424	62	26	422	49	21	213	28	13
Predicted to be damaging	204	17	10	195	17	8	100	10	5

Table 9. The number of candidate genes resulting from bioinformatic data analysis in the affected pair of siblings in the Swedish family during different modes of filtering. Filtering against databases of known variants (dbSNP 131) and application of the recessive model for genes present in both siblings reduced the number of genes more than 150-fold in all cases and almost 350-fold for the homozygous model. Damage prediction from PolyPhen 2 further reduced the gene count to a total of ten and five genes that overlapped between the two siblings under the recessive and the homozygous models, respectively. The *ADK* gene was present in the final list of five genes. Abbreviations are as follows: Dom, ≥ 1 heterozygous or homozygous variant; Rec, ≥ 1 homozygous or ≥ 2 heterozygous variants; Hom, ≥ 1 homozygous variant; NS, nonsynonymous variant; SS, splice-site variant; I, coding indel.

In collaboration with other groups, four additional children from two families were identified. All subjects had similar biochemical and clinical phenotypes including frontal bossing and mutations in the *ADK* gene.

A defective phosphorylation of adenosine to adenosyl-monophosphate (AMP) would be expected to also result in elevated adenosine. Therefore, we validated the functional significance of the genetic defect by confirming elevated urinary excretion of adenosine in the Swedish cases. We also demonstrated reduced activities of recombinant adenosine kinase enzymes carrying the different patient-derived mutations.

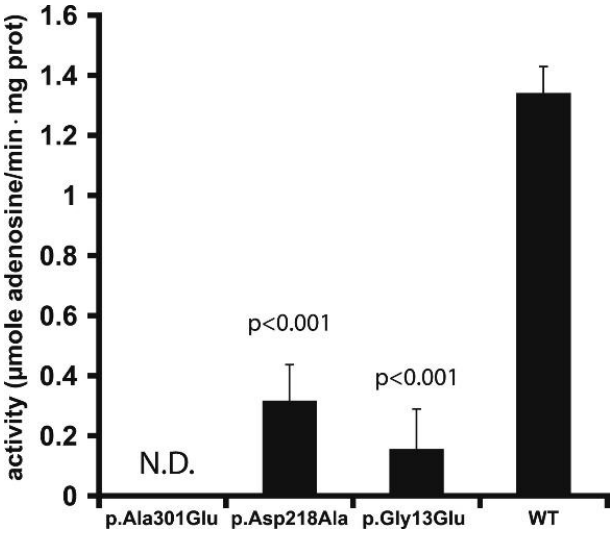


Figure 27. Adenosine Kinase Activity from recombinant Wild-Type and mutant enzymes. Recombinant forms of wild-type ADK and ADK containing each of the mutations detected in individuals with ADK deficiency were expressed in *E. coli*, purified, and tested for their capacity to phosphorylate adenosine. The graph shows the mean value + 1 standard deviation of three independent measurements carried out with 5 µM adenosine. All mutants displayed significantly impaired activity in comparison to the wild-type enzyme (two-tailed Student's t test). N.D. denotes not detectable; WT denotes wild-type. p.Ala301Glu are from Swedish patients, p.Asp218Ala and p.Gly13Glu are from the Malaysian patients.

5.4 RAPID PULSED WHOLE GENOME SEQUENCING

5.4.1 PAPER V

Massive parallel DNA sequencing provides a novel diagnostic opportunity, provided that the technology is adapted to healthcare standards and is customized to the specific clinical situation. Science for Life Laboratory (SciLifeLab) is a Swedish national center for large-scale molecular biosciences with focus on health and environmental research, a joint effort between Karolinska Institutet, the Royal Institute of Technology (KTH), Stockholm University and Uppsala University. Our group has worked extensively to establish a collaboration between the Karolinska University Hospital and the SciLifeLab Clinical Genomics facility in Stockholm. Bioinformatic tools and workflows have been established, enabling implementation of whole genome sequencing (WGS) into healthcare.

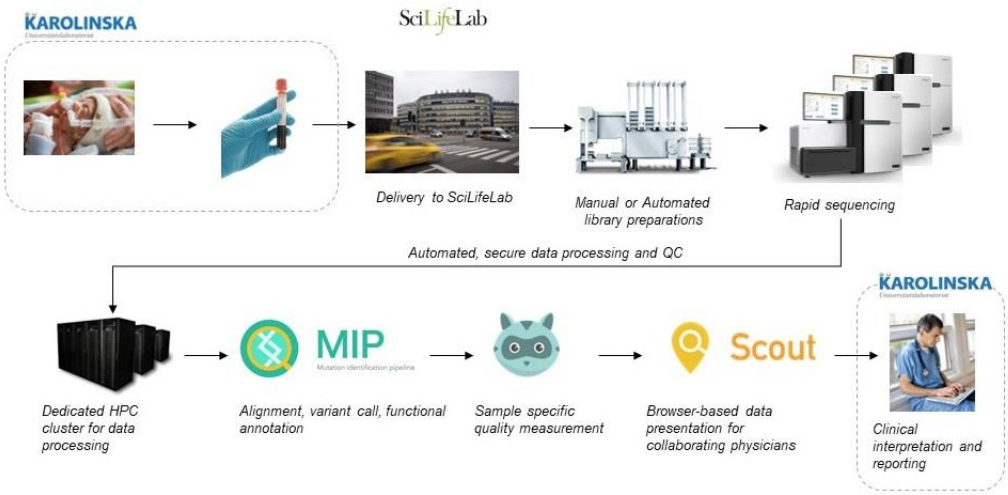


Figure 28. The workflow for WGS analysis in the SciLifeLab – Karolinska collaboration

We decided to perform a proof-of-concept study, to illustrate the potential benefit of rapid WGS in acutely presenting neonates with suspected IEM. We used a conceptionally new approach where we analyzed pulsed sequence data at different time intervals, shortening the procedure to 15-36 hours.

Three patients were selected, two had known diagnoses and were retrospectively analyzed (Propionic acidemia, PA and Pyruvate dehydrogenase deficiency, PDHD) while the third patient was recruited from the neonatal intensive care unit (NICU) at Karolinska due to a suspected metabolic disease. Genomic DNA was extracted from blood and sequenced.

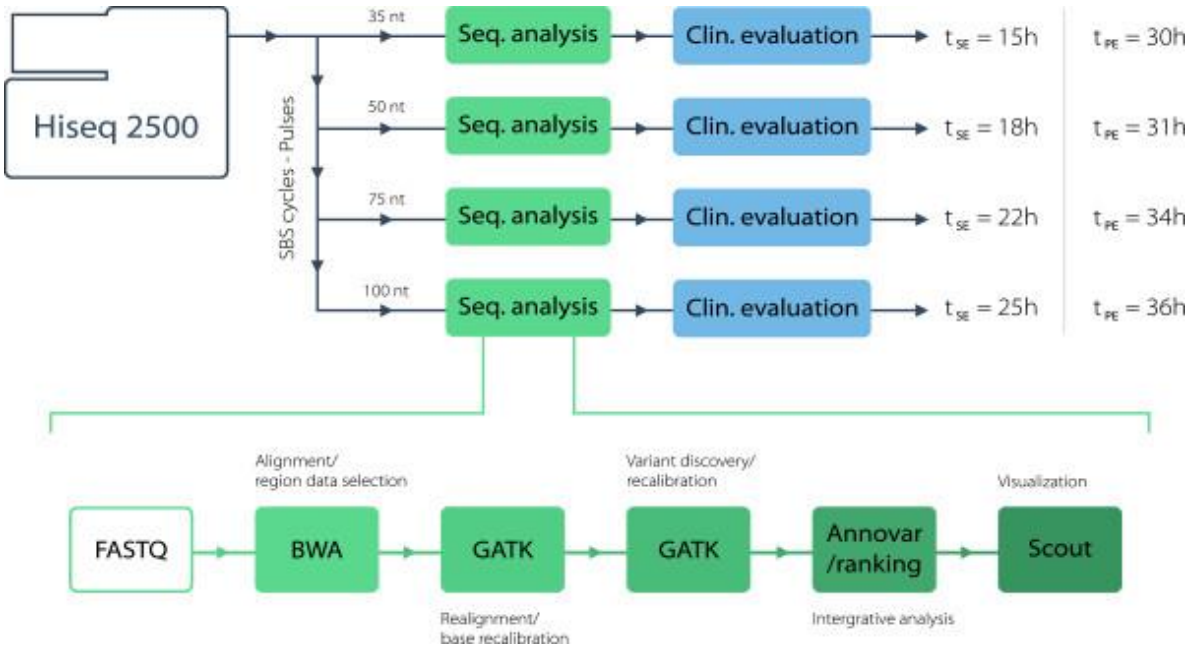


Figure 29. Summary of steps used in the pulsed automated bioinformatic analysis, resulting in a final clinical diagnosis within 36 hours. SBS: sequencing by synthesis, nt: nucleotides, Seq: sequencing, Clin: clinical, SE: single-end pulse, PE: paired-end pulse.

After sequencing data was filtered against 474 genes known to be associated with inborn errors of metabolism. The patient with PA could be correctly diagnosed at the first pulse (single end short reads, 30 nucleotides) after 15 hours. In the patient with PDHD the correct diagnosis could be made at the second pulse (single end short reads, 50 nucleotides). In the third patient no abnormalities were noted after completion at 36 hours (paired-end long reads, 100 nucleotides), and the child also had normal findings in the extensive metabolic biochemical investigations carried out in parallel.

6 DISCUSSION

Our center, CMMS, is a specialized clinic for diagnosis of IEM and related rare diseases, investigating patients from all over Sweden. We have established a cross-disciplinary structure consisting of clinicians from different specialties (pediatrics, neurology, endocrinology, clinical chemistry, clinical genetics), biochemists, analytical chemists, molecular biologists and bioinformaticians, providing state-of-the art laboratory investigations and expert advice on all aspects of IEM. Since 1965, CMMS also runs the nation-wide neonatal screening program ("PKU test"), currently offering testing for 25 different treatable conditions to all ≈120 000 Swedish newborns yearly.

CMMS has worked in close collaboration with the Clinical Genomics facility at Science for Life Laboratory (SciLifeLab) in Stockholm since its start. SciLifeLab is a national, academic center for large-scale bioscience with advanced infrastructure and expertise for e.g., whole genome sequencing, that was established in 2010. A range of bioinformatic tools and workflows have been developed allowing rapid, quality assured, comprehensive, clinical-grade analyses of WGS data for diagnosis of rare inherited diseases. IEM was particularly suitable for initial clinical implementation, due to the multidisciplinary organization of CMMS where clinical specialists work closely together with experts in laboratory medicine and experimental science. By restricting analyses to rare variants in genes relevant for each patient's individual disease presentation, and by putting genome data into context with clinical information and biochemical findings, a manageable number of variants can be generated for rapid evaluation by the diagnostic team and translated all the way to individualized treatment. The concept has evolved into a cross-clinic collaboration including Clinical Genetics and Clinical Immunology enabling sharing of genome data, collaboration around unclear cases and diagnostics across a broad range of rare diseases. A new landscape of monogenic disorders is thus emerging.

Up to the end of 2019 we had analyzed more than 3000 patients using WGS, with an overall diagnostic yield of 38% across more than 700 different genes. Around 850 of these patients were investigated primarily for IEM. Our latest strategy involves a new protocol for diagnostics of mitochondrial diseases where we from a muscle biopsy can obtain mitochondrial biochemical analysis, light- and electron microscopic studies and, by extracting DNA directly from the muscle specimen, simultaneously obtain the sequence of both the nuclear and mitochondrial DNA from a single sample. This strategy can be employed for muscular dystrophies and other monogenic disorders where information of muscle morphology and/or biochemistry from muscle tissue is relevant. In the future we are planning to include gene expression analysis by performing sequencing of RNA to increase the diagnostic yield even further.

The major part of my thesis project was carried out before clinical WGS became routinely available. Linkage analysis and gene-by-gene sequencing were the methods of choice but these have since become mostly obsolete. However, detailed and thorough clinical

investigations remain essential both in order to make sense of large datasets, and to understand the mechanistic implications of encountered mutations.

Paper I and II. The disorder sarcoplasmic body myopathy is now renamed myoglobinopathy after finding the causative genetic defect almost 40 years after its original description. To date six families have been diagnosed with this rare muscle dystrophy. If our assumption that the mutation has arisen in a mutational hotspot is true, more families are likely to emerge. I have had the privilege of seeing patients at all disease stages and follow them from presymptomatic to onset, progression and, in some cases, to death. Even if no treatment currently exists, the finding of the mutation has made presymptomatic diagnosis without muscle biopsy and prenatal diagnosis possible. Several mutation-free babies are now born from descendants of the family in paper I.

Paper III. We describe the first two Swedish families with spinocerebellar ataxia type 4, constituting the third and fourth families reported. Detailed clinical characterization expands the phenotype of this rare disorder significantly. Besides ataxia and peripheral neuropathy affected subjects suffer from striking dysautonomia. Motor neuron signs, eye movement abnormalities, dystonia and chorea were also found in some patients. The two families in this study have a similar phenotype and share the same haplotype on chromosome 16. Since they originate from the same area in southern Sweden, we suspect that the families may be related and extended haplotype analysis to investigate kinship will be performed. It is also possible that the family of Scandinavian origin described by Flanigan et al in 1996 is related to the families characterized in paper III. Recently, we identified a likely causative gene in our families. Investigations are ongoing to validate this finding.

Paper IV. Since our publication in 2011, in total 19 cases of ADK deficiency have been published (85). Methionine elevation is a characteristic finding but can be intermittent. Therapy with methionine restricted food seems to ameliorate the biochemical and liver phenotype to some extent, while the effect on seizures and other neurological symptoms is unclear (86). To investigate whether the neurological symptoms were due to hepatic encephalopathy, Sandau et al (87) constructed mice with selective knockout of ADK in the brain. These experiments showed that diminished expression of ADK in the brain led to progressive seizures, learning disability and reduced synaptic plasticity in mouse brain. Interestingly, adenosine also has endogenous anticonvulsant and neuroprotective properties and ADK is a major enzyme for adenosine removal. The effects of ADK are complex since overexpression of ADK in astrocytes can provoke seizures and blockage of ADK expression can prevent seizures (88). Since primary or secondary phenotypic alterations in astrocytes is now believed to be the cause of, or contributing to, epileptogenesis (89) modulation of astrocyte adenosine metabolism could be a potential target for treatment.

Paper V. Rapid whole genome sequencing has an advantage over exome sequencing in that there is no time-consuming library preparation step required. Rapid whole genome sequencing is a new technology that will improve diagnosis in the NICU department, saving

babies' lives. As the technology continuously improves and can be made at lower cost there is also a growing interest in population-based screening in the newborn period (90).

7 FUTURE PERSPECTIVES

7.1 MYOGLOBINOPATHY

The patients affected by myoglobinopathy have repeatedly asked if there might be treatment available in the future. I have kept their hope for this opportunity alive and I truly believe a treatment strategy could be devised.

I see two main roads for designing a treatment. The damage to muscle cells and tissue seems to be caused by defective oxidation through a toxic gain of function rather than a loss of function defect in oxygen binding, NOS regulation etc. If oxidative damage could somehow be prevented the disease progression could possibly be prevented or slowed. A second possibility would be to use siRNA or antisense oligonucleotides to reduce expression of the mutant allele. Development of such a treatment requires a number of experiments (91). Tests in cell cultures and one or more animal models must be performed. Then on- and off-target effects must be addressed, preferably by a combination of Southern and Western blot and RNAseq. Another obstacle is the problem of delivering enough oligonucleotides to the muscle *in vivo*, which must be assessed by measurements of MB RNA and protein from muscle biopsies. There are several recent examples of successful development of treatment based on selective gene silencing. One example is the development of Givosiran for treatment of acute intermittent porphyria(92). The clinical trials leading up to approval of this novel drug were done in collaboration with the Swedish Porphyria Centre at CMMS.

7.2 SPINOCEREBELLAR ATAXIA TYPE 4

In this autosomal dominant disorder, we have described two affected families and a third possible family is under investigation. The disease could be common in Sweden among patients with undiagnosed ataxias. Analysis of the custom capture performed has thus far not identified the causative mutation, but we are currently investigating a likely causative gene in the region on chromosome 16q22.1. Elucidating the genetic background will add another solved spinocerebellar ataxia to the list and make genetic testing possible. If the molecular mechanisms can be clarified it will likely shed new light and deeper understanding on disease pathogenesis, which in turn could result in ways to modulate the process and to develop treatment.

7.3 ADENOSINE KINASE DEFICIENCY

Further research in this disorder and other conditions affecting adenosine metabolism will likely shed new light on the mechanisms involved in epilepsy.

7.4 RAPID WHOLE GENOME SEQUENCING

In our center, CMMS, we are already performing WGS in infants acutely sick from a suspected metabolic disorder where we deliver results within one week, and sometimes even faster. The time until results are obtained will continue to improve as all aspects of the process become more evolved, including the sequencing itself, computational speed,

improved automation through bioinformatics and as education and recruitment of staff involved in the whole chain increases. Rapid, acute WGS-based diagnostics has many additional potential uses, apart from finding IEM in newborns. Rare genetic diseases can be found across all clinical specialties and can present at essentially all ages. In order to implement rapid WGS for diagnostics across all these potential scenarios, healthcare needs to adapt by promoting cross-disciplinary collaboration such that genome data is used in a responsible way and put into context relevant for each individual patient.

8 SVENSK SAMMANFATTNING

Denna avhandling handlar om att förbättra diagnos och behandling av personer som drabbas av sällsynta neurologiska sjukdomar. Diagnos före behandling är en princip som ofta lärs ut till medicinstudenter. Men hur ska man göra om diagnos inte kan ställas på grund av att det saknas antingen resurser eller kunskap eller om sjukdomen inte ens finns beskriven? De sjukdomar som beskrivs i denna avhandling är alla orsakade av defekt i en enda gen. Generna är huvudsakligen belägna i kromosomerna i våra cellers kärnor och utgör ritningen för hur våra kroppar är konstruerade. Man tror att det finns cirka 20 000 gener i vår arvsmassa och de översätts till ett ännu större antal proteiner, eller äggviteämnen, som tar hand om viktiga kemiska eller stödjande uppgifter i kroppen. En gen består av en genetisk sekvens, vilken utgör en kod bestående av fyra tecken, A, G, C och T. Hos människan är den genetiska koden cirka 3 miljarder tecken lång. Vid sjukdomar som orsakas av ett enda fel i en gen, så kallade monogena sjukdomar, har det uppstått ett fel i denna kod som i sin tur orsakar fel i funktionen av ett eller flera proteiner. Antalet sjukdomar där den genetiska bakgrunden och de molekylära mekanismerna är kända har ökat snabbt tack vare utveckling av nya metoder för att fastställa den genetiska koden. Numera finns möjlighet att utläsa den genetiska koden i alla 20 000 gener samtidigt i en och samma analys. Det finns fortfarande många monogena sjukdomar där man inte lyckats hitta den genetiska förklaringen. I mitt doktorandprojekt har jag försökt finna den genetiska orsaken till tre olika sällsynta sjukdomar, och i två av fallen lyckades detta. Jag och mina kolleger har även lyckats visa vilka effekter det blir i proteinerna som respektive gener kodar för. När det gäller den tredje sjukdomen har vi ännu inte nått hela vägen fram till en genetisk förklaring, men vi är övertygande om att arbetet kommer att lyckas så småningom.

Framgångarna beror mycket på två olika saker, tillgång modern utrustning för storskaliga genetiska analyser och de omfattande resurser som finns tillgängliga på min arbetsplats, Centrum för medfödda metabola sjukdomar, CMMS.

Delarbete I är en detaljerad beskrivning av nio personer i en familj med en sällsynt muskelsjukdom, sarkoplasmatisk inklusionskroppsmyopati. Den första beskrivningen av sjukdomen var i en svensk familj och den publicerades för 40 år sedan. Delarbete II handlar om den genetiska orsaken till sjukdomen där vi visar att orsaken är en förändring i genen som kodar för myoglobin. I delarbete II beskrivs ytterligare 5 familjer som visade sig ha exakt samma förändring i myoglobingenen. I delarbete II påvisar vi också att skadorna på musklerna orsakas av avvikande oxidation.

Delarbete III ger en detaljerad klinisk beskrivning av 2 familjer som drabbats av en sjukdom som orsakar störd funktion i olika delar av nervsystemet. De drabbade personerna har störd balans och koordination, ataxi, beroende på att lillhjärnan påverkas. De som har sjukdomen har även polyneuropati och autonom dysfunktion. Polyneuropati är en störd funktion i de nerver som förmedlar signaler från hjärnan och ryggmärgen ut till övriga kroppen och det autonoma nervsystemet styr olika kroppsfunktioner som inte är direkt viljestyrda som till

exempel hjärtrytm, tarmfunktion, svettning mm. Genom en så kallad kopplingsanalys, där man undersöker vilka kromosomdelar som finns hos de sjuka respektive hos de friska i samma familj, kunde vi dra slutsatsen att sjukdomen orsakas av gen som ligger i kromosom 16 och att det är samma sjukdom, Spinocerebellär ataxi typ 4, som tidigare beskrivits två gånger i två olika familjer i Utah och i Tyskland. Ytterligare studier pågår, men vi har ännu inte kunnat hitta den orsakande genen och har därför inte kunnat klarlägga vad som orsakar sjukdomssymtomen.

Delarbete IV handlar om den kliniska, biokemiska och genetiska kartläggningen av en hittills okänd sjukdom som medför avvikelser i de biokemiska processerna i kroppen, metabolismen, sjukdomen kallas nu adenosinkinasbrist. De två syskonen med denna sjukdom som vi undersökte hade en ovanlig biokemisk avvikelse, förhöjt metionin. Efter att ha uteslutit alla kända orsaker till högt metionin kunde vi visa att syskonen led av en tidigare okänd defekt i den så kallade metionincykeln, där en rad viktiga processer sker. Adenosinkinas är ett protein som hör till gruppen enzymer, dvs proteiner som har som uppgift att omvandla ämnen. Vid adenosinkinasbrist ansamlas inte bara metionin utan även nedbrytningen av adenosin påverkas, vilket får en mängd konsekvenser som i sin tur orsakar sjukdom.

Delarbete V handlar om hur man kan gå till väga för att snabbt ställa genetisk diagnos hos akut sjuka spädbarn på intensivvårdsavdelningar med hjälp av specialanpassad helgenomsekvensering. Spädbarn med störningar i metabolismen svarar inte sällan på behandling, förutsatt att rätt diagnos kan ställas innan permanenta skador på centrala nervsystemet och andra organ har uppträtt. I denna studie visar vi hur en genetisk diagnos kan ställas på 15–18 timmar.

9 ACKNOWLEDGEMENTS

None of the work in this thesis would have been possible without the contribution from all the patients. Some of you are now like my second family. Thank you!

Anna Wedell, my main supervisor, colleague, boss and friend, for all your encouragement. Your extensive knowledge in several research areas and your ability to quickly get to the point has been an inspiration and support. I also want to thank you for giving me freedom to pursue the detours I can't resist.

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