INTERNATIONAL DIABETIC NEUROPATHY CONSORTIUM

ANNUAL REPORT 2017-2018

AARHUS
UNIVERSITY
DEPARTMENT OF CLINICAL MEDICINE

novo nordisk fonden

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TABLE OF CONTENTS

> Diabetic neuropathy is common, but also complicated. It needs to be studied in more detail.

PREFACE

It is a pleasure for me to distribute this third annual report of the International Diabetic Neuropathy Consortium (IDNC).

IDNC represents a unique collaboration between four universities (Aarhus University, the University of Southern Denmark, the University of Oxford and the University of Michigan, Ann Arbor). In this consortium, we are exploring mechanisms giving rise to diabetic neuropathy and pain.

Diabetic neuropathy is one of the main complications of diabetes. Nevertheless, neuropathy has been overshadowed by other diabetic complications. The challenges in diabetic neuropathