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PERIPHERAL NEUROPATHY AND ALTERED COBALAMIN METABOLISM IN PARKINSON'S DISEASE AND OTHER MOVEMENT DISORDERS

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PERIPHERAL NEUROPATHY AND ALTERED COBALAMIN METABOLISM IN PARKINSON'S DISEASE AND OTHER MOVEMENT DISORDERS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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POPULAR SCIENCE SUMMARY OF THE THESIS

Parkinson's disease is a common neurological disorder that is expected to affect 2-3% of the population over 65 years. In this disease, we know that there is a lack of a signal substance named dopamine. Dopamine can be likened to the oil we use for a bicycle chain, movements can be carried out smoothly when the brain has good access to dopamine, but when there is a lack of dopamine, movements become smaller, hesitant, and more tremulous. This poverty of movement is a typical symptom of Parkinson's disease. However, we also know a number of other symptoms that can be bothersome for patients suffering from this disease, which not necessarily are visible for the environment. Obstipation, depression, memory complaints, and impaired sense of smell are symptoms that are common. As for now, there is no treatment available that slows down the disease process in Parkinson's disease. Instead, our treatments aim to ease symptoms, often by trying to restore dopamine levels in the brain.

The nerve cells present in the brain form, together with the spinal cord, part of what we call the *central nervous system*. The nerve fibers that travel from the central nervous system, out to the arms, legs and inner organs, instead form part of what we call the *peripheral nervous system*. These peripheral nerve fibers serve an important purpose by transmitting different signals, both to and from the central nervous system. These signals can, for example, carry sensory information, such as pain, touch and temperature, or information to our muscles when we want to initiate movements. In recent years, studies have shown that these nerve cells, outside of the brain, also can be affected in Parkinson's disease.

In two studies of this thesis, I have investigated the peripheral nervous system in patients with Parkinson's disease. We have used different methods to study the function and appearance of peripheral nerve fibers, and compared our findings to controls. In Study I, we could see that patients with Parkinson's disease showed clinical signs of disturbed function of peripheral nerve fibers to a higher degree compared to controls. We could also see, from blood samples, that the patients showed abnormalities related to vitamin B12. In Study IV, I expand my thoughts surrounding vitamin B12 also to the central nervous system. In this study I describe a patient with Parkinson's disease who suffered from a unique genetic condition related to the body's handling of vitamin B12.

In Study II we investigated if a disturbance of peripheral nerve fibers could explain a symptom that sometimes is seen in Parkinson's disease, so-called "restless legs". Patients who suffer from restless legs often describe a discomfort in the legs, "like pins and needles", that appears at night or when still. As an example, restless legs can make a visit to the cinema, sitting in a chair for a long time, very troubling. In our study, we could not find that restless legs in patients with Parkinson could be explained by a dysfunction of peripheral nerve fibers. This finding could instead tell us that symptoms of restless legs may respond to the treatment we use outside of Parkinson's disease, and that these symptoms not necessarily have to follow the same progressive path as other symptoms in Parkinson's disease.

In Study II, we used a special microscopic technique to photograph the small nerve fibers that are present in the cornea of the human eye. We also measured the ability of single nerves, of arms and legs, to transmit nerve signals. We could see that changes in some of these examinations, to some extent, could tell us how far a patient was in her/his Parkinson. I discuss further in this thesis if the examination of the well-being of peripheral nerves could be a way to monitor how Parkinson's disease progresses over time.

In Study III and V, we looked at two very rare hereditary disorders. Gaucher disease is expected to occur in one in 47 000 births and gives rise to symptoms from the bone, inner organs, and blood. We know that this disease also has a genetic kinship with Parkinson's disease. It has been suggested that the disease also can affect the peripheral nerves and contribute to pain. We investigated patients with Gaucher disease followed in Stockholm and outside of Luleå, in the northern parts of Sweden. We could see that some patients, with a certain form of the disease, had possible signs of a dysfunction of the thinnest peripheral nerve fibers. I discuss in my thesis on possible implications of our finding, including thoughts on pain. There is however, an uncertainty in our findings because the number of patients that were examined is small, given the rarity of the disease.

In Study V, we describe two families affected by a rare hereditary disorder that gives rise to stiffness and weakness of the legs. Often these symptoms lead to the need for walking aid in order to be able to walk safely. We investigated family members with different methods, including neurological examination, measurement of signal transmission in peripheral nerves, and analysis of cerebrospinal fluid. We could show how the disease can differ both between and within families with the same disease. We also discuss the results from the cerebrospinal fluid analysis.

In summary, in this thesis I want to shed light on the peripheral nervous system from different angles in Parkinson's disease, and other more rare genetic disorders. I propose that our findings may motivate new studies that investigate whether the well-being of the peripheral nervous system could be used as a marker that reflects what goes on in the brain of patients with Parkinson's disease. Such an accessible marker could be a valuable tool in future treatment studies. Furthermore, I propose we actively monitor blood levels of vitamin B12 in the care of patients with Parkinson's disease.

ABSTRACT

Peripheral neuropathy is a disorder of the peripheral nervous system (PNS) that may carry different underlying aetiologies. Studies have suggested PNS involvement may be prevalent in neurological diseases that traditionally have been regarded primarily as disorders of the central nervous system (CNS), including the neurodegenerative disorder Parkinson's disease (PD).

In PD, an increased prevalence of large and/or small fiber neuropathy has been reported. Underlying associations with biochemical signs of disturbed vitamin B12 (cobalamin) metabolism have been suggested, and proposed to be driven by chronic exposure to treatment with levodopa. However, peripheral neuropathy as an intrinsic disease feature has also been suggested, possibly driven by peripheral neurodegeneration associated with PD.

We investigated the prevalence of clinical signs of peripheral neuropathy in a levodopa treated population followed at Karolinska University Hospital. Assessments included biochemical, genetic, and clinical evaluations. We observed a significantly higher prevalence of clinical signs indicative of a peripheral neuropathy, as assessed by the Utah Early Neuropathy Scale (UENS), in PD patients relative to controls. Furthermore, an association between UENS scores and plasma levels of homocysteine, an amino acid involved in the cobalamin dependent methionine cycle, was demonstrated.(Study I)

In Study II we explored if Restless legs syndrome (RLS) may constitute a clinical expression of peripheral neuropathy in patients with PD and co-existent RLS. PNS assessments included both functional and structural evaluations of large and small fibers. More specifically, we employed the UENS, nerve conduction studies, quantitative sensory testing, and a novel method visualising the small fibers of the corneal subbasal nerve plexus, *in vivo* corneal confocal microscopy. An association between PNS assessments and the clinical expression of RLS, in the setting of PD, could not be demonstrated. This finding argues against a peripheral degenerative aetiology of RLS in this context. However, associations between PNS assessments and direct or indirect measures of disease burden in PD were demonstrated. This may possibly support the notion that PNS pathology, to some extent, could reflect ongoing CNS pathology in PD.

Previous studies have highlighted a possible role of altered cobalamin metabolism also in the setting of CNS manifestations in PD. In Study IV we characterized an adult patient with PD demonstrating a rare biochemical alteration, related to the cobalamin dependent metabolic pathway that takes place in the mitochondrial compartment. We present the underlying genetic variants, including a novel variant, presumed to drive this alteration, and discuss possible clinical implications.

Gaucher disease (GD) is a hereditary lysosomal storage disorder that, to some extent, shares genetic background with PD. An increased prevalence of both small and large fiber neuropathy has been associated with GD. We examined patients with GD type 1 followed at

Karolinska University Hospital and patients with the Norrbottnian GD type 3 followed at Sunderby Region Hospital. We assessed symptoms and clinical signs compatible with a peripheral neuropathy, followed by electrodiagnostic testing in selected cases. Acknowledging small sample size, we believe our study may support the notion that small fiber neuropathy could represent an inherent disease feature in GD type 1, but argues against this being a prevalent finding in Norrbottnian GD type 3. We suggest that the recognition of an ongoing small fiber neuropathy in this disease may harbour treatment implications with regard to pain management.(Study III)

Hereditary spastic paraparesis (HSP) constitutes a group of genetic movement disorders with vast phenotypic and genotypic hetereogeneity. We characterized the clinical phenotype, PNS involvement, and cerebrospinal fluid findings in two families with HSP-*KIF5A.* We confirm previous reports of inter- and intrafamilial variability of the clinical phenotype. Furthermore, we argue that elevated cerebrospinal fluid neurofilament light chain is not a mandatory finding in this upper motor neuron disease.(Study V)

In conclusion, I suggest monitoring and treatment of biochemical signs of altered cobalamin metabolism in PD may serve a protective role with regard to the PNS. I speculate that PNS pathology in PD may reflect both levodopa mediated effects and manifestations inherent to the disease itself, possibly with a predilection for large and small fibers respectively. Thus, I believe future studies addressing the potential biomarker role of PNS assessments, as a surrogate marker of general disease progression in PD, are warranted. Such studies must account for the possibility of clouding effects related to altered cobalamin metabolism mediated by levodopa.

LIST OF SCIENTIFIC PAPERS

I. **Andréasson M**, Brodin L, Laffita-Mesa JM, Svenningsson P. Correlations between methionine cycle metabolism, COMT genotype, and polyneuropathy in L-dopa treated Parkinson's disease: a preliminary cross-sectional study. *J Parkinsons Dis* 2017:7(4):619-628

II. **Andréasson M**, Lagali N, Badian RA, Utheim TP, Scarpa F, Colonna A, Allgeier S, Bartschat A, Köhler B, Mikut R, Reichert KM, Solders G, Samuelsson K, Zetterberg H, Blennow K, Svenningsson P. Parkinson's disease with restless legs syndrome – an in vivo corneal confocal microscopy study. *NPJ Parkinsons Dis* 2021:7(1):4

III. **Andréasson M**, Solders G, Björkvall CK, Machaczka M, Svenningsson P. Polyneuropathy in Gaucher disease type 1 and 3 – a descriptive case series. *Sci Rep* 2019:9(1):15358

IV. **Andréasson M**, Zetterström RH, von Döbeln U, Wedell A, Svenningsson P. *MCEE* mutations in an adult patient with Parkinson's disease, dementia, stroke and elevated levels of methylmalonic acid. *Int J Mol Sci* 2019:20(11):2631

V. **Andréasson M**, Lagerstedt-Robinson K, Samuelsson K, Solders G, Blennow K, Paucar M, Svenningsson P. Altered CSF levels of monoamines in hereditary spastic paraparesis 10: A case series. *Neurol Genet* 2019:5(4):e344

SCIENTIFIC PAPERS NOT INCLUDED IN THE THESIS

I. Sundgren M, **Andréasson M**, Svenningsson P, Noori RM, Johansson A. Does information from the Parkinson KinetiGraph™ (PKG) influence the neurologist's treatment decisions? – An observational study in routine clinical care of people with Parkinson's disease. *J Pers Med* 2021:11(6):519

II. **Andréasson M**, Svenningsson P. Update on alpha-synuclein-based biomarker approaches in the skin, submandibular gland, gastrointestinal tract, and biofluids. *Curr Opin Neurol* 2021:34(4):572-577 (Review article)

III. Badian RA, **Andréasson M**, Svenningsson P, Utheim TP, Lagali N. The pattern of the inferocentral whorl region of the corneal subbasal nerve plexus is altered with age. *Ocul Surf* 2021:22:204-212

IV. Badian RA, Allgeier S, Scarpa F, **Andréasson M**, Bartschat A, Mikut R, Colonna A, Bellisario M, Utheim TP, Köhler B, Svenningsson P, Lagali N. Wide-field mosaics of the corneal subbasal nerve plexus in Parkinson's disease using in vivo confocal microscopy. *Sci Data* 2021:8(1):306

CONTENTS

LIST OF ABBREVIATIONS

AdoCbl	Adenosylcobalamin
ALS	Amyotrophic lateral sclerosis
BBB	Blood-brain-barrier
CMT2	Charcot-Marie-Tooth disease type 2
CNBD	Corneal nerve branch density
CNFL	Corneal nerve fiber length
CNS	Central nervous system
Cobalamin	Vitamin B12
COMT	Catechol-O-methyltransferase
CSF	Cerebrospinal fluid
ENeG-Ix	Electroneurography-index
GCase	Glucocerebrosidase
Hcy	Homocysteine
HSP	Hereditary spastic paraplegia
HVA	Homovanillic acid
H&Y	Hoehn and Yahr
IEM	Inborn errors of metabolism
IENFD	Intraepidermal nerve fiber density
IVCCM	*In vivo* corneal confocal microscopy
L-Dopa	Levodopa
LRRK2	Leucine-rich repeat kinase 2
MCE	Methylmalonyl-CoA epimerase
MeCbl	Methylcobalamin
MHPG	3-methoxy-4-hydroxyphenylglycol
mH&Y	Modified Hoehn and Yahr
MMA	Methylmalonic acid
MMA-uria	Methylmalonic aciduria
MoCA	Montreal Cognitive Assessment
mSST	Modified severity scoring tool
MUT	Methylmalonyl-CoA mutase

NCS	Nerve conduction studies
NfL	Neurofilament light chain
PD	Parkinson's disease
PNS	Peripheral nervous system
p-α-syn	Phosphorylated alpha-synuclein
QST	Quantitative sensory testing
RLS	Restless legs syndrome
SAM	S-adenosylmethionine
SNP	Single nucleotide polymorphism
SPRS	Spastic paraplegia rating scale
THF	Tetrahydrofolate
UENS	Utah Early Neuropathy Scale
WGS	Whole genome sequencing
α-syn	Alpha-synuclein
5-CH_3THF	5-methyltetrahydrofolate
5-HIAA	5-hydroxyindoleacetic acid

1 INTRODUCTION

In this thesis, the peripheral nervous system (PNS) and vitamin B12 (cobalamin) metabolism have been explored in the clinical setting of an ongoing neurological disease. More specifically, this work focuses on clinical and pathophysiological aspects of the abovementioned systems in relation to movement disorders.

In the following segment, a background on the studied diseases and related pathophysiology will be provided. Furthermore, an overview of relevant previous research, which laid the foundation for the aims of this thesis, will be delineated.

1.1 THE PERIPHERAL NERVOUS SYSTEM

1.1.1 Structural overview

The PNS comprises motor, sensory, and autonomic nerve fibers that are responsible for communicating input/output to/from their respective cell bodies. These cell bodies are either located in the PNS or in the central nervous system (CNS), depending on cell type.[1]

Peripheral nerve fibers are further subdivided, based on their size and degree of myelination, into three different groups: A-, B-, and C-fibers. A-fibers are myelinated fibers, serving both sensory and motor signalling, and can be further subdivided on the basis of fiber size and conduction velocity. B-fibers are small myelinated fibers, responsible for autonomic signalling at the preganglionic level. Lastly, C-fibers are the smallest fibers, being unmyelinated with slow conduction velocity, and responsible for sensory and postganglionic sympathetic function.[1, 2] A summary of distinctive physiological and possible clinical correlates of small and large peripheral fibers, relevant for this thesis, is shown in Table 1.[1, 3, 4]

Fiber group	Size (Ø) and velocity	Function	Myelin	Clinical correlates
Large fibers	*20 µm and 120 m/s*			
Aα		Motor	Yes	Weakness, atrophy
		Sensory		Proprioceptive loss, imbalance
Aβ		Sensory	Yes	Reduced sensation to touch, pressure and vibration
Small fibers				
Aδ		Sensory	Thinly	Pain, abnormal cold sensation
C		Sensory	No	Pain, abnormal heat sensation
		Autonomic		sympathetic dysfunction
	0.3 µm and <2 m/s			

Table 1. Features of small and large peripheral nerve fibers. Possible clinical correlates in the context of peripheral nerve disease are also shown. Based on the original Erlanger Gasser classification.[3, 4]

1.1.2 Peripheral neuropathy

1.1.2.1 *Classification and terminology*

Peripheral neuropathy represents a disorder of the PNS that results in clinical signs, symptoms and objective pathology of the peripheral nerve/s/. Depending on the number and location of affected nerves, clinical terminology includes the affection of a single nerve (mononeuropathy), nerve root or plexus (radiculopathy or plexopathy), and multiple nerves (polyneuropathy or multiple mononeuropathy).[5]

Neuropathies are further phenotypically classified based on size, function (sensory, motor or autonomic) and distribution of the affected nerve fibers. Thus, small and large fiber neuropathies are two well-recognized clinical entities, affecting thinly myelinated Aδ- and unmyelinated C-fibers, and myelinated Aα- and Aβ-fibers, respectively. A combined small and large fiber neuropathy may also occur.[6]

With regard to myelinated fibers, electrodiagnostic testing, including nerve conductions studies (NCS), can further discern whether a large fiber neuropathy primarily affects the myelin, referred to as a demyelinating neuropathy, or the axon, an axonal neuropathy, or both, a mixed neuropathy. Lastly, the distribution of nerve fiber involvement, with regard to symmetry and whether the neuropathy follows a length or non-length dependent pattern, is often assessed to further aid in the clinical characterization. Of note, a non-length dependent sensory involvement is often indicative of a sensory neuronopathy, thus reflecting pathology primarily localized to the cell body at the level of the dorsal root ganglion.[6]

Exemplifying the discussed terminology, peripheral neuropathy attributed to diabetes mellitus may thus present as a symmetrical length dependent sensorimotor polyneuropathy, affecting small and large fibers, with both axonal and demyelinating features on NCS.[7]

This thesis is primarily focused on peripheral neuropathy manifested as a polyneuropathy, in the context of an ongoing neurological disease.

1.1.2.2 *Aetiology and epidemiology*

Multiple aetiologies have been associated with peripheral neuropathy. Broadly, these include infectious, inflammatory, systemic disease, toxic, hereditary, nutritional, and metabolic conditions. The clinical characterization of a polyneuropathy can aid in the diagnostic process, given that associations exist between different phenotypic presentations and specific aetiologies.[8] Diabetes mellitus is often reported as the most common underlying cause, however, in a significant proportion of patients a clear aetiology cannot be demonstrated, thus rendering a diagnosis of idiopathic polyneuropathy in many patients.[9-11]

The significant heterogeneity with regard to aetiology, clinical presentation, and symptom severity, together with varying availability of diagnostic methodology, have complicated the conduction of prevalence and incidence studies on peripheral neuropathy in the general population. An age-standardized prevalence of definite polyneuropathy was reported to be 4.0% in a Dutch study, which employed comprehensive diagnostic methodology.[11] A systematic review, assessing 29 epidemiological studies, reported a prevalence of

polyneuropathy between 1 and 3% in the general population. Notably, both studies observed a higher prevalence in the elderly.[12]

1.1.2.3 *Clinical aspects*

Given the complexity of the PNS and the numerous underlying aetiologies associated with disorders of the PNS, the clinical expression of a peripheral neuropathy can vary significantly. Clinically, the length dependent involvement of large sensory fibers may translate into proprioceptive and sensory loss, by the patient perceived as imbalance together with paraesthesia and numbness of hands and feet. The concurrent involvement of large motor fibers confers the addition of motor weakness. Differently, small fiber neuropathy may primarily be reflected by pain, insensitivity to cold and heat, dysaesthesia, and autonomic disturbances such as anhidrosis and disruption of bowel and bladder emptying. Moreover, the temporal profile of disease evolution may differ. An acute or subacute onset is often seen in inflammatory neuropathies, whilst insidious progression often is seen in diabetes mellitus or hereditary conditions.[8]

Importantly, a negative impact on different quality of life measures has been demonstrated in peripheral neuropathy of different aetiologies.[13, 14]

1.1.3 Evaluating the peripheral nervous system

Several methods are used in clinical practice, and in the context of clinical studies, for the structural and functional assessment of the PNS. In this segment, some available methods to assess small and large fibers will be discussed with regard to feasibility and limitations. Several additional methods exist, including sural nerve biopsy, but a full coverage of existing methods does not fall within the scope of this thesis.

1.1.3.1 *Clinical rating scales*

Clinical rating scales are based on standardized neurological examinations, generally not requiring special equipment outside of the setting of a regular clinical visit. However, different scales may be focused on detecting different types of nerve fiber pathology.

The Utah Early Neuropathy Scale (UENS) is regarded as sensitive for the detection of an early sensory predominant length dependent polyneuropathy. The scale carries a significant emphasis on sensation to pin-prick, as a clinical reflection of small fiber affection. The standardized examination is focused on signs arising from the lower extremities and requires a tuning fork, reflex hammer, and a safety pin. The scale is not time-consuming and can be done with easiness at a regular visit.[15]

The Toronto Clinical Scoring System is a rating scale that evaluates both clinical signs and patient reported symptoms indicative of a peripheral neuropathy. It has been validated in the setting of diabetic polyneuropathy, in which rating scores were demonstrated to correlate with NCS and large fiber densities assessed from sural nerve biopsies.[16]

As with clinical rating scales used in other disease conditions, the subjective nature of the assessments constitutes a main limitation.

1.1.3.2 *Objective assessment of large fibers*

Sensory and motor NCS are objective electrodiagnostic measures of large fiber function. Briefly, electrical stimulation of a peripheral nerve is performed and a surface electrode detects the corresponding elicited sensory or muscle action potential. The study provides information on nerve conduction velocity together with the amplitude of the compound muscle action potential and sensory nerve action potential. Deviations in these parameters may support the presence of demyelinating and/or axonal pathology respectively. The patient may experience slight discomfort and the assessment usually requires a separate visit to a neurophysiological facility with calibrated equipment. Importantly, NCS are unable to evaluate small fiber function.[17]

Moreover, it has been suggested that sensory NCS are less sensitive in detecting pathology restricted to distal large fiber endings that form part of the cutaneous Meissner's corpuscles, mainly located in glabrous skin of fingers and toes. These mechanoreceptors (Aβ-fibers), sensitive to tactile stimulation, are not selectively assessed when applying electrical stimulation, and thus distal large fiber pathology may go undetected.[18] As an alternative, punch biopsies from glabrous skin, with the morphological visualization of Meissner's corpuscles and their large fiber endings, have been employed to detect this type of selective large fiber neuropathy in different pathological conditions.[19]

1.1.3.3 *Objective assessment of small fibers*

With regard to small fiber function, several different objective methods exist with varying limitations and advantages.

The quantitative sudomotor axon reflex test assesses the postganglionic sympathetic small C-fibers innervating sweat glands. The test is based on the local stimulation of muscarinergic sweat gland receptors with acetylcholine. The generated sweat production is quantified and interpreted as an indirect measure of postganglionic C-fiber function. The method is time-consuming and not readily available for clinical use.[20] More recently, the Sudoscan, a faster and simpler method aimed at assessing C-fiber function has emerged, and has been used in clinical studies. With this method, the sweat gland is activated by a applying a low-voltage current to the skin. When activated, the glands start producing sweat, resulting in a flow of chloride ions. By measuring this ion flow, the conductance, an indirect quantification of sweat gland function can be determined. The method is less time-consuming, confers no discomfort to the patient, and requires less preparation. Sudoscan has been demonstrated to be able to detect diabetic neuropathy, in which obtained data correlated with clinical symptoms and signs.[21]

Quantitative sensory testing (QST) is a readily available method for assessing small fiber function. Briefly, thermal stimuli are applied to hands and feet with the patient instructed to report any perceived changes in temperature. Accordingly, thresholds for warmth and cold can be quantified. The results may be interpreted as a reflection of C- and Aδ-fiber function respectively. However, the test entails a subjective component and thus requires adequate cooperation of the patient. Furthermore, the test does not discriminate between central and peripheral somatosensory dysfunction since the entire tract, from cortex to peripheral nerve, is assessed.[22]

Finally, structural quantification of intraepidermal nerve fiber density (IENFD) is a well-established and sensitive method for the detection of cutaneous small fiber neuropathy. The method requires a 3-5 mm skin punch biopsy followed by pathological examination. Using immunohistochemical detection with antibodies carrying neuronal specificity (PGP9.5), the quantification of small nerve fibers visible in the epidermis is performed, generating a numerical value of the IENFD.(Figure 1) Quantification of IENFD using skin biopsy is considered a gold-standard assessment of small fiber neuropathy.[23] However, this method confers a purely structural measurement, which not necessarily is equivalent to a measure of small fiber *function*.

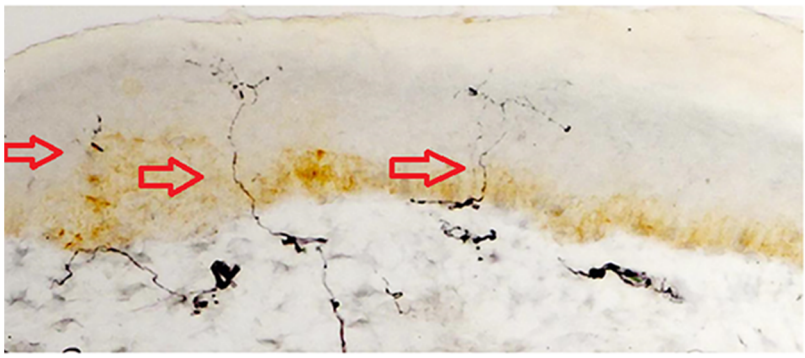

Figure 1. Skin biopsy showing three intraepidermal small nerve fibers (red arrows). This figure is a derivative of an image by Alam et al.[24] Licensed under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

In vivo corneal confocal microscopy (IVCCM) is a more recent method of structural small fiber assessment. More specifically, the method enables the visualization of the corneal small fibers that form the subbasal nerve plexus. The method is quick and non-invasive but requires patient cooperation and an experienced investigator. The microscopic recording of images of the subbasal nerve plexus enables the quantification of different nerve fiber parameters, including the corneal nerve fiber length (CNFL) and corneal nerve branch density (CNBD).(Figure 2) These corneal parameters have been demonstrated to correlate with other objective assessments of peripheral neuropathy in diabetes mellitus.[25] The total area of the imaged nerve plexus used for analysis, and the extent of manual and/or automatic methods used to calculate the abovementioned parameters, are not standardized and thus differ between studies. These aspects will be discussed later in this thesis.

1.1.3.4 *Summary*

To summarize, both functional and structural assessments of peripheral small and large fibers are available, carrying different advantages and limitations. Depending on the hypothesis and what patient group is being evaluated, a decision on which method/s to use can be taken.

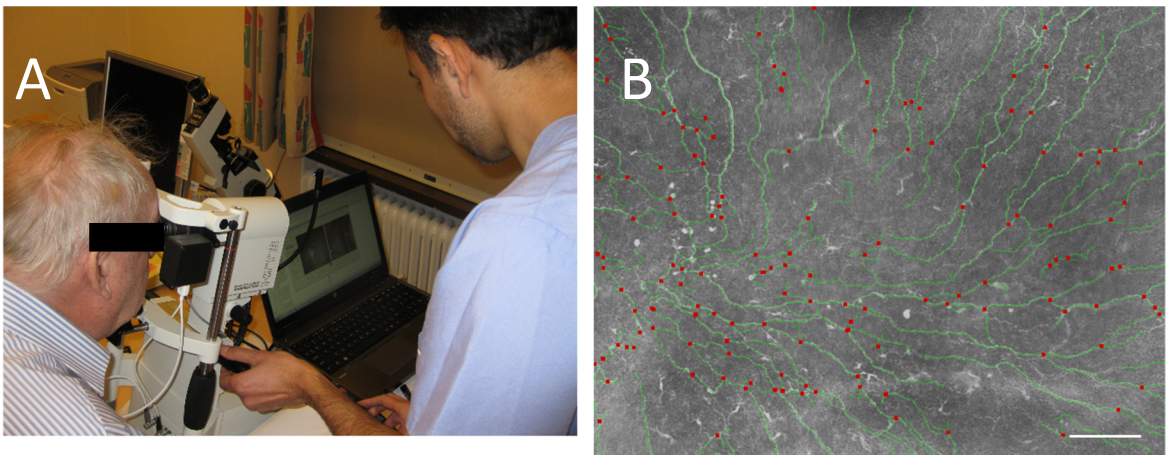

Figure 2. (A) *In vivo* corneal confocal microscopy - a non-invasive tool to image the corneal subbasal nerve plexus. (B) Magnified region of the nerve plexus showing nerve paths (green) and branching points (red). Scale bar = 100 µm. Fig 2A: Courtesy of Professor Lagali. Subjects in the image have approved its reproduction. Fig 2B: Image by Andréasson et al.[26] Licensed under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

1.2 THE ROLE OF COBALAMIN

Cobalamin is a water-soluble B vitamin, essential for cellular function throughout the human body. Main dietary sources include, among others, the intake of milk, cheese, and meat.[27, 28] Common causes of deficiency include autoimmune atrophic gastritis, malabsorption, drug interactions, dietary deficiency, and less commonly, inborn errors of metabolism (IEM).[28]

In this segment, we will review the two main cellular pathways dependent on cobalamin, and discuss possible clinical implications of altered cobalamin metabolism.

1.2.1 Intracellular uptake and the methionine cycle

Following a series of transport steps in the gastrointestinal tract, including binding to intrinsic factor, cobalamin enters the systemic circulation mainly bound to the protein haptocorrin, making it unavailable to most tissues. The remaining 10-30% of cobalamin in the systemic circulation is bound to the transport protein transcobalamin, which instead enables cellular uptake in various tissues.[29] In the cell, cobalamin makes its first stop in the lysosome where transcobalamin is degraded and free cobalamin is made available for lysosomal release. It is thought that at least two lysosomal transport proteins, LMBD1 and ABCD4, are essential for the release of cobalamin into the cell cytosol.[30, 31]

Through a series of intracellular metabolic steps, cobalamin is converted to its two active forms, adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl). MeCbl is essential for maintaining the cytosolic methionine cycle, which will now be discussed further.[29]

The methionine cycle is a cytosolic process with the main purpose of maintaining a cellular methylation capacity through the continuous remethylation of homocysteine (Hcy) to methionine. Methionine is an essential compound given that it can be converted to S-adenosyl-methionine (SAM). SAM is an important donor of methyl groups, vital to several physiological biochemical reactions including methylation of proteins, catecholamines, and DNA.[32]

As shown in Figure 3, the remethylation of Hcy, mediated by the enzyme methionine synthase, is dependent on MeCbl in conjunction with 5-methyltetrahydrofolate (5-CH_3THF). In other words, folate metabolism is linked to the methionine cycle. Besides participation in the methionine cycle, folate is also vital for other cellular activities, including DNA synthesis through metabolism of tetrahydrofolate (THF). Importantly, the availability of THF is dependent on the continuous demethylation of 5-CH_3THF back to THF. Thus, in the setting of MeCbl deficiency, folate may be trapped in the 5-CH_3THF state since MeCbl is necessary for its demethylation. This in turn may have clinical implications reflected by impaired folate dependent DNA synthesis and associated anaemia.[32]

Studying Figure 3 again, a second possible route in the cytosolic handling of Hcy is reflected by the transsulfuration pathway. This pathway enables the generation of cysteine from Hcy and is regulated by the enzyme cystathionine beta-synthase. Importantly, this enzymatic step is irreversible and vitamin B6 serves as a co-factor for the enzyme, thus linking another B vitamin to the methionine cycle.[33]

In conclusion, in the setting of cytosolic cobalamin deficiency, an increase of Hcy is expected together with a reduction of SAM. Moreover, MeCbl deficiency may contribute to functional folate deficiency by means of reduced demethylation of 5-CH_3THF, thus impairing pathways dependent on THF. Likewise, folate deficiency may contribute to an increase of Hcy given the resulting reduced capacity of remethylation of Hcy.(Figure 3)

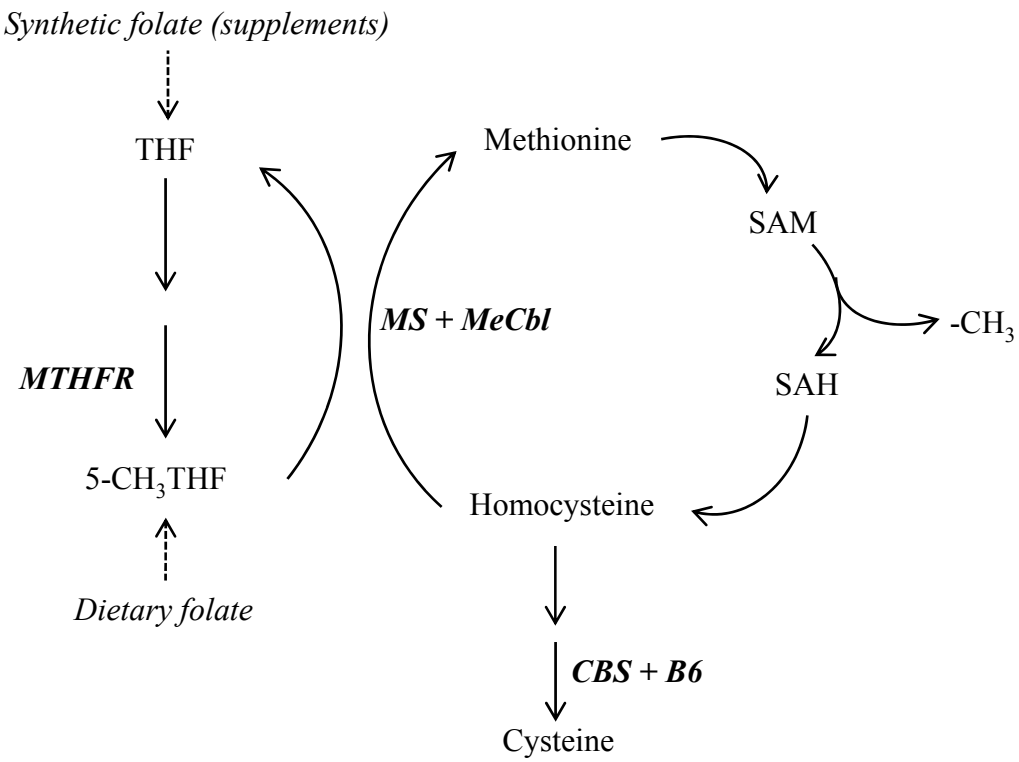

Figure 3. The methionine cycle takes place in the cytosol and upholds a physiological methylation capacity through the continuous remethylation of homocysteine to methionine. For this process, MeCbl and 5-CH_3THF are needed. Abbreviations: THF – tetrahydrofolate; MTHFR – methylenetetrahydrofolate reductase; MS – methionine synthase; SAM - S-adenosyl-methionine; SAH – S-adenosyl-homocysteine; CBS – cystathionine beta-synthase; MeCbl – methylcobalamin; 5-CH_3THF - 5-methyltetrahydrofolate.

1.2.2 Mitochondrial cobalamin metabolism

Besides the important role in upholding methylation capacity through the cytosolic methionine cycle, cobalamin is also involved in mitochondrial metabolism by means of AdoCbl.[32]

The role of AdoCbl in the mitochondrion is related to metabolic pathways degrading cholesterol, odd-chain fatty acids, and branched amino acids. These pathways result in the common downstream metabolite propionyl-CoA. In the mitochondrial compartment, AdoCbl functions as a co-factor for the enzyme methylmalonyl-CoA mutase (MUT), which participates in the catabolic propionyl-CoA pathway that ultimately generates succinyl-CoA. In turn, succinyl-CoA constitutes an important metabolite of the Krebs cycle.[28, 31] (Figure 4)

Reviewing Figure 4 conclusions can be made. Thus, in the setting of mitochondrial cobalamin deficiency, an increase in serum levels of methylmalonic acid (MMA) may occur, together with an accumulation of propionyl-CoA.[28, 34, 35]

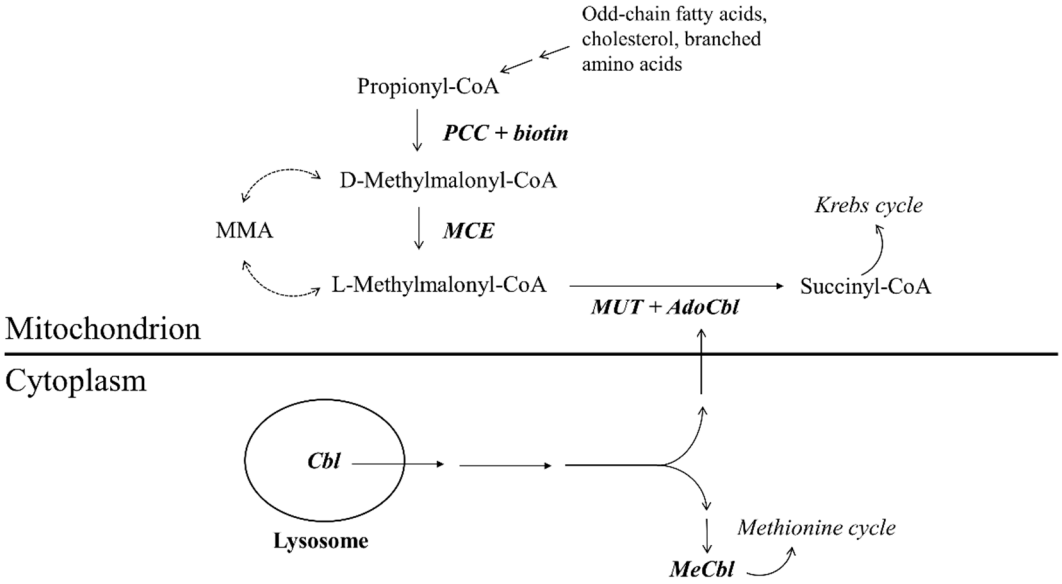

Figure 4. Cobalamin leaves the lysosome and participates in mitochondrial metabolism in the form of AdoCbl. Abbreviations: Cbl- cobalamin; MeCbl – methylcobalamin; AdoCbl – adenosylcobalamin; MUT – methylmalonyl-CoA mutase; MMA – methylmalonic acid; MCE – methylmalonyl-CoA epimerase; PCC – propionyl-CoA carboxylase. Figure by Andréasson et al.[36]

To summarize, in the setting of a general cobalamin deficiency, both mitochondrial and cytosolic cobalamin dependent pathways may be affected. In turn, this may manifest biochemically as increased levels of Hcy and MMA in combination with functional folate deficiency.

1.2.3 Neurological aspects of cobalamin deficiency

Besides the well characterized haematological abnormalities associated with cobalamin deficiency, several associated neurological conditions have been described without consistent correlation to the severity of haematological findings.[37] Neurological signs and symptoms

associated with cobalamin deficiency may reflect both PNS and CNS involvement. Neuropsychiatric symptoms[38], myeloneuropathy[37, 39], myelopathy[37, 39], and peripheral neuropathy[37, 40] have been described in the literature.

With regard to myelopathy, the involvement of the posterior and lateral columns is typical.[39, 41] Clinically, this may translate into proprioceptive loss and upper motor neuron signs of the lower limbs, thus making the clinical detection of a co-existent peripheral neuropathy challenging. Reports on isolated peripheral neuropathy associated with cobalamin deficiency are rare.[37, 40] Characteristic features of peripheral neuropathy, in the setting of cobalamin deficiency, include a sensory axonal process, involvement of both upper and lower extremities, and a subacute onset. [40, 42] Although treatment response to cobalamin substitution has been described with regard to CNS symptoms, less convincing support exists with respect to the PNS.[39, 40]

Several IEM affecting different steps of the intracellular cobalamin pathways have been described. Depending on what stage of the intracellular handling of cobalamin is affected, such metabolic disturbances may be attributed selectively to MeCbl and/or AdoCbl.[29-31] Clinical correlates of selective AdoCbl deficiency are mainly reflected by descriptions of children with IEM affecting the mitochondrial compartment. In these children, different phenotypes encompassing cognitive impairment, stroke, movement disorders, and asymptomatic presentations have been described, sometimes in the setting of acute metabolic decompensation with acidosis.[43, 44]

In conclusion, ample evidence supports the notion that cobalamin deficiency may be associated with CNS and/or PNS disease.

1.3 MOVEMENT DISORDERS

Movement disorders constitute a large heterogeneous group of neurological diseases, mainly characterized by an excess and/or poverty of movements. Importantly, the different clinical phenotypes, within the scope of movement disorders, vary considerably and may encompass several additional neurological and non-neurological features.[45]

In this segment, an introduction to the movement disorders explored in this thesis will be provided.

1.3.1 Parkinson's disease

1.3.1.1 *Epidemiology*

After Alzheimer's disease, PD is the most common neurodegenerative disorder, affecting 2-3% of the global population aged over 65. Considering age being the greatest risk factor for disease development, an increasing prevalence and incidence of PD are to be expected in the coming future.[46] The disease is overrepresented among male populations and onset is less common before the age of 50.[47, 48]

Beyond age and gender, several additional risk factors have been associated with the development of PD. These include environmental exposures, such as pesticides[49], positive family history[50], and the presence of specific genetic variants[51] that confer increased risk.

Negative associations between coffee consumption[52] and smoking[52, 53], and PD, have also been shown. Importantly however, smoking in the setting of established PD has been associated with a negative impact on disease progression in terms of cognitive decline.[54]

1.3.1.2 *Clinical aspects*

Clinically, PD may present with both an excess of movement, such as tremor, as well as poverty of movement manifested by bradykinesia. Together with bradykinesia, the presence of tremor and/or rigidity forms the basis of a clinical diagnosis, as outlined in established diagnostic criteria.[55] Besides motor features, several non-motor symptoms are common and supportive of a diagnosis. These may include sleep disturbances, hyposmia, autonomic dysfunction, cognitive impairment, and psychiatric symptoms. Importantly, these non-motor symptoms have been shown to significantly affect quality of life measures.[56, 57] Moreover, both the clinical expression and progression of disease vary considerable between patients. Thus, motor and non-motor features may progress more rapidly in one patient relative to another, although both share the same clinical diagnosis of PD.[58]

Granting that clinical history and neurological examination constitute the diagnostic cornerstones in PD, different ancillary tests have been proposed and evaluated in studies with regard to diagnostic and prognostic properties. Some of these include structural and functional imaging of the brain and the nigrostriatal pathway[59], biochemical assessments of cerebrospinal fluid (CSF)[60] and other body fluids[61], and neuropathological evaluation of tissue biopsies[62, 63]. However, a robust biomarker of disease progression, which is essential in order to detect disease modifying effects in future clinical trials, is lacking.

As for now, only symptomatic treatment options are available for the care of patients with PD. These include pharmacological and non-pharmacological options. With regard to motor symptoms, treatment aims to restore the functional consequences of the striatal dopamine deficiency associated with the disease. The main pharmacological treatment options include levodopa (L-dopa), dopamine agonists and enzyme inhibitors. L-dopa and dopamine agonists pass the blood-brain-barrier (BBB), after which L-dopa is decarboxylated to active dopamine. Both agents act by stimulating striatal dopamine receptors with varying affinities for different receptors. Enzyme inhibitors confer their effect by reducing the metabolism of dopamine and L-dopa, thus enhancing dopamine availability in the brain. Of interest for this thesis, catechol-O-methyltransferase (COMT) is an enzyme that methylates L-dopa in a SAM dependent reaction, both in the brain and in peripheral blood. This methylation precludes the generation of dopamine. Consequently, treatment with COMT inhibitors may reduce this SAM dependent methylation and thus increase the flux of L-dopa that is converted to dopamine.

Several non-motor symptoms, such as depression, cognitive impairment, and obstipation, may respond to non-dopaminergic agents commonly used in settings outside of PD.[46, 64]

In conclusion, significant clinical heterogeneity with regard to disease presentation and progression is common in PD. An individually tailored treatment plan, accounting for this heterogeneity, is essential in order to minimize drug side-effects and to address the symptoms perceived most bothersome by the individual patient.

1.3.1.3 *Pathogenesis*

The pathogenesis of PD is not fully understood. However, several pathophysiological mechanisms have been identified and supported by clinical and preclinical evidence. The main neuropathological findings in PD consist of the degeneration of dopaminergic neurons of the midbrain and the deposition of intracytoplasmic aggregates of the protein alpha-synuclein (α-syn), named Lewy bodies, in neurons throughout the brainstem.[65, 66] It has been postulated that clinical disease progression is mirrored by the neuropathological spread of Lewy bodies within and subsequently outside of the brainstem, finally reaching the cerebral cortex.[56, 65] Accordingly, striatal dopamine deficiency, secondary to the degeneration of the dopaminergic pathway projecting from the substantia nigra to the striatum, is characteristic of the disease and regarded causative of many of its motor symptoms. However, several non-motor symptoms may instead be attributed to pathology and neurotransmitter deficiencies in cell populations outside of the substantia nigra.

Whether Lewy bodies are merely the result of upstream disease mechanisms or a disease generator itself, is debated. Nevertheless, the presence of Lewy bodies, together with midbrain neurodegeneration, is considered a neuropathological hallmark of PD.[67]

1.3.1.4 *Peripheral neuropathy and alpha-synuclein pathology in Parkinson's disease – what is known?*

The presence of PD-associated pathology in the PNS has been known for a long time. In 1960, a neuropathological autopsy study in patients with, what was then referred to as, "*idiopathic paralytic agitans*", reported the microscopic presence of Lewy bodies in the autonomic peripheral nervous system.[68]

Evidence of peripheral neuropathy in PD has emerged in the last two decades, highlighted by a seminal skin biopsy study in 2006 that demonstrated a significant autonomic small fiber denervation (sympathetic C-fibers) relative to controls.[69] These findings were further extended by Nolano et al, demonstrating a cutaneous somatosensory small fiber neuropathy, in which the severity of small fiber denervation (as well as involvement of Meissner's corpuscles) exhibited an asymmetry congruent with the side clinically more affected by parkinsonism.[70] Moreover, a sensory large fiber axonal neuropathy, as assessed by sural NCS, was demonstrated in L-dopa treated PD patients in 2004.[71]

Several ensuing studies have repeated the above findings, reporting an increased prevalence of both small and large fiber neuropathy in PD, with prevalence rates as high as 55 %.[72] The phenotypic descriptions of large fiber neuropathy, in the setting of PD, have been reported as symmetric, length dependent, sensory or sensorimotor, and axonal, as assessed by clinical examination and NCS.[73-76] However, with regard to small fiber neuropathy, as assessed by skin biopsy, asymmetric and both length and non-length dependent patterns of autonomic and somatosensory denervation have been demonstrated.[19, 69, 77, 78]

Associations with altered methionine cycle metabolism

Considering L-dopa is a substrate for SAM dependent methylation by COMT, chronic L-dopa exposure has been suggested to put extra pressure on the methionine cycle and its remethylation capacity, in order to uphold production of methionine.[79] In turn, this pressure

has been suggested to result in an increased demand for MeCbl and 5-CH_3THF, compounds necessary for the remethylation of Hcy to methionine.(Figure 3)

Indeed, associations between peripheral neuropathy, and altered levels of Hcy, MMA, cobalamin, folate, and duration of L-dopa exposure have been reported.[71, 72, 74-76, 80] Furthermore, a possible stabilizing therapeutic effect of B-vitamin supplementation has been suggested in previous studies, although not in any randomized controlled treatment study.[73, 81] Whether the associations between peripheral neuropathy and altered methionine cycle metabolism reflect causal effects have thus not been fully elucidated. Besides the previously described neurological consequences of cobalamin deficiency, which include peripheral neuropathy, toxic effects of homocysteine have been suggested to contribute to the observed associations. Indeed, hyperhomocysteinemia has been associated with endothelial dysfunction and oxidative stress, and proposed as a risk factor for vascular disease.[79, 82]

With regard to vitamin B6, a co-factor for the transsulfuration of Hcy (Figure 3), associations between deficiency and L-dopa dose, as well as the development of large fiber neuropathy have been suggested.[83, 84] Vitamin B6 is necessary for the decarboxylation of L-dopa to dopamine. Since dopamine cannot pass the BBB, most L-dopa formulations are combined with carbidopa, an inhibitor of active vitamin B6, in order to reduce decarboxylation of L-dopa in the systemic circulation. Consequently, an enhanced supply of non-decarboxylated L-dopa is made available to the CNS. However, the irreversible bond between carbidopa and active vitamin B6 has been suggested to increase the risk of systemic vitamin B6 deficiency, as reported in smaller studies.[85] Outside the setting of PD, both excess and deficient levels of vitamin B6 have been associated with peripheral neuropathy.[86]

On the contrary, an absence of convincing associations between small fiber neuropathy, and altered methionine cycle metabolites or L-dopa exposure, have also been reported.[78] Furthermore, an increased prevalence of cutaneous and corneal small fiber neuropathy has been demonstrated in PD patients naïve to L-dopa[19, 69], or with minimum exposure[87]. These reports thus argue against L-dopa and alterations of the methionine cycle as causative factors for the development of peripheral neuropathy. More specifically, Nolano et al demonstrated somatosensory and autonomic denervation in skin biopsies from both L-dopa treated and L-dopa naïve patients. The study also demonstrated a reduced density of Meissner's corpuscles in glabrous skin from the fingertip, suggested to reflect a distal sensory large fiber neuropathy. Notably, all these patients exhibited normal NCS and the detected cutaneous large fiber denervation was more pronounced in patients with L-dopa treatment.[19]

Pathological alpha-synuclein deposits in cutaneous nerve fibres

In recent years, detection of α-syn pathology outside of the CNS has been explored in several studies. Deposits of phosphorylated α-syn (p-α-syn) have been demonstrated in cutaneous small nerve fibers, predominantly autonomic C-fibers but also in somatosensory small fibers.[78, 88, 89] Interestingly, the detection of these deposits has been reported to follow a length dependent gradient, with the highest yield seen in biopsies taken from the neck.[78, 90] Moreover, fiber selectivity with regard p-α-syn deposits has been demonstrated and suggested to confer discriminatory properties within the group of synucleinopathies. Thus, a predilection for deposits in autonomic fibers has been demonstrated in PD, while Multiple

system atrophy, another synucleinopathy, has been associated with the involvement of somatosensory fibers.[62, 91] Overall, several studies have concluded that the detection of cutaneous p-α-syn has high specificity for an underlying synucleinopathy.[88, 92, 93]

As described with regard to neuropathological findings in the CNS, the role and significance of p-α-syn deposits in peripheral nerves of PD patients are not fully elucidated. However, the, to some extent, demonstrated associations between localization of p-α-syn deposits and ongoing denervation[62] may argue against an epiphenomenon.

Patterns of peripheral denervation

A symmetric length dependent pattern of denervation is commonly seen in polyneuropathy of other causes, such as diabetes. In PD, several studies have reported a length dependent (distal to proximal) pattern of denervation both with regard to small[19, 78] and large[73, 74] fibers. However, Doppler et al observed that a subtype of intraepidermal small fibers (substance P positive) demonstrated denervation in a non-length dependent pattern, akin to what is expected for a sensory neuronopathy.[78] Furthermore, a reversed length dependent (proximal to distal) gradient of cutaneous small fiber denervation was suggested in a longitudinal study, where the cervical IENFD was proposed as a possible marker of disease progression.[94]

Clinically, PD typically presents with asymmetric motor symptoms in terms of parkinsonism, and this asymmetry has been shown to be stable over time.[95] Moreover, neuropathological studies have demonstrated an asymmetric loss of neurons in the substantia nigra, reflective of the clinical symptoms.[96] With regard to the PNS, several studies have supported an asymmetric distribution of cutaneous small fiber neuropathy in PD, with a more pronounced reduction of IENFD in skin biopsies taken from the side more affected by parkinsonism.[19, 70, 77, 97]Thus, peripheral neurodegeneration in PD has been speculated to echo the asymmetric neurodegeneration seen in the CNS.[19]

Contribution to symptom burden

Studies addressing the contribution of peripheral neuropathy to the overall PD symptom burden are few. However, the presence of peripheral neuropathy has been proposed to constitute a marker of a more aggressive PD phenotype, correlating with more pronounced motor and non-motor symptoms.[98] An association between the presence of peripheral neuropathy and an increased risk of falls has also been reported.[99] Moreover, a more recent comprehensive study was able to demonstrate an objective negative influence of peripheral neuropathy on gait, as assessed by wearable technology.[100]

In summary, small and large fiber neuropathy has frequently been demonstrated in PD. Whether these findings are reflective of a peripheral neurodegenerative process primarily targeting small fibers, an effect of chronic L-dopa exposure primarily targeting large fibers, or a combination, is not fully understood. Nevertheless, the detection of peripheral neuropathy in PD appears to harbour both clinical and possibly diagnostic implications.

1.3.2 Restless legs syndrome

1.3.2.1 *Epidemiology*

Restless legs syndrome (RLS) is a common neurological disorder, with an estimated adult prevalence of 5-8.8%[101] fulfilling clinical criteria, and 2-3%[102] with clinically significant symptoms. The disease is more frequent among women, often presents between ages 30 to 40, and the prevalence increases with age.[101]

RLS has been associated with neurological disorders as well as other systemic conditions. These include periodic limb movement disorder[103], pregnancy[104], iron deficiency anaemia[105], and chronic kidney disease[106]. Importantly, RLS has been demonstrated to have an impact on several quality of life measures, thus emphasising the importance of diagnosis and treatment.[107]

1.3.2.2 *Clinical aspects*

RLS is regarded as a neurological disease with a circadian pattern of symptom expression, characterized by an urge to move the legs in order to reduce a perceived discomfort in the lower limbs. As described by the International Restless Legs Syndrome Study Group in 2014, diagnosis is based on five clinical criteria, preferably assessed by oral history. Thus, besides the urge to move the legs, a clinical diagnosis requires the presence of worsening of symptoms during night-time and inactivity, as well as symptom relief induced by movement.[108] The name of the disease stems back to the Swedish neurologist Karl-Axel Ekbom, who compiled and described a series of patients with the syndrome in 1945.[109]

Ruling out clinical mimics of RLS is an important key to correct diagnosis and treatment. This is of certain relevance when diagnosing RLS in the setting of co-existent PD. The clinical expression of RLS in PD has been characterized, in contrast to idiopathic RLS, by weaker heredity, more pronounced unilaterality of symptoms, older age at presentation, and symptom onset more often occurring after PD diagnosis.[110-112] Moreover, some symptoms specific for PD may mimic RLS, such as wearing-off phenomenon, dystonia, and akathisia.[111, 113]

A possible association between RLS and PD has been explored in several studies and rendered conflicting data. Data on prevalence of RLS in PD thus vary considerably, including prevalence rates reported as both higher and equal to controls.[111, 114-119] Notably, in studies evaluating PD patients naïve to dopaminergic treatment, the prevalence of RLS has been reported as not significantly higher compared to controls.[120] These conflicting data have been attributed partly to the presence of RLS mimics in PD. In this context, ancillary testing to consolidate the diagnosis of RLS in PD has been proposed, including the sensory suggested immobilization test.[121, 122]

Treatment of RLS includes iron substitution if ferritin levels are in the lower reference range. In the absence of low iron levels, main pharmacologic options include different dopaminergic agents, α2δ-ligands and opioids (oxycodone-naloxone).[123-125] An important clinical aspect in the treatment of RLS is the risk of augmentation, that is the emergence of symptoms earlier in the day together with spreading of symptoms to other body parts and a need for increased

medication dosage. The established association between dopaminergic treatment and risk of augmentation has suggested the use of α2δ-ligands as a first line of treatment.[126, 127]

1.3.2.3 *Pathogenesis*

The pathogenesis of RLS is not fully understood, but pathophysiological mechanisms have been postulated. An underlying disturbance of cerebral iron metabolism, with reduced cerebral iron levels has been supported by neuropathological[128, 129], imaging[130, 131], and CSF[132] studies, together with the observed treatment response to iron. Interestingly, PD, in contrast to RLS, is a disorder characterized by increased cerebral iron levels.[131] Considering the treatment response to dopaminergic agents, altered dopaminergic signalling from the brain to the spinal cord has also been suggested to be implicated in RLS.[133] Genetic risk factors have been reported, but a clear understanding of their pathogenic role remains.[134]

Furthermore, some studies have suggested an increased prevalence of RLS in populations with peripheral neuropathy. Studying patients with diabetic neuropathy, a high prevalence of RLS (33.3%) was demonstrated and was shown to associate with small fiber affection as assessed by skin biopsy.[135] Hattan et al assessed the prevalence of RLS in patients with an established diagnosis of peripheral neuropathy, demonstrating an increase among patients with a hereditary polyneuropathy relative to controls.[136] In contrast, studies have also argued against an association between RLS and peripheral neuropathy.[137]

1.3.3 Hereditary spastic paraplegia

1.3.3.1 *Epidemiology, inheritance, and terminology*

Hereditary spastic paraplegia (HSP) constitutes a large group of hereditary progressive neurodegenerative movement disorders. Given a pronounced heterogeneity with regard to underlying genetic mechanisms and clinical presentation, epidemiological studies assessing the disease group as a whole have been difficult. However, a Norwegian population study, based on 2.68 million people, reported a HSP prevalence of 7.4/100 000 with a majority reporting disease onset before the age of 40 and no detected gender differences.[138]

The inheritance in HSP includes autosomal dominant, autosomal recessive, mitochondrial, and X-linked patterns. The different HSP subtypes have traditionally been assigned the name SPG (spastic paraplegia gene) followed by a number, indicating when the associated gene or locus were first discovered. As of today, more than 80 subtypes of HSP have been described and associated with different genes or loci. Importantly, in some of these subtypes the causative protein or gene have not been identified or fully confirmed.[139]

Partly in light of the above, a more uniform nomenclature has been proposed in general for genetically determined movement disorders. The naming of the predominant phenotype followed by the underlying proved causative gene has thus been recommended.[140] As an example, the formerly assigned subtype SPG10 is in this thesis therefore instead referred to as HSP-*KIF5A*.

1.3.3.2 *Clinical aspects and pathogenesis*

The different HSP subtypes vary considerably in clinical course and phenotype. However, all share the presence of a slowly progressive lower limb weakness with associated spasticity, diminished vibration sensation of the lower limbs, and bladder disturbances. When additional neurological features are present, eg peripheral neuropathy, cognitive impairment, and/or parkinsonism, the phenotype is regarded as complicated HSP.[139]

As for now, no curative or disease-modifying treatments exist for HSP. Symptomatic treatment focuses on ameliorating symptoms of spasticity, pain, and bladder disturbances with pharmacological agents and physical therapy.

With regard to pathogenesis, it has been suggested that some pathophysiological mechanisms may be common for HSP, despite the vast number of subtypes.[141] As an example, axonal transport of cargo to and from the neuronal cell body is dependent on binding to microtubules. In both HSP-*KIF5A* and HSP-*SPAST*, the underlying pathogenic gene variants are expected to compromise microtubule dependent activity.[142, 143] An insufficient communication between the cell body and axonal terminals, due to impaired axonal transport, is also fitting with the clinical picture of a length dependent upper motor neuron pathology primarily resulting in lower limb weakness.

1.3.3.3 *Phenotypic and genotypic heterogeneity*

The molecular diagnostic evaluation of patients with suspected HSP is a challenge and has historically relied on a detailed phenotypic characterization, followed by a targeted genetic testing based on previously reported genotype-phenotype correlations. Such targeted genetic testing has also been guided by the reports on varying prevalence between HSP subtypes, with HSP-*SPAST* accounting for the majority of autosomal dominant cases.[144, 145]

With the advent of next generation sequencing more comprehensive genetic analyses are possible. Gene panels, in which a panel of HSP associated genes are tested, as well as whole exome or genome sequencing followed by filtered analysis of genes associated with HSP, are becoming more available. In turn, this has resulted in publications of more comprehensive genotype-phenotype correlations in different HSP subtypes. Indeed, significant phenotypic overlaps between different HSP subtypes, as well as with other neurodegenerative disorders such as hereditary ataxia, have been demonstrated.[141]

More specifically, pathogenic variants in *KIF5A* have been associated with both Charcot-Marie-Tooth type 2 (CMT2), a hereditary polyneuropathy, and HSP-*KIF5A*. This phenotypic variability has been reported as a spectrum, where a varying degree of combined PNS and CNS involvement may be present. Moreover, an intrafamilial phenotypic variability in patients with the same pathogenic variant in *KIF5A* has also been described.[146]

1.4 GAUCHER DISEASE

1.4.1 Epidemiology and pathogenesis

Gaucher disease (GD) is an autosomal recessive disorder caused by insufficient activity of the lysosomal enzyme glucocerebrosidase (GCase). The enzyme is encoded by the *GBA1* gene and crucial for the cellular metabolism of glycosphingolipids. Glucosylceramide is a glycosphingolipid that is generated during the degradation of cell membranes, and consequently a substrate for GCase, together with glucosylsphingosine. In GD, the reduced GCase activity leads to the accumulation of glucosylceramide primarily in macrophages that reside in different tissues, so called "Gaucher cells". These alterations in glycosphingolipid metabolism are considered central to disease pathogenesis.[147, 148]

GD is a rare disease with a reported birth frequency of 1:47000 births in the general population of Sweden[149], slightly more common than 1:57000 births reported in an Australian population study.[150] This may partly be explained by a well described cluster of GD patients in the northern part of Sweden, referred to as Norrbottnian GD type 3 (GD3).[151]

1.4.2 Clinical aspects

The clinical presentation, progression, and severity of GD vary why a subclassification based on clinical phenotype often is employed.

GD type 1 (GD1) is typically characterized by pronounced visceral, skeletal, and haematological manifestations, and patients can be diagnosed both in child- and adulthood.[148] GD type 2 (GD2) and GD3 exhibit additional CNS involvement, where patients with GD2 seldom survive early childhood. Distinct features of the CNS involvement in GD3 include predominant horizontal supranuclear gaze palsy, myoclonic epilepsy, spasticity, ataxia, and dementia. Patients in this group have been reported to show a more variable disease progression and severity, and often survive into adulthood.[147, 152]

The underlying pathogenic variants in the *GBA1* gene have been demonstrated to correlate, to some extent, with disease phenotype. Thus, homozygosity for the L444P variant has been associated with GD3, while the presence of one allele carrying the N370S variant is considered protective against GD3.[153]

Existing treatments for GD are targeted to mitigate the effects of reduced GCase activity. With enzyme replacement therapy, recombinant GCase is infused intravenously on a regular basis in order to uphold cellular GCase activity. With substrate reduction therapy, an oral agent is administered and acts by reducing levels of glucosylceramide, the substrate to GCase, through upstream mechanisms. Importantly, these therapies have demonstrated effects on haematological and/or visceral symptoms, but no treatment effect has been shown with regard to neurological symptoms.[154]

1.4.3 Peripheral nervous system involvement and cobalamin

Involvement of the PNS in GD, not related to mechanical nerve or medullar compression, has been under increasing investigation. However, early case reports also exist, including a female patient with GD and peripheral neuropathy in association with cryoglobulinemia from

1978[155], and a female patient on enzyme replacement therapy with a reported sensory axonal peripheral neuropathy in association with anti-sulfatide antibodies from 1995.[156]

In the last two decades, several studies evaluating involvement of the PNS in GD1 have been carried out. A Dutch study from 2008 explored the prevalence of neurological symptoms in a retrospective cohort of 75 patients with GD1. Four patients demonstrated an electrodiagnostically confirmed diagnosis of peripheral polyneuropathy and three patients exhibited a mononeuropathy.[157] Similar findings were reported in a French cross-sectional study from 2010, including a cohort of 105 patients with GD1. Five percent of these patients carried a diagnosis of peripheral neuropathy, although three of these patients also exhibited an associated gammopathy. The authors proposed a separate neurological phenotype in GD1, with possibly unique neuropathology, making this disease subtype more prone to develop peripheral neuropathy.[158]

Prospective studies exploring, among other endpoints, PNS involvement in GD1 have been carried out. A Spanish study from 2007 included 31 patients classified as GD1. 15 of these patients underwent electrodiagnostic evaluation of which 12 exhibited sensory and/or motor abnormalities of primarily axonal type.[159] A larger prospective study from 2010 evaluated the prevalence and incidence of peripheral neuropathy over a 2-year period in 103 patients with GD1. 11 patients (10.7%) demonstrated clinical and electrodiagnostic findings compatible with a symptomatic large fiber polyneuropathy at baseline. An additional six cases were observed during the 2 year observation time, and the neuropathies were mainly described as axonal and sensorimotor. Of note, two of the patients exhibited a concurrent gammopathy, and the neuropathic patients were older and had significantly higher levels of Hcy and MMA.[160] Furthermore, studying a group of GD1 patients reporting pain, a high prevalence of small fiber neuropathy, as assessed by skin biopsy and QST, has also been reported. Notably, a considerable proportion of these patients presented a non-length dependent cutaneous denervation pattern.[161]

The mechanisms underlying peripheral neuropathy in GD1 are not fully understood. An intrinsic disease feature, possibly reflecting disturbed lipid accumulation at the level of the sensory dorsal root ganglia has been hypothesized.[160] Moreover, peripheral neuropathy as a complication to treatment with substrate reduction therapy[162] has also been suggested. Studies assessing the PNS in GD3 are lacking.

Considering the lysosomal dysfunction intrinsic to GD, and the role of the lysosome in cobalamin metabolism, an increased risk of cobalamin deficiency in GD has been speculated.[163] However, studies are scarce and although a high incidence of low serum levels of cobalamin have been demonstrated, these levels were not significantly different from controls.[164] Furthermore, a study on cultured skin fibroblasts from GD patients could not demonstrate significant metabolic abnormalities with regard to the intracellular handling of cobalamin.[165]

1.4.4 Genetic associations with Parkinson's disease

In recent years, the presence of heterozygous or homozygous variants in the *GBA1* gene has been established as the most common genetic risk factor for the development of PD, with a reported 5- to 6-fold risk increase.[51, 166] Furthermore, decreased activity of GCase in dried

blood spots has been demonstrated not only in PD patients with *GBA1* variants, but also in sporadic PD.[167] The underlying mechanisms driving the association between PD and the presence of *GBA1* variants are not fully understood. However, in vitro and animal studies suggest an interaction between GCase and α-syn, resulting in further lysosomal dysfunction and an increased aggregation of pathological α-syn.[168, 169]

1.5 SUMMARY

Movement disorders are often viewed as disorders of the CNS. Various methods to assess the more accessible PNS are available, and have begun to shed further light on possible implications of detecting PNS involvement in some of these disorders. Altered cobalamin metabolism has been associated with neurological signs and symptoms arising from both the CNS and PNS, and possible implications in PD have been suggested.

In view of the above, the research aims of this thesis were formulated.

2 AIMS

The general aim of this thesis was to investigate different aspects of the PNS and the integrity of the methionine cycle in the setting of an ongoing movement disorder.

The major specific aims were:

A. To explore the prevalence of clinical signs of peripheral neuropathy, and its possible associations with altered cobalamin metabolism, in a Swedish L-dopa treated PD population.

B. To investigate whether RLS is a possible clinical expression of small fiber neuropathy in PD, using novel corneal confocal microscopy methodology.

C. To perform a clinical, genetic, and metabolic characterization of an adult patient with PD and biochemical alterations in the AdoCbl dependent mitochondrial pathway.

D. To explore the prevalence of peripheral polyneuropathy in the rare lysosomal storage disorder Gaucher disease, including a group of patients with the rare Norrbottnian subtype.

E. To perform a clinical, genetic and biochemical CSF characterization of two families with HSP-*KIF5A*.

3 MATERIALS AND METHODS

All papers included in this thesis are based on studies involving the participation of patients and controls. In study I, II, IV, and V participants were included from the Stockholm region, with the majority being patients, and accompanying persons, followed at the outpatient clinics at the Department of Neurology, Karolinska University Hospital and Center for Neurology, Academic Specialist Center. In study III, participants were invited to participate in conjunction with clinical visits at the Department of Haematology, Karolinska University Hospital and the Department of Medicine, Sunderby Regional Hospital.

Below, the main methodology used in carrying out the work for this thesis will be described.

3.1 CLINICAL ASSESSMENTS

In this segment, the main clinical scales and evaluations will be presented. For more detailed information, with regard to assessments and inclusion/exclusion criteria, the reader is referred to the individual papers included in this thesis.[26, 36, 170-172]

3.1.1 Diagnosis and evaluation of disease burden

3.1.1.1 *Parkinson's disease*

Throughout this thesis, established clinical diagnostic criteria have been used in order to consolidate the study diagnosis of PD, with certainty levels as described in the individual studies. The presence of bradykinesia in combination with resting tremor and/or rigidity constitutes a hallmark of these criteria.[55, 173]

In order to quantify the burden of disease, different clinical scales have been applied. An established measure of the global burden of motor symptoms in PD is the Hoehn and Yahr (H&Y) scale.[174] This scale aims to measure the distribution of motor involvement and its resulting functional impact on balance, gait and postural stability. Significant impairment of postural reflexes, often expressed clinically as dependency on walking aid, corresponds to H&Y stage 3.0, which may be regarded as a milestone with regard to axial motor progression. In addition, a modified version of the scale, mH&Y, has been widely used in PD research.[175] This scale aims to enable a more granular evaluation of motor burden, especially with respect to balance as shown in Table 2. H&Y and mH&Y were used in study I and II respectively.

Assessment of cognition, in study I, IV, and V, was carried out with the Montreal Cognitive Assessment (MoCA), a scale proposed sensitive for the detection of mild cognitive impairment.[176]

Hoehn and Yahr	Modified Hoehn and Yahr
1. Unilateral involvement only, usually with minimal or no functional disability	**1.0** Unilateral involvement only
	1.5 Unilateral and axial involvement
2. Bilateral or midline involvement without impairment of balance	**2.0** Bilateral involvement without impairment of balance
3. Bilateral disease; mild to moderate disability with impaired postural reflexes; physically independent	**2.5** Mild bilateral disease with recovery on pull test
	3.0 Mild to moderate bilateral disease; some postural instability; physically independent
4. Severely disabling disease; still able to walk or stand unassisted	**4.0** Severe disability; still able to walk or stand unassisted
5. Confinement to bed or wheelchair unless aided	**5.0** Wheelchair bound or bedridden unless aided

Table 2. The original and modified Hoehn & Yahr rating scale.[174, 175]

3.1.1.2 *Restless legs syndrome*

In Study II, diagnosis of RLS relied on established clinical diagnostic criteria. These criteria are solely based on oral history and not on clinical signs.[108] In order to further consolidate the diagnosis of RLS, in the context of co-existent PD, the sensory suggested immobilization test was carried out.[121, 122] This test assesses the sensory discomfort reported by the patient when observed in a recumbent position and instructed to lie still for an hour (between 8 and 9 PM). Every 10 minutes the subject is asked to report the discomfort perceived in the lower limbs using a visual analogue scale. A mean leg discomfort score >11 has previously been proposed as supportive of RLS diagnosis in PD.[121]

The severity of RLS symptoms was quantified with the International Restless Legs Syndrome Study Group Rating scale, a patient questionnaire assessing the subjective impact of RLS symptoms during the previous week.[177]

3.1.1.3 *Gaucher disease*

All included patients with GD1 and GD3 in Study III had a previously confirmed molecular and genetic diagnosis. Disease severity was evaluated with oral history and revision of medical records. The modified Severity Scoring Tool (mSST) was applied in the GD3 population. This clinical rating scale was established to monitor disease progression in GD with neurological involvement. The scoring tool encompasses 12 domains reflecting various neurological signs and symptom history. Each domain is given a separate weighted score with a maximum total score of 36 for all included domains.[178]

3.1.1.4 *Hereditary spastic paraplegia*

All patients included in Study V had an established clinico-genetic diagnosis of HSP-*KIF5A*. Assessments consisted of several clinical rating scales, including the Spastic Paraplegia Rating Scale (SPRS). This scale addresses neurological signs and implications of spasticity, as well as historical symptoms including pain and autonomic symptoms.[179] Moreover,

additional rating scales were used in order to capture non-pyramidal signs and symptoms, as described in the paper.

3.1.2 Assessing the peripheral nervous system

The clinical evaluation of signs compatible with peripheral neuropathy was performed, throughout study I, II, and III, with the UENS.[15] This scale is considered sensitive for the detection of an early sensory predominant length dependent neuropathy.[15]

Clinical rating scales constituted the sole assessments of the peripheral nervous system in study I, while Study II, III and V included more objective assessments as described in the ensuing segments. In Study III patient reported symptoms indicative of peripheral neuropathy were assessed by oral history.

3.2 NEUROPHYSIOLOGY

In Study II, III and V collaborative work was undertaken with the Departments of Neurophysiology at Karolinska University Hospital, Sunderby Regional Hospital and Umeå University Hospital. In Study III, NCS and QST were performed primarily in GD patients reporting symptoms compatible with a peripheral neuropathy and/or a UENS score ≥ 4. This cut-off was chosen based on previous descriptions of small fiber neuropathy in this disease.[161]

3.2.1 Nerve conduction studies

Sensory and motor NCS were performed in upper and lower extremities, with the aim of detecting possible axonal or demyelinating large fiber pathology. In Study II, a separate electroneurography-index (ENeG-Ix) was determined for each participant. The ENeG-Ix is calculated from twelve different parameters, derived from the testing of both sensory and motor nerves. Briefly, the index for each participant represents the degree of deviation from normal controls standardized for age and height. The ENeG-Ix has thus been postulated to constitute a composite measure of large fiber function.[180]

The choice of tested nerves and details on used equipment are further detailed in the individual papers.

3.2.2 Quantitative sensory testing

Perception thresholds for cold and warmth in hand and foot were determined, as an indirect measure of small fiber function (Aδ- and C-fibers respectively). Details on used equipment and the number of examined extremities are further detailed in the individual papers.

3.3 IN VIVO CORNEAL CONFOCAL MICROSCOPY

IVCCM is a non-invasive method that was used for Study II. Briefly, by using a confocal microscope the small Aδ- and C-fibers of the central corneal subbasal nerve plexus were visualized and recorded. From the recorded images, subsequent automatic generation of large two-dimensional mosaic images were generated. From these mosaics, further automatic algorithms enabled the quantification of mean values of nerve fiber parameters, including corneal nerve fiber length (CNFL, the total length of all nerve fibers in a mosaic image,

[mm/mm^2]), and corneal nerve branch density (CNBD, the total number of branching points of nerve fibers in a mosaic image, [no/mm^2]).[181-184]

A more detailed description of the above methodology is provided in the corresponding paper.

3.4 BIOCHEMICAL ANALYSES

In all studies, biochemical blood analyses were performed according to clinical routine. Below, some of these are highlighted for their importance in understanding the aims and discussion of the present thesis.

3.4.1 Integrity of the methionine cycle

Plasma or serum levels of Hcy, MMA, cobalamin and folate were analysed (Study I, II, III and IV) as a way of assessing the status of the methionine cycle. Alterations in the levels of these compounds may all reflect direct and/or indirect deficiencies of vitamin B6, B9 and B12.[28]

3.4.2 Plasma and cerebrospinal fluid neurofilament light chain

Levels of neurofilament light chain (NfL), a marker of axonal degeneration, were measured in plasma (Study II) and CSF (Study V) in collaboration with Sahlgrenska University Hospital, Gothenburg. An in-house ELISA method and a single molecular array assay were used to determine NfL levels in CSF and plasma respectively.[185, 186] Of note, an influence of both age and male sex on CSF levels of NfL has previously been demonstrated.[187, 188] Further details are outlined in the respective papers.

3.4.3 Monoamine metabolites in cerebrospinal fluid

In Study V, frozen CSF samples were transported to Sahlgrenska University Hospital, Gothenburg, for measurement of monoamine metabolites. High-performance liquid chromatography was performed to separate homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) with subsequent electrochemical detection and quantification.[189]

3.5 GENETIC ANALYSES

In Study I, DNA was primarily extracted from peripheral blood mononuclear cells isolated from whole blood. Genotyping of predetermined single nucleotide polymorphisms (SNPs) in the *COMT* and *MTHFR* genes was performed with commercial TaqMan assays. This work was carried out by co-authors at Svenningsson lab, Karolinska Institutet. Further details are outlined in the paper.

In study V, whole genome sequencing (WGS) of DNA isolated from blood was performed at the Center for Inherited Metabolic Diseases, Karolinska University Hospital. The obtained WGS data were filtered in order to only analyze genes associated with IEM. The two detected variants in the *MCEE* gene were confirmed through Sanger sequencing. Subsequently, the

biallelic localization of these variants was confirmed through targeted Sanger sequencing of the *MCEE* gene in the proband's two sons.[190]

3.6 STATISTICAL ANALYSES

Statistical analysis is an important tool to present data in an understandable way. Furthermore, statistical calculations are needed to test the hypotheses and research questions that have been postulated during the planning of the respective studies.

IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was used for the analyses in Study II and III. Statistica version 13 (TIBCO Software Inc., Palo Alto, USA) was used for Study I.

3.6.1 Descriptive analyses

The descriptive data collected for this thesis included various categorical and numerical variables. Categorical nominal variables were primarily presented as proportions. Ordinal and numerical variables were presented as medians and/or means with interquartile ranges and standard deviations respectively.

3.6.2 Comparative and correlation analyses

Numerical variables were tested for normality with the Shapiro-Wilk test and by the assessment of skewness. If meeting assumptions for parametric testing, the independent *T*-test was used for comparisons between groups. If not, non-parametric testing with the Mann-Whitney *U*-test was employed. Comparisons between more than two groups were performed with one-way analysis of variance when meeting the requirements for parametric testing, including the assumption of homoscedasticity. If not, Kruskal-Wallis *H*-test was used. Categorical nominal variables were compared between groups by using Chi-square and Fisher's exact test.

Non-parametric Spearman's rank order correlation was primarily used to study associations between variables throughout this thesis.

3.7 ETHICAL CONSIDERATIONS

All studies were approved by the regional ethical board of Stockholm. All participants were given written and oral information about the study design, study purpose and possible implications of participation. Care was taken to answer all questions that arose during this conversation. Information was given with regard to appropriate measures in the event of unexpected biochemical or clinical findings. Written and oral informed consent was thereafter obtained from all participants.

Reference numbers for the individual ethical applications and decisions are outlined in respective papers.

4 RESULTS

In this chapter, the main results of the studies included in this thesis will be presented.

4.1 PLASMA HOMOCYSTEINE LEVELS ASSOCIATE WITH CLINICAL SIGNS OF PERIPHERAL NEUROPATHY IN L-DOPA TREATED PARKINSON'S DISEASE

Study I included 33 PD patients with ongoing L-dopa treatment and 16 controls, all fulfilling exclusion and inclusion study criteria. The two groups were comparable with respect to age but a gender difference was evident, as demonstrated by an overrepresentation of males in the patient population. 16/33 patients fulfilled the study diagnosis of peripheral neuropathy (UENS score > 4) compared to none in the control group. An overview of the clinical and demographic characteristics of the control and PD group, subdivided with regard to the presence of a study diagnosis of peripheral neuropathy, is shown in Table 3.

	PD+peripheral neuropathy (n=16)	PD-peripheral neuropathy (n=17)	Controls (n=16)	*P*
Age (y)	65.3 (3.66)	64.7 (2.69)	64.6 (5.94)	0.41[a,1], 0.65[b,1], 0.38[c,1]
Sex male (%)	12 (75)	14 (82)	5 (31.3)	0.61[a,2], **<0.05**[b-c,2]
Smoker (%)	2 (12.5)	2 (11.8)	1 (6.25)	0.95[a,2], 0.54[b,2], 0.58[c,2]
Disease duration (y)	10.8 (5.13)	7.94 (5.10)	-	0.16[a,1]
B-vitamin subst. (%)	4 (25)	7 (41.2)	3 (18.8)	0.32[a,2], 0.67[b,2], 0.16[c,2]
Daily L-dopa dose (mg)	814 (491)	773 (558)	-	0.32[a,1]
Hoehn and Yahr (stage)	2.56 (0.892)	1.88 (0.485)	-	**0.0094**[a,1]
COMT-inhibitor (%)	8 (50)	4 (23.5)	-	0.11[a,2]
LCIG (%)	2 (12.5)	3 (17.6)	-	0.68[a,2]

Table 3. Main demographic and clinical characteristics of the participants. Numerical variables expressed as mean (standard deviation). [a]comparing PD with and without a study diagnosis of peripheral neuropathy. [b]comparing PD with a study diagnosis of peripheral neuropathy to controls. [c]comparing PD without a study diagnosis of peripheral neuropathy to controls. [1]Mann-Whitney U-test. [2]chi-square test

The UENS score was higher in PD patients (n=33) compared to controls (n=16), (mean UENS score 5.0 vs 2.38, $p = 0.0027$). In the patient group, a significant correlation was demonstrated between UENS scores and Hcy-levels ($rho = 0.40$, $p = 0.022$), as shown in Figure 5. PD patients with a study diagnosis of peripheral neuropathy demonstrated higher Hcy levels relative to controls ($p = 0.0020$), but not to PD patients without a study diagnosis of peripheral neuropathy ($p = 0.080$).

No significant associations were demonstrated between UENS scores and disease duration or LEDD ($p = 0.20$ and $p = 0.34$ respectively).

In a subgroup analysis, PD patients with (n=11) and without (n=22) any ongoing vitamin B supplementation were compared. A significant increase in UENS scores ($p = 0.035$) and Hcy

levels (p = 0.0025) were demonstrated in the non-supplemented group relative to the supplemented group.

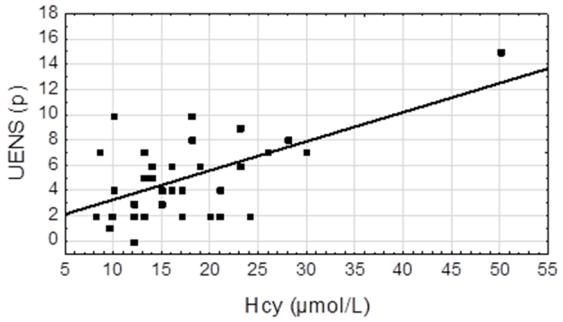

Figure 5. Significant correlation between UENS scores and plasma levels of homocysteine among PD patients (n=33). Spearman's rank order correlation; *rho* = 0.40, p = 0.022. Reprinted from paper 1[170] with permission from IOS press.

4.2 LOW ACTIVITY COMT GENOTYPE MORE FREQUENT IN PARKINSON'S DISEASE PATIENTS WITH CLINICAL SIGNS OF PERIPHERAL NEUROPATHY

Genotyping of SNPs in the *COMT* (rs4680) and *MTHFR* (rs1801131; rs1801131) genes was performed in all subjects in Study I. No significant difference in the distribution of the two tested *MTHFR* SNPs was demonstrated between PD patients with and without clinical signs of peripheral neuropathy (p = ns). A significant difference in the distribution of the *COMT* SNP was demonstrated as illustrated in Table 4, favouring the A/A low activity variant in PD patients with clinical signs of peripheral neuropathy.

	PD+peripheral neuropathy (n=16)	PD-peripheral neuropathy (n=17)
COMT A158G: n (%)		
A/A	8 (50.0)	3 (17.6)
A/G	4 (25.0)	12 (70.6)
G/G	4 (25.0)	2 (11.8)

Table 4. *COMT* genotypes in patients with and without a study diagnosis of peripheral neuropathy, demonstrating a significant difference in the distribution. p = 0.042 (Fisher's exact test), p = 0.032 (chi-square test).

No significant difference with regard to treatment with COMT-inhibitors was demonstrated between PD patients with and without a study diagnosis of peripheral neuropathy (p = 0.11).

4.3 NO EVIDENCE FOR SMALL FIBER NEUROPATHY UNDERLYING RLS IN PARKINSON'S DISEASE

Results from Study I formed the basis for Study II, in which we sought to study a potential clinical expression of peripheral neuropathy in PD, using a more robust methodology.

The study included PD patients with (n=21) and without (n=21) a clinical diagnosis of co-existent RLS. An age- and sex-matched control group (n=13) was also included. An overview of the study population is outlined in Table 5.

	PD+RLS (n=21)	PD-RLS (n=21)	CL (n=13)	*P*
Age (y)	69.4 (5.9)	69.1 (6.0)	69.7 (6.6)	0.93[3]
Male/female	15/6	15/6	9/4	0.99[1]
Smoking (n, %yes)	3 (14.3)	2 (9.5)	2 (15.4)	0.89[2]
RLS heredity (n, %yes)	8 (38.1)	4 (19.0)	1 (7.7)	0.14[2]
B12 or multivitamins (n, %yes)	13 (61.9)	12 (57.1)	3 (23.1)	0.068[1]
B6 or multivitamins (n, %yes)	3 (14.3)	5 (23,8)	3 (23.1)	0.76[2]
p-NfL (pg/ml)	6.3 (3.8)	6.2 (4.2)	6.1 (4.1)	0.95[3]
PD-specific variables				
Motor duration (y)	7.9 (4.1)	7.5 (4.4)	-	0.66[4]
L-dopa duration (y)	5.1 (3.8)	4.6 (4.6)	-	0.42[4]
mH&Y (stage)	2.3 (0.4)	2.1 (0.5)	-	**0.039**[4]
LEDD (mg)	725 (360)	684 (308)	-	0.96[4]
No L-dopa treatment (n, %yes)	1 (4.8)	1 (4.8)	-	1.0[2]
RLS-specific variables				
RLS duration (y)	10.6 (11.3)	-	-	-
IRLS (p)	18.5 (7.9)	-	-	-
Positive SIT test (n, %yes)	17 (81.0)	-	-	-

Table 5. Main demographic and clinical characteristics of the participants in study II. Numerical variables expressed as mean (standard deviation). Abbreviations: PD+RLS – Parkinson's disease with restless legs syndrome; PD-RLS – Parkinson's disease without restless legs syndrome; CL – controls; B12/B6 or multivitamins – participants reporting intake of either multivitamins or vitamin B12/B6; MMA – methylmalonic acid; NfL – neurofilament light chain; mH&Y – modified Hoehn and Yahr; LEDD – levodopa equivalent daily dose; IRLS - International Restless Legs Syndrome Study Group Rating Scale; SIT – suggested immobilization test. [1]Chi-square test; [2]Fisher's exact test; [3]Kruskal-Wallis H-test; [4]Mann-Whitney U-test.

The groups were well matched with regard to age and sex. A majority (81%) of patients in the PD+RLS group demonstrated a positive sensory suggested immobilization test. RLS symptoms were severe as assessed by the IRLS. B-vitamin supplementation was common, reaching over 60 % in the whole PD group. No significant differences were seen when comparing levels of methionine cycle metabolites between groups (p = ns), with PD patients exhibiting median levels within reference range.

The main results of peripheral nerve assessments are shown in Table 6. Overall, no significant group differences were demonstrated with regard to small and large peripheral nerve fibers as assessed by IVCCM, QST and NCS. Clinical signs of peripheral neuropathy (UENS) neither differed between the two PD groups. However, the UENS score was higher in both PD groups compared to controls, echoing the findings from Study I.

	PD+RLS (n=21)	PD-RLS (n=21)	CL (n=13)	P^1
Clinical rating scale				
UENS (p)	5.8 (4.1)	6.1 (2.4)	2.7 (2.8)	**0.001**
Neurophysiology				
ENeG-Ix	-0.70 (0.95)	-0.64 (0.82)	-0.66 (0.64)	0.82
WT hand (° C)	2.6 (1.7)	2.4 (1.0)	2.4 (1.7)	0.84
CT hand (° C)	2.5 (1.9)	1.9 (1.1)	1.7 (0.47)	0.74
WT foot (° C)	10.9 (4.1)	10.8 (4.5)	9.7 (4.2)	0.71
CT foot (° C)	8.5 (10.0)	7.7 (6.9)	5.0 (2.8)	0.54
IVCCM				
CNFL (mm/mm^2)	17.5 (3.8)	16.9 (3.1)	17.6 (3.9)	0.81
CNBD (no/mm^2)	105 (34.6)	106 (38.7)	111 (36.6)	0.92

Table 6. Neurophysiological, corneal and clinical assessments of peripheral nerves. Numerical variables expressed as mean (standard deviation).
Abbreviations: PD+RLS – Parkinson's disease with restless legs syndrome; PD-RLS – Parkinson's disease without restless legs syndrome; CL – controls; UENS – Utah Early Neuropathy Scale; ENeG-Ix – electroneurography index; WT – warmth threshold; CT – cold threshold; IVCCM – *in vivo* corneal confocal microscopy; CNFL – corneal nerve fiber length; CNBD – corneal nerve branch density;
[1]all analyses performed with Kruskal-Wallis H-test except CNFL, in which one-way ANOVA was used.
This table is a derivative of Andréasson et al.[26]

4.4 SMALL AND LARGE FIBER PATHOLOGY MAY ASSOCIATE WITH MEASURES OF DISEASE BURDEN IN PARKINSON'S DISEASE

In Study II, a subgroup analysis was performed in the whole patient population (n=42), examining potential direct or indirect markers of disease severity, including mH&Y, duration of L-dopa treatment, disease duration and p-NfL. Significant age- and sex-adjusted associations were demonstrated between CNBD and the duration of L-dopa treatment (*rho* = -0.36, $p = 0.022$); ENeG-Ix and p-NfL (*rho* = -0.51, $p = 0.001$); UENS and p-NfL (*rho* = 0.35, $p = 0.026$); and between warmth/cold thresholds of the hand and mH&Y (*rho* = 0.35, $p = 0.028$ and *rho* = 0.37, $p = 0.019$ respectively).(Figure 6) An age-adjusted association was demonstrated between CNFL and the duration of L-dopa treatment (*rho* = -0.34, $p = 0.031$).

Adjustments for multiple comparisons were not performed in the correlation analyses given their exploratory nature.

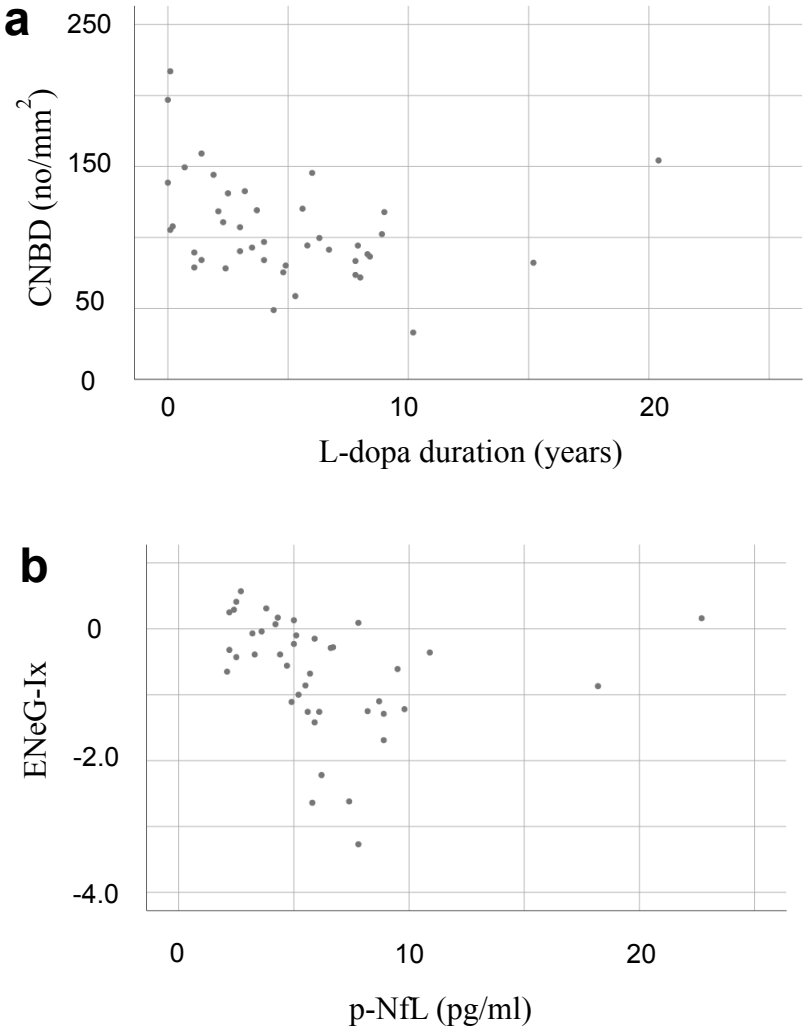

Figure 6. The duration of L-dopa therapy associates with CNBD (a), and the ENeG-Ix associates with p-NfL (b). Partial Spearman's rank order correlation adjusted for age and sex; *rho* = -0.36, *p* = 0.022 and *rho* = -0.51, *p* = 0.001 respectively. Plots show unadjusted data points.
Figure by Andréasson et al.[26] Licensed under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

4.5 ALTERED COBALAMIN METABOLISM IN THE SETTING OF PARKINSON'S DISEASE WITH PROMINENT CNS MANIFESTATIONS

In Study IV, the biochemical, genetic, and clinical phenotype of a 78-year-old patient with PD was described. Besides PD, the patient suffered from cardiovascular disease including cardiomyopathy, stroke, and renal failure. Clinically, a clear L-dopa motor response was present at diagnosis. Over time, a marked progressive cognitive impairment developed as reflected by a final assessed MoCA score of 5/30. Normal markers of tau, phosphorylated tau, and amyloid-β42 were demonstrated in CSF.

A longstanding elevation of s-MMA, non-responsive to high-dose cobalamin substitution, had repeatedly been noted in the patient's medical record, finally prompting an expanded investigation. Biochemical metabolic assessment revealed an intermittent methylmalonic aciduria (MMA-uria) and increased levels of propionyl-carnitine in plasma.(Table 7)

Biochemical analyses	2005	2011	2013	2015	2016
	Apr, Dec	Mar	Mar, Oct	Oct, Dec	Jan, Jun
MMA (µmol/l) [<0,40]	**8.1**[a], **11**[a]	**180**[a]	-, **35**[a]	**9.9**[b], **16**[b,e]	-, **18**[b]
p-Hcy (µmol/l) [5,0-15][c], [5,0-20][d]			**17**[c], **18**[c]	**26**[d], **24**[d,e]	-, **32**[c]
s-cobalamin (pmol/l) [150-650]	510, 460	420	-, 410		
s-folate (nmol/l) [7-40]	>40, -	>40			
p-propionyl-carnitine (µmol/l) [<1,3]					-, **13**
u-MMA (mmol/mol creatinine) [<10]				9.0, 5.5	**10, 60**
p-creatinine (µmol/l) [<100]	-, **124**	**117**	**119**, **133**	-, **189**	**159**, -

Table 7. Biochemical evolution of metabolic markers in blood and urine, demonstrating intermittent MMA-uria and signs of alterations in the AdoCbl dependent mitochondrial pathway.
Abbreviations: MMA–methylmalonic acid; Hcy – homocysteine.
[a]serum; [b]plasma; [c, d]different homocysteine reference intervals; [e]measured after treatment with high dose hydroxycobalamin. This table is a derivative of Andréasson et al.[36] Licensed under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

The biochemical profile raised suspicion of a selective disturbance of mitochondrial cobalamin metabolism why a WGS was performed. Subsequent filtering of generated data towards genes associated with IEM was carried out. Two variants were detected, and later confirmed with Sanger sequencing, in the *MCEE* gene; c.139C>T (p.Arg47X) and c.419delA (p.Lys140fs). The first variant has previously been described and reported to be associated with methylmalonyl-CoA epimerase (MCE) deficiency, when present in homozygous state.[191] The latter variant is novel and is predicted to result in a premature stop codon in the resulting transcript.(Figure 7) After targeted Sanger sequencing in the patient's two neurologically unaffected sons, compound heterozygosity in the proband was concluded.

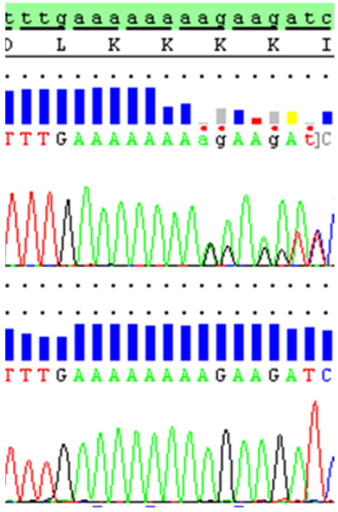

Figure 7. Chromatogram showing normal reference sequence in bottom. A deletion of A (adenine) occurs at position 419 as shown in patient's sequence in top. This figure is by Andréasson et al.[26] Licensed under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

4.6 SUBCLINICAL SMALL FIBER NEUROPATHY MAY BE A POSSIBLE FEATURE OF GAUCHER DISEASE TYPE 1

In Study III a total of 19 patients with GD1 (n=8) and GD3 (n=11) were included. A significantly higher median age was evident in the GD1 group (54 y vs 39 y, $p = 0.021$). No patient demonstrated biochemical signs indicative of diabetes mellitus or the presence of a monoclonal gammopathy.

Five patients with GD1 reported neuropathic symptoms and/or exhibited an UENS score ≥4. Following electrodiagnostic and QST, a large and small fiber neuropathy was deemed present in three and two patients respectively. After acknowledging other possible aetiologies (alcohol toxicity, exposure to chemotherapy, folate deficiency, and treatment with miglustat), a total of two patients with GD1 were considered to exhibit a subclinical small fiber neuropathy in which a disease manifestation of GD was considered a possibility.(Table 8)

Age/sex (y/M,F)	**Age at Dx** (y)	***GBA1* genotype**	**Therapy**	**Alcohol**[b]	**Symptoms**	**UENS**	**NCS**	**QST**
1[a]. 52/F	48	c.604C>T/ c.1226A>G	ERT	No	Yes	6	-	-
2. 61/M	55	c.1226A>G/ c.1226A>G	ERT	No	No	4	Normal	SFN (C-fibers)
3. 52/M	3	c.437C>T/ c.1226A>G	ERT	No	No	0	-	-
4. 29/F	3	c.798C>G/ c.1040T>G	ERT	No	No	0	-	-
5. 73/M	60	c.721G>A/ c.1226A>G	ERT	Yes	Yes	11	Mild demyelinating motor PNP	No SFN
6. 64/F	54	c.1226A>G/ c.1226A>G	SRT	No	Yes	4	Mild demyelinating motor PNP	No SFN
7. 48/M	34	c.1226A>G/ c.1448T>C	ERT	No	No	3	Normal[c]	No SFN[c]
8. 55/M	14	c.1226A>G/ c.1226A>G	None	No	No	5	Normal	SFN (C-fibers)

Table 8. Clinical and demographic characteristics of patients with GD type 1, showing clinical and neurophysiological support for a subclinical SFN in Pt 2 + Pt 8 and a symptomatic large fiber PNP in Pt 1, Pt 5 and Pt 6. Abbreviations: y – years; Dx – diagnosis; Symptoms – reported symptoms of polyneuropathy; UENS – Utah Early Neuropathy Scale; NCS – nerve conduction studies; QST – quantitative sensory testing; ERT – enzyme replacement therapy; SRT – substrate reduction therapy; PNP – polyneuropathy; SFN – small fiber neuropathy. Footnotes: [a]Established chemotherapy induced PNP, [b]≥168 (M) or ≥108 (F) g alcohol/week[192], [c]Electrodiagnostic testing performed 9.5 months after baseline.

In the group with GD3, all but one were classified as the Norrbottnian subtype, exhibiting L444P homozygosity. Although three patients reported symptoms suggestive of peripheral neuropathy, after electrodiagnostic testing and QST, a mild large fiber neuropathy could only

be confirmed in one. However, in this patient a likely explanatory co-existent condition was present. Two patients without symptoms were not available for neurophysiogical evaluation.

In all, no certain case of large or small fiber neuropathy that could be ascribed as a disease manifestation of GD3 was demonstrated.

4.7 FUNCTIONAL COBALAMIN DEFICIENCY APPEARS NOT ASSOCIATED WITH PERIPHERAL NEUROPATHY IN GAUCHER DISEASE

Six patients in the whole GD group exhibited mild elevations of Hcy. In two of these patients, an associated mild folate deficiency and increased serum levels of MMA (1.12 μmol/L, [<0.4 μmol/L]) were demonstrated respectively. The patient with biochemical signs of functional cobalamin deficiency, as expressed by elevated MMA, had a diagnosis of GD3 and exhibited no clinical signs or symptoms compatible with a peripheral neuropathy. The patient with folate deficiency demonstrated a symptomatic large fiber neuropathy, and also reported alcohol overconsumption.

Of note, six patients were receiving cobalamin supplementation why a previous diagnosis of functional cobalamin deficiency is possible. Unfortunately, the original indication for this supplementation was not available.

4.8 INTRA- AND INTERFAMILIAL PHENOTYPIC VARIABILITY IN HSP-*KIF5A*

In Study V, four patients with a confirmed clinical and genetic diagnosis of HSP-*KIF5A* were characterized.

The patients came from two families and carried heterozygous variants in the neuronal kinesin heavy chain gene, *KIF5A,* which have previously been described; c.767A>G (p.Asn256Ser) and c.967C>T (p.Arg323Trp).[193, 194] Pedigrees of the two families were compatible with an autosomal dominant inheritance.

Structured clinical examination and electrodiagnostic testing revealed a complicated HSP phenotype with varying intra- and interfamilial symptom severity and presentation. Symptom onset spanned from childhood to adulthood. A mild to moderate polyneuropathy was present in all patients, with varying degree of small fiber involvement and demyelinating large fiber features in one patient. A summary of the clinical phenotypes is shown in Table 9.

4.9 ALTERED CEREBROSPINAL FLUID LEVELS OF MONOAMINE METABOLITES IN HSP-*KIF5A*

Three patients underwent CSF analysis including the determination of levels of monoamine metabolites. HVA levels, a dopamine metabolite, were increased in all patients. Two patients demonstrated reduced levels of MHPG, a norepinephrine metabolite. No alterations in the levels of 5-HIAA, a serotonin metabolite, were present.

None of the assessed patients had a history of psychiatric disease or ongoing psychiatric medication.

4.10 CEREBROSPINAL FLUID NEUROFILAMENT LIGHT CHAIN IN HSP-*KIF5A*

CSF levels of NfL were increased in one of the three assessed patients in Study V. This patient (B II:1) exhibited the longest disease duration, highest SPRS score and a moderate polyneuropathy at the time of CSF sampling.

The main results from the CSF analyses are shown below in Table 10.

Pt.	NfL (pg/ml) [<560 (30-39 y)] [<1850 (>60 y)]	HVA (nmol/l) [40-170]	5-HIAA (nmol/l) [50-170]	MHPG (nmol/l) [65-140]
A I:1	-	-	-	-
A II:1	-	-	-	-
A III:1	517	208*	141	38*
B II:1	2285*	237*	107	87
B III:1	432	272*	101	60*

Table 10. Results of CSF analyses in three patients with HSP-*KIF5A*. Elevation of NfL in the patient with highest disease burden (B II:1) and altered levels of downstream metabolites of dopamine and norepinephrine (HVA and MHPG) were observed.

Pt.	Age of onset (y)	Presenting symptoms	Age at study inclusion (y)	Genotype	MoCA	SPRS	Pyramidal signs	NCS and QST
A I:1	50-60*	Impaired gait	Died at age 90	-	-	-	-	-
A II:1	33	Impaired gait	67	c.767A>G	28	11	Hyperreflexia, spastic gait, Babinski sign	Mild mixed sensorimotor PNP + small fibers (C and Aδ)
A III:1	34	Impaired gait, leg cramps	45	c.767A>G	28	19	Hyperreflexia, ankle clonus, spastic gait, Babinski sign	Mild axonal sensory PNP
B II:1	26	Impaired gait, imbalance	66	c.967C>T	26	26	Pronounced scissor gait, equivocal Babinski sign	Moderate axonal sensorimotor PNP
B III:1	Childhood	Impaired gait, paresthesia	32	c.967C>T	30	7	Spastic gait, ankle clonus, equivocal Babinski sign	Moderate axonal sensorimotor PNP + small fibers (Aδ)

Table 9. Electrodiagnostic, genetic and clinical features of two families with HSP-*KIF5A*.
Abbreviations: MoCA – Montreal Cognitive Assessment, SPRS – Spastic Paraplegia Rating Scale, NCS – nerve conduction studies, QST – quantitative sensory testing, PNP – polyneuropathy.
*Clinical data based on historical account.

5 DISCUSSION

Caring for patients suffering from a neurodegenerative disease involves the interaction with both the patient and persons close to her/him. In PD, given the vast heterogeneity with regard to rate of disease progression and symptom burden, regular discussions surrounding prognosis and expectations are important and necessary. Careful repeated assessments during clinical visits, which include neurological examination and identification of troublesome symptoms, are regularly performed in order to aid in the diagnostic and prognostic process, as well as in the formulation of a treatment plan.

Carrying out this thesis, I have met many patients, with their families, who have shared thoughts and worries surrounding their neurological disease. These meetings have taught me, among many things, that what the neurologist sees as a troublesome symptom does not always correspond to what is perceived as worrying by the patient or her/his family. As an example, a large-amplitude rest tremor may be very salient for the observing neurologist, but instead a depression or severe obstipation may constitute the patient's main limitation in daily life.

In this thesis, with the aim of seeing the whole patient, I have explored parts of the nervous system that are not always assessed in the routine clinical care of patients with movement disorders. In this chapter I will discuss some of my main findings, relate to previous studies and present possible implications for future research.

5.1 PERIPHERAL NEUROPATHY IN PARKINSON'S DISEASE – INTRINSIC DISEASE FEATURE OR L-DOPA MEDIATED SIDE-EFFECT?

Study I and II demonstrated an increased UENS score in PD patients relative to controls. The UENS encompasses features that may be indicative of both large and small fiber affection, such as test of proprioception and pin-prick sensation respectively. However, this rating scale is purely based on clinical signs, and does not assess possible correlating symptoms such as pain, paresthesia or imbalance.[15] Thus, it should be acknowledged that our finding of increased UENS scores among PD patients does not inform us if these patients exhibited a clinical or subclinical neuropathy. These results are still in line with previous studies that have employed clinical rating scales as part of their PNS assessment.[72, 75] More specifically, Rajabally et al also employed the UENS and reported a mean UENS score of 4.43 in the PD group compared to 2.7 in the control group.[75] These figures are in agreement with the mean values obtained for study I (mean UENS score 5.0 vs 2.38).

In Study II, a higher UENS score was present in the two assessed PD groups relative to controls (Table 6), but we could not confirm these differences when employing NCS, QST and IVCCM. There may be several explanations for this discrepancy. Firstly, Study II was not, unlike Study I, designed to explore the prevalence of peripheral neuropathy in PD in comparison to controls, but rather to investigate the presence of small fiber pathology in PD patients with co-existent RLS. Thus, the control group was smaller (n=13) and may have contributed to the absence of such findings. Secondly, UENS is a bilateral examination of the

lower limbs, while the data for NCS and QST reported in Study II represented unilateral measurements. Given the repeated reports of asymmetric presentations of peripheral cutaneous neuropathy in PD[19, 70, 77, 97], this factor may have influenced our findings since we did not target our testing towards the side predominantly affected by parkinsonism.

Thirdly, a direct implication of Study I was an increased attention, with regard to biochemical monitoring of vitamin B levels, in the routine clinical care of PD patients at Karolinska University hospital and Center for Neurology. This may, in part, be reflected by the fact that 33.3% of PD patients were taking B vitamin supplements in Study I, compared to 59.5% of the PD population in Study II. Thus, if B vitamin supplementation would confer a protective role, this could also have influenced our results.

Lastly, as stated in the introduction, NCS may fail to detect selective affection of distal large fiber endings supplying the cutaneous Meissner's corpuscles. Indeed, reports exist of normal sensory NCS with detectable cutaneous distal large fiber pathology in the setting of PD.[19]

The IVCCM results will be discussed further in the coming segments.

5.1.1 Support for mechanisms related to the methionine cycle

Study I demonstrated associations between Hcy levels and UENS scores among PD patients, and the PD patients fulfilling a study diagnosis of peripheral neuropathy also demonstrated higher Hcy levels relative to controls. These findings are in agreement with a previous report observing higher Hcy levels in PD with peripheral neuropathy compared to PD without neuropathy.[72] Similar findings have also been reported by others.[71, 74, 76]

In Study I, L-dopa treatment was part of the inclusion criteria. Unfortunately, we did not have a comprehensive measure of total L-dopa exposure over time, instead the daily L-dopa dose was used as a surrogate and did not associate with UENS scores. However, previous studies have suggested a relationship between accumulated L-dopa exposure and peripheral neuropathy.[72, 74, 76, 80]

Considering a possible association between alterations in the methionine cycle and the development of peripheral neuropathy, interest has focused towards a putative protective role of COMT-inhibitors, a treatment often used in PD. Given that L-dopa is a substrate for methylation via SAM in conjunction with COMT, we can expect that the administration of L-dopa may result in an increase in Hcy.[79](Figure 3) Consequently, it has been suggested that co-administration of a COMT inhibitor might suppress this mechanism. However, conflicting reports exist on the role of COMT inhibitors in the context of peripheral neuropathy in PD. Some studies have suggested a protective role[74, 195] while others have not been able to demonstrate such association.[72] Our results from Study I could not support a protective role, observing no difference with regard to use of COMT inhibitors between patients with/without a study diagnosis of peripheral neuropathy.

However, in Study 1 we observed a different distribution of *COMT* genotypes between patients with/without a study diagnosis of peripheral neuropathy, possibly favouring the low

activity genotype (A/A) in neuropathic patients.(Table 4) The role of this finding is unclear. It is well-established that COMT is expressed in the brain and has an important role in the catabolism of catecholamines such as dopamine, norepinephrine, and epinephrine. Moreover, COMT is also expressed in several peripheral tissues.[196] It can only be speculated whether the low activity genotype, through altered catechol metabolism, may confer an increased risk for pathology of the peripheral nerve. However, possible underlying mechanisms are hard to delineate why this remains speculative and studies using larger samples are needed to replicate and further investigate this finding from Study I.

In conclusion, previous reports, together with the findings from study I, support an association between clinical signs of peripheral neuropathy and biochemical alterations of the methionine cycle. Of note, conclusions from Study I, and the referenced studies in this segment, have mainly been based on L-dopa treated populations.

5.1.2 Support for an intrinsic disease feature

Studying populations naïve to treatment with L-dopa brings the possibility to discern the putative contributory role of L-dopa exposure to the development of peripheral neuropathy in PD. Overall, findings of large fiber neuropathy in L-dopa naïve patients are rare, although this has been reported in distal large fiber endings.[19] On the contrary, numerous reports have observed an evident cutaneous small fiber neuropathy in patients with no L-dopa exposure[19, 69] and in patients with absent associations with methionine cycle metabolites.[78]

In summary, ample reports have demonstrated cutaneous small fiber neuropathy in L-dopa naïve patients, and repeated reports have demonstrated an asymmetric cutaneous denervation pattern that mirrors co-existent parkinsonism[19, 70, 77, 97]. Furthermore, the presence of α-syn pathology in cutaneous small fibers[78, 88, 89] is now a well-established feature of PD. Considering that the pathological hallmark of PD, the degeneration of nigrostriatal neurons, involves pathology of small unmyelinated nerve fibers[197], it is tempting to suggest that peripheral small fiber neuropathy also may be intrinsic to PD. Moreover, this concept would not preclude the possibility that parallel pathology of large myelinated fibers may be attributed to altered methionine cycle metabolism.

5.2 IVCCM APPEARS NOT SUITABLE AS A DIAGNOSTIC TOOL IN PARKINSON'S DISEASE, POSSIBLE MARKER OF DISEASE PROGRESSION?

5.2.1 Corneal parameters do not discriminate Parkinson's disease from controls

In Study II, two corneal parameters (CNBD and CNFL), known to be reduced in early diabetic peripheral neuropathy[25], were assessed. Neither CNBD nor CNFL could discriminate patients from controls in our study. Importantly, alluding to the previous discussion, no significant intraindividual differences were demonstrated between eyes (p = ns), thus arguing against the presence of an asymmetric corneal neuropathy.

Previous studies employing IVCCM in PD have reported conflicting results. An increase in CNFL and CNBD relative to controls has been reported in patients with moderate PD.[198] On the contrary, a decrease in the same parameters has been demonstrated in other studies[199, 200], including one performed in recently diagnosed patients[87] with minimal L-dopa exposure. Furthermore, a large study included patients with varying degree of cognitive impairment, demonstrating an increase in CNBD but no change in CNFL relative to controls.[201]

The conflicting results generated from these studies may have different explanations. Individual images of the subbasal nerve plexus, obtained by IVCCM, are very small and account for less than 1 % of the central cornea.[181] Previous studies have therefore often manually selected several images to use for further analysis.

In our study, we recorded multiple images encompassing wide-field areas of the subbasal nerve plexus. Using machine-based algorithms, large two-dimensional mosaics, composed of the recorded images that had been merged together, were generated. Importantly, both the generation of the mosaic and the subsequent nerve tracing with quantification of CNFL and CNBD were fully automated, using machine-based algorithms. The advantages of this methodology are twofold. Firstly, by employing automatic methodology we avoided bias known to be associated with the manual selection of images.[181] Secondly, the total size of the depicted area was very large, the mean area of a mosaic image was 7.7 mm^2, which is significantly larger than what is obtained from a single microscopic image (0.16 mm^2). We believe these factors significantly increased the representability and validity of our images and subsequent findings.

Previous referenced studies have selected three[199, 200], four to six[198, 201] or four to eight[87] images per eye for subsequent analysis with semi- or fully automated quantification methods. This methodology corresponds to the visualization of a relatively small region of the subbasal nerve plexus. In our study, the mean mosaic size was 48 times the individual image size, representing an order of magnitude larger area of imaged and quantified nerves, relative to prior studies.

Given the robust methodology used in Study II, including large depiction areas with fully-automated methodology, we believe our results are reliable. Thus, IVCCM with quantification of CNFL and CNBD does not seem to discriminate patients with moderate PD from controls. The absence of asymmetry may further support this.

5.2.2 The role of IVCCM as a putative tool to monitor disease progression in Parkinson's disease needs further evaluation

Studying all PD patients from Study II (n=42), significant associations between corneal parameters (CNBD and CNFL) and the duration of L-dopa treatment were present. One could argue this might be indicative of an L-dopa mediated toxicity affecting small nerve fibers. However, we speculate whether the duration of L-dopa therapy also can be viewed as an indirect marker of disease burden. Generally, L-dopa therapy is initiated when a patient has accumulated significant motor symptoms that cannot be fully controlled with other agents

such as dopamine agonists or enzyme inhibitors, indicating a significant progression of disease.

In our study, we also found associations between NfL, a marker of axonal damage, and two other PNS assessments; UENS and ENeG-Ix. Furthermore, an association between mH&Y stage and temperature thresholds of the hand was also present. Combined, these findings may suggest that small and/or large fiber monitoring may be of interest in PD. It should be acknowledged however, that adjustments for multiple comparisons were not performed in this exploratory subgroup analysis.

Previous studies have also suggested associations between corneal parameters and different clinical features, including autonomic symptoms and motor signs[198], as well as cognition and H&Y stage.[201] Of note, a few longitudinal studies assessing cutaneous small fiber neuropathy in PD have suggested IENFD as a potential objective marker of disease progression.[94, 202]

In conclusion, our findings do not support IVCCM as a primary diagnostic tool in PD, but in light of previous studies, our findings suggest IVCCM as a tool to monitor disease progression may merit further longitudinal studies.

5.3 RLS APPEARS NOT TO BE A PHENOTYPIC EXPRESSION OF SMALL FIBER NEUROPATHY IN PARKINSONS' DISEASE

Given previous reports of small fiber neuropathy associated with PD, the main aim of Study II was to investigate a possible clinical expression of this manifestation, namely RLS.

Our study could not demonstrate an underlying small or large fiber neuropathy to be associated with RLS in our PD population. Importantly, given the mimics of RLS often present in PD, we consolidated the RLS diagnosis with the sensory suggested immobilization test.[121, 122] We believe this further validated our main finding, that peripheral neuropathy is not causative of RLS in this group of patients. This finding is in line with one previous study that solely assessed the PNS using a clinical rating scale.[203]

Some implications of our finding may be relevant. PD is considered a progressive neurodegenerative disorder. Assuming RLS in PD is not an intrinsic disease feature, we may assume that the evolution of RLS in this setting will not follow a progressive degenerative path. In other words, treatment, treatment response, and prognosis of RLS in PD may be favourable and akin to what is seen in idiopathic RLS. A clinical consequence could thus be the need for vigilance with regard to treatment of RLS in PD, employing similar strategies used in idiopathic RLS, which include the use of α2δ-ligands.

However, it should be acknowledged that neurodegenerative CNS mechanisms underlying RLS in the setting of PD may be implicated and were not assessed in our study.

5.4 ALTERED COBALAMIN METABOLISM IN PARKINSON'S DISEASE – A SHORT VISIT TO THE CNS

So far, I have discussed aspects of altered cobalamin metabolism in relation to the PNS in PD. Study IV allows me to expand this discussion a bit further in this segment, in which the CNS will be a focus.

Implications of cobalamin deficiency and Hcy elevation with regard to CNS manifestations in PD have been explored in several studies. A longitudinal study in early untreated PD patients suggested cobalamin and Hcy levels as predictors of the progression of gait difficulties and cognition respectively.[204] Similar findings have been reported in another longitudinal study, suggesting Hcy levels may predict the development of cognitive worsening and progression of motor symptoms.[205] As with the PNS, a clear understanding of pathophysiological mechanisms has not been fully appreciated. However, toxicity related to Hcy, including increased oxidative stress and endothelial dysfunction, has been suggested, and possibly supported by the demonstration of hyperhomocysteinemia as a risk factor for ischaemic lesions of the striatum in PD.[79, 206]

Moreover, preclinical evidence exists, suggesting AdoCbl interacts with the enzyme Leucine-Rich Repeat Kinase 2 (LRRK2).[207] Of note, different heterozygous variants in the *LRRK2* gene are established both as risk factors for PD and causative of monogenic PD.[208, 209] LRRK2 is a kinase that is expressed in the brain, and pathogenic variants in the underlying gene have been associated with an increased kinase activity and secondary neurotoxicity. A preclinical study demonstrated that AdoCbl bind LRRK2 and inhibits its activity and associated toxicity.[207]

Thus, the abovementioned studies stress the existence of different cobalamin dependent mechanisms that possibly are of importance in PD.

In Study IV, an adult patient with PD and the progressive development of significant cognitive impairment was described. The biochemical and genetic characterization was indicative of an MMA-uria with underlying compound heterozygous variants in the *MCEE* gene.(Table 6) The *MCEE* gene encodes the protein MCE which is implicated in the AdoCbl dependent mitochondrial metabolism of odd-chain fatty acids, cholesterol, and branched amino acids.[34](Figure 4) The localization of the MCE dependent reaction, upstream of the interaction between AdoCbl and MUT, explains why this condition does not respond to cobalamin substitution. MCE deficiency has previously been reported in the paediatric setting with varying clinical presentations, including metabolic acidosis and asymptomatic descriptions.[210, 211] The presence of asymptomatic patients exhibiting genetic and biochemical alterations compatible with MCE deficiency has questioned the clinical relevance of this condition.

Interestingly, MMA-uria of other genetic causes, pertinent to the AdoCbl dependent mitochondrial pathway, has been associated with stroke of the basal ganglia, cognitive impairment, as well as movement disorders including ataxia and tremor.[212] Indeed, the patient

we describe exhibited, besides PD and cognitive decline, a history of repeated stroke including visible ischaemic lesions of the basal ganglia on imaging.

Previous reports of MCE deficiency in the adult patient are not available, thus conclusions with regard to possible clinical implications of the detected metabolic alteration described in Study IV are difficult to make. However, studies in rats have suggested excitotoxic effects induced by MMA, both when assessing rats receiving intrastriatal injections of MMA and in cultured embryonic striatal rat neurons.[213, 214] Furthermore, it can be hypothesized that the upstream increase of propionyl-CoA, secondary to MCE deficiency, may alter the urea cycle with resulting hyperammonemia, which in turn may exert toxic effect on the CNS.[215]

In conclusion, previous studies support an association between altered cobalamin metabolism and progression of CNS manifestations in PD. The clinical relevance of our finding of MCE deficiency in an adult PD patient is of unknown significance as for now. However, our findings call for vigilance when confronted with unexpected findings in the biochemical monitoring of methionine cycle metabolites in PD, especially if non-responsive to cobalamin substitution therapy.

5.5 SMALL FIBER NEUROPATHY – A POSSIBLE LATE-ONSET FEATURE OF GAUCHER DISEASE?

5.5.1 Subclinical small fiber neuropathy in GD1

GD1 has, until recently, been viewed as a disorder not associated with neurological involvement. Now, we know that the presence of pathogenic variants in the underlying *GBA1* gene constitutes a strong risk factor for the development of PD, both in the heterozygous and homozygous state.[51, 166] Given the reports suggesting peripheral neuropathy also may be associated with the disease, Study III was carried out and will be discussed in the following segment.

Of the eight GD1 patients assessed, three demonstrated signs and symptoms compatible with a symptomatic large fiber neuropathy. However, possible aetiologies were identified in all three. Importantly, one patient (pt. 6) was receiving substrate reduction therapy with miglustat.(Table 8) In an early open-label trial with this drug, two of 28 patients developed a symptomatic peripheral neuropathy and were withdrawn from the study.[162] Moreover, in a smaller trial, paraesthesia was a frequent adverse event.[216] Overall, we could not conclude that any of the GD1 patients exhibited a large fiber neuropathy as an inherent disease manifestation. A previous comprehensive study demonstrated a 10.7% prevalence of large fiber neuropathy in a larger sample of patients with GD1.[160] Given the small sample in our study, it is plausible that our sample size does not permit firm conclusions with regard to large fiber neuropathy. However, we also note the report by Devigili et al, in which GD1 patients with pain were assessed with a comprehensive battery of PNS testing. None of the 25 patients who underwent NCS demonstrated evidence of a large fiber neuropathy.[161]

Two GD1 patients in our study demonstrated neurological signs and findings on QST indicative of a subclinical small fiber neuropathy, in which no other explanatory cause was identified. These findings are in line with what was reported in the previously referenced study by Devigili et al, in which NCS also were normal. A majority of the patients in that study demonstrated abnormalities on QST, including high cold thresholds, and a reduction of IENFD was observed in 19 of 21 biopsied patients.[161]

Given the rare number of reports assessing small fiber function in GD1, the findings from Study III may further support the notion of small fiber neuropathy as possible disease manifestation of GD1.

5.5.2 Peripheral neuropathy not a common finding in GD3

There are not many previous studies assessing the PNS in GD3. Motor NCS were carried out in eleven young patients in a study from 1980 without any reported peripheral neuropathy.[151] In the eleven patients included in Study III, no case of large or small fiber neuropathy could be ascribed as a disease manifestation. It should be acknowledged that two patients were not available for electrodiagnostic testing, of which one received treatment with miglustat.

It may appear surprising that these patients, who suffer from significant symptoms arising from the CNS, would be spared from PNS involvement. We note that these patients were much younger (median age 39 years) than the patients with GD1. Furthermore, the two GD1 patients with subclinical small fiber neuropathy were aged 55 and 61 at the time of testing. It can thus be speculated if peripheral neuropathy in GD may represent a late-onset feature, akin to what has been described with PD, in which the pathophysiological mechanisms may differ from what is seen with other disease manifestations. Another possibility could be that the Norrbottnian GD3 carries a unique phenotype not associated with PNS involvement.

5.5.3 Possible clinical implications?

Given what has been demonstrated in previous reports, in combination with the findings from Study III, I believe attention to the possible presence of a peripheral neuropathy, when caring for a patient with GD1, is warranted. In particular, when a patient reports symptoms of pain, a more thorough history with regard to pain quality and characteristics may guide the physician to the search for a possible underlying small fiber neuropathy. In turn, this may carry great meaning for the patient, considering the different treatment strategies employed in neuropathic pain.

5.6 PERIPHERAL NEUROPATHY IN HEREDITARY SPASTIC PARAPLEGIA – POSSIBLE DIAGNOSTIC CLUE?

Recognizing the clinical history and signs of a slowly progressive upper motor neuron involvement of the lower limbs constitutes a first step to consider HSP as a differential diagnosis. Together with family history and the exclusion of metabolic and structural lesions of the spinal cord, a suspicion of an underlying HSP may then be further considered.

Given the vast number of HSP subtypes, the ensuing search for a molecular diagnosis is challenging. A strategic first approach may be the targeted testing for specific HSP subtypes based on reported prevalence. In family A, in Study V, targeted testing for pathogenic variants in the *SPAST*, *ATL1,* and *REEP* genes was first performed, given the reports of their relatively high frequencies in HSP with autosomal dominant pattern.[144] A reassessment of the clinical phenotype, including electrodiagnostic testing, then guided our search towards *KIF5A* in light of its phenotypic overlap with CMT2.[146]

Although next generation sequencing nowadays is starting to replace targeted testing of specific genes, I believe the importance of a comprehensive phenotypic characterization still is of great importance. The reasons for this, I believe, are twofold. Firstly, findings of genetic variants of unknown significance are likely to increase with the use of WGS or whole exome sequencing. Consequently, a phenotypic description compatible with previous reports associated with the specific gene, may aid in the assessment of a variant's pathogenicity. Secondly, although pain in HSP often may be attributed to spasticity, assessing the whole patient may reduce the risk of not acknowledging, as an example, a contributory painful peripheral neuropathy.

In conclusion, the increasing use of next generation sequencing with its applications is promising and should spark us as neurologists to continue carrying out comprehensive phenotypic characterizations for both diagnostic and patient care purposes.

5.6.1 Elevated cerebrospinal fluid neurofilament light chain not a mandatory finding in HSP-*KIF5A*

In the clinical setting, HSP is often viewed as a slowly progressive upper motor neuron disorder, primarily affecting the longest axons responsible for motor function of the legs. Other disorders of the upper motor neuron, such as amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis, may sometimes be a differential diagnosis to consider in the clinical setting. The temporal evolution and the progressive spread of symptoms may aid in the diagnostic process, however biochemical biomarkers have also been suggested.

NfL is a protein that forms part of the cytoskeletal structure of neuronal axons and cell bodies. In the setting of neuroaxonal damage, an elevation of this protein is considered a consequence.[217] Indeed, elevated CSF levels of NfL have been reported to carry both diagnostic and prognostic properties in the setting of ALS.[218] Furthermore, a study comparing serum levels of NfL in HSP, ALS, and controls reported an intermediate elevation of NfL in HSP patients relative to ALS patients and controls.[219]

After the conduction of our Study V, a large study, comprising different subtypes of HSP, was published with the aim to compare CSF levels of NfL between patients and controls. This study demonstrated, on a group level, higher NfL levels in the HSP group relative to controls, controlling both for age and sex, proposing NfL as possible diagnostic biomarker. Moreover, this study could not demonstrate a clear influence of disease severity or duration to NfL levels, arguing against NfL as a marker of disease progression.[220] Similar findings were

reported in a study evaluating serum levels of NfL in HSP, demonstrating higher levels in HSP relative to controls.[219] Of note, only one patient with HSP-*KIF5A* was included in these two studies combined.

Back to the clinical setting, I believe the findings from our study, although with small sample size in comparison, carry some valuable information. We hypothesized, given the existing preclinical support for a disturbed axonal transport of neurofilaments in HSP-*KIF5A*[221, 222], that a CSF elevation of NfL would be present. Considering only one of the three patients demonstrated an elevation, I believe our findings stress the importance of not translating results based on group levels to the individual patient. Thus, NfL elevation is not mandatory in HSP-*KIF5A* and should rather be used as a supportive feature.

5.7 LIMITATIONS

Some limitations pertinent to the studies of this thesis have already been discussed separately in this discussion. In this segment, I will describe additional main limitations that are important to acknowledge when interpreting my conclusions. For a more detailed discussion on limitations related to each study, the reader is also referred to the original papers.

5.7.1 Study I and II

The main limitation of Study I was the definition of a study diagnosis of peripheral neuropathy, which was based solely on clinical signs as assessed by a standardized clinical rating scale. Although the scale has been used in the setting of PD before[75], the scale does not discriminate between a subclinical and a symptomatic peripheral neuropathy. Furthermore, sensory findings attributed to disruptions in central pathways cannot be excluded. The exclusion criteria used for this study were not as strict as in Study II. Thus, the inclusion of both patients and controls with undiagnosed rarer conditions relevant for the development of peripheral neuropathy is a possibility.

The populations in Study I were not sex-matched. Although no significant differences in UENS scores and Hcy-levels, with regard to sex, were demonstrated within patient and control groups, this is a factor that must be acknowledged and might have influenced our results.

I learnt many lessons from Study I and as a consequence, an attempt was made to improve the design of Study II. Thus, stricter inclusion/exclusion criteria were used and a more comprehensive battery of PNS assessments was employed. The limitations of these methods have been discussed further in section 5.1. Moreover, the inclusion of additional small fiber assessments with higher sensitivity, such as skin biopsies with calculation of IENFD, would have added further validity to our findings.

Study II included a relatively small control group (n=13) which may have influenced the comparisons between PD and controls as previously discussed. However, the main purpose

of this study was to investigate and compare the two PD groups, stratified by the co-existence of RLS.

5.7.2 Study III

The sample sizes both with regard to GD1 and GD3 were small. Furthermore, the study did not include a control group. Together, these factors weaken the possibility to draw firm conclusions. However, considering the rarity of the disease, especially with regard to GD3, I believe the presented case series still confers important data with possible implications for the design of future longitudinal studies. Furthermore, QST is a functional measure that cannot separate peripheral from central somatosensory pathways. Thus, considering the skeletal manifestations, such as kyphosis, often associated with GD, an influence on the QST results by means of mechanical myelopathy cannot be excluded.

As in Study I and II, a more comprehensive structural assessment of small fibers, including IENFD, might have strengthened the validity of our results.

5.7.3 Study V

The small sample size constitutes the main limitation of this study and precludes any firm conclusions with regard to the findings of altered monoamine metabolism. Moreover, in one of the patients, CSF had been stored since 2012 in a -80°C freezer. A possible influence on the subsequent CSF analysis, related to long storage time, is a possibility.

6 CONCLUSIONS

Based on the results generated from this thesis, and in the light of previous reports, I believe the following conclusions can be made.

1. Clinical signs of peripheral neuropathy are prevalent in L-dopa treated PD.

2. Associations between clinical signs of peripheral neuropathy and elevated homocysteine in PD exist, but potential causality is not fully established.

3. Active monitoring of cobalamin, folate, homocysteine, and MMA levels is warranted in PD, including treatment when signs of deficiency.

4. When confronted with biochemical alterations of cobalamin metabolism, non-responsive to substitution therapy, an expanded metabolic investigation may harbour both scientific, and clinical, insights and implications.

5. PD with co-existent RLS appears not to constitute an inherent manifestation of PD-associated peripheral neuropathy. This may support a non-neurodegenerative explanation for RLS in this setting, possibly carrying favourable implications with regard to treatment and prognosis.

6. Small fiber neuropathy may be associated with GD1, but larger studies are needed to draw firm conclusions with regard to prevalence and possible contribution to symptom burden.

7. Unexplained pain in a patient with GD1 may encourage further assessment of peripheral small fibers.

8. Clinical intra- and interfamilial heterogeneity is common in HSP and should motivate the neurologist to always perform a careful phenotypic characterization in a patient with suspected HSP.

9. Normal CSF levels of NfL do not exclude a diagnosis of HSP.

7 FUTURE ASPECTS

As for now, no disease modifying treatment exists for PD. A major requisite for a clinical trial, aiming to demonstrate a disease modifying effect, is the access to a robust measurable marker of disease progression. In line with the suggested notion, that small fiber pathology is an inherent disease manifestation, and large fiber pathology may be attributed to altered cobalamin metabolism, I believe future studies evaluating the biomarker potential of objective small fiber assessments are warranted. Such assessments should include investigation of cutaneous denervation, detection of cutaneous p-α-syn deposits and possibly IVCCM. Indeed, a few longitudinal studies have suggested IENFD as a possible marker of disease progression in PD.[94, 202]

If this notion holds true, that PNS pathology reflects CNS pathology in PD, I believe three major obstacles need to be accounted for, before employing PNS pathology as an outcome measure in a clinical trial. Firstly, effects of altered cobalamin metabolism may influence the results, contributing with PNS pathology of non-degenerative source. Such effects might be accounted for by introducing standardized prophylactic vitamin B supplementation as part of the study protocol. Secondly, given the reported asymmetry of PNS pathology in PD, care must be taken to account for this to ensure consistent assessments over time. Thirdly, a disease modifying treatment that selectively targets the CNS would not allow its evaluation by PNS assessments.

This third obstacle allows me to elaborate on my next thought on future directions - to what extent may small and/or large fiber neuropathy contribute to the total symptom burden experienced by the patient? Our study argued against a contributory role in the development of RLS, however additional avenues in this regard could be visited. These may include evaluating the selective role of the PNS in pain, autonomic dysfunction and gait difficulties[100] in PD. The demonstration of a contributory role of the PNS in these aspects may serve as an incentive for future disease modifying treatments to target both the CNS and PNS.

The rarity of Gaucher disease complicates the conduction of large scales studies. I believe international collaboration in the setting of a multicentre study is necessary to enable more firm conclusions with regard to the PNS in this condition. Such studies should include both functional and structural assessment of small and large fibers, and the presence of miglustat exposure needs to be accounted for. Besides IENFD, employing IVCCM in this setting may be a novel objective approach that has not previously been systematically explored. Furthermore, sural nerve biopsies might shed light on possible pathophysiological mechanisms, such as a potential role of glucosylceramide accumulation in the peripheral nerve.

I would like to conclude by alluding to my previously stated general aim of *seeing* the whole patient. I believe the design and conduction of future studies, should also be guided by the expressed difficulties of living with a neurological disease that we as neurologists obtain when *listening* to patients and their families.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Parkinsons sjukdom är en vanlig neurologisk sjukdom som tros drabba 2-3% av befolkningen över 65 år. Vid Parkinson vet vi att det råder brist i hjärnan på ett signalämne som heter dopamin. Dopamin kan liknas med smörjmedlet i en cykelkedja; har hjärnan god tillgång på dopamin kan vi smidigt utföra rörelser i olika delar av kroppen. Vid brist på dopamin kan det istället utvecklas små, tröga och ibland hackiga rörelser. Detta rörelsearmod är ett typiskt symtom som drabbar patienter med sjukdomen. Men vi vet också att det finns många andra symtom som utgör ett besvär för patienter, och inte nödvändigtvis syns för omgivningen. Dessa kan till exempel vara förstoppning, depression, minnessvårigheter och nedsatt luktsinne. I dagens läge har vi ingen sjukdomsbromsande behandling, utan all behandling syftar till att lindra symtom genom att med mediciner försöka återställa bristen på dopamin.

Nervcellerna innanför skallbenet, i hjärnan, utgör tillsammans med ryggmärgen det vi kallar för det *centrala nervsystemet*. Alla de nervtrådar som löper ut från hjärnan till armar, ben och inre organ utgör det vi kallar för det *perifera nervsystemet*. Dessa perifera nervtrådar är viktiga för att förmedla olika sinnesintryck såsom smärta, och känsel för beröring och temperatur till hjärnan. Vi vet också att denna typ av nervtrådar förmedlar signaler från hjärnan till våra muskler för att initiera rörelser, och till svettkörtlar för att producera svett. På senare tid har forskning visat att även nervceller utanför hjärnan kan påverkas vid Parkinsons sjukdom.

I den här avhandlingen har jag i två studier tittat på hur det perifera nervsystemet mår vid Parkinsons sjukdom. Vi har med olika metoder undersökt de perifera nervtrådarnas funktion och utseende, och jämfört med hur det ser ut hos kontrollpersoner. Vi kunde se att patienter med Parkinsons sjukdom uppvisade kliniska tecken på störd funktion av de perifera nervtrådarna i högre utsträckning jämfört med kontrollpersoner. Vi kunde också se i blodprov, från dessa patienter, att det fanns tecken på störd omsättning av vitamin B12. I studie IV utvecklar jag rollen av störd omsättning av vitamin B12 även till det centrala nervsystemet. Jag beskriver där en patient med Parkinson som drabbats av en unik genetisk störning som rör omsättningen av vitamin B12.

I studie II undersökte vi om störd funktion i de perifera nervtrådarna kunde kopplas till ett särskilt symtom som ibland ses hos patienter med Parkinsons sjukdom, så kallade ”rastlösa ben”. Vid rastlösa ben beskriver patienter att de besväras av ett obehag i benen, ”såsom myror”, som ofta uppträder kvällstid och vid inaktivitet. De rastlösa benen kan till exempel göra ett långt stillasittande teaterbesök mycket besvärligt. I vår studie kunde vi se att rastlösa ben hos Parkinsonpatienter inte kan förklaras av en störd funktion i de perifera nervtrådarna. Detta fynd skulle kunna tala för att dessa besvär istället kan svara på den sedvanliga behandling vi brukar ge vid detta tillstånd när man inte har Parkinson, och att dessa besvär inte nödvändigtvis behöver följa ett progressivt förlopp som övriga symtom vid Parkinsons sjukdom.

I Studie II använde vi oss av en särskild mikroskopisk teknik där vi fotograferade de allra tunnaste nervtrådarna som finns i ögats hornhinna. Vi mätte också nervledningsförmågan hos enskilda nerver i armar och ben. Vi kunde se att vissa förändringar i dessa undersökningar kunde kopplas till olika mått på hur långt gången Parkinson den undersökte patienten hade. Jag resonerar i min avhandling om huruvida denna typ av mått på det perifera nervsystemets mående skulle kunna användas som en framtida markör för att följa hur Parkinsonsjukdomen utvecklas över tid.

I Studie III och V tittar jag på två mycket ovanliga ärftliga sjukdomar. Sjukdomen Gaucher uppskattas drabba 1 på 47 000 födslar. Sjukdomen kan ge många symtom från skelettet, bukorganen och blodbilden. Vi vet att denna sjukdom har ett visst genetiskt släktskap med Parkinsons sjukdom. Sjukdomen har även föreslagits kunna drabba perifera nervtrådar och bidra till smärta. Vi undersökte patienter med denna sjukdom dels här i Stockholm, dels vid Sunderby sjukhus utanför Luleå. Vi kunde se att vissa patienter, med en särskild typ av sjukdomen, hade möjliga tecken på en störning i de tunnaste perifera nervtrådarna. Jag resonerar i min avhandling om möjliga innebörder av detta fynd såsom utveckling av smärta. Det finns dock en osäkerhet rörande vårt fynd då antalet undersökta patienter var få, givet den ovanliga sjukdomen.

I studie V kartlägger jag två familjer som drabbats av en ovanlig ärftlig sjukdom som medför svaghet och stelhet i benen. Ofta innebär denna svaghet att gånghjälpmedel behövs för att ta sig fram på ett säkert sätt. Vi undersökte dessa familjemedlemmar med olika metoder inkluderande kroppslig undersökning, mätning av ledningsförmåga i perifera nerver och analys av ryggmärgsvätskan. Vi kunde bekräfta att det kan finnas en stor variation på hur symtombilden ser ut, både mellan och inom familjer med samma sjukdom. Vi diskuterar även de fynd vi hittade i ryggmärgsvätskan.

Sammantaget vill min avhandling belysa det perifera nervsystemet ur olika aspekter vid Parkinsons sjukdom, och mer ovanliga genetiska sjukdomar. Jag föreslår att våra fynd motiverar nya studier som undersöker om det perifera nervsystemets mående kan användas som en markör för hur Parkinsonsjukdomen fortlöper i hjärnan. En sådan lättillgänglig markör skulle kunna vara ett värdefullt verktyg i framtida behandlingsstudier. Jag föreslår också att vi aktivt kontrollerar vitamin B12 nivåer i vår vård av Parkinsonpatienter.

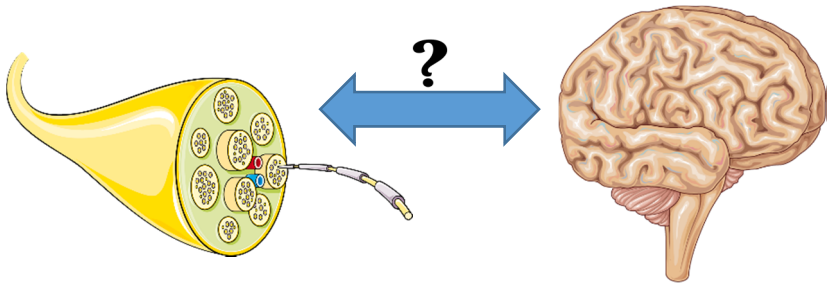

Figure 7. Kan det perifera nervernas mående säga oss något om vad som händer i hjärnan vid Parkinsons sjukdom?

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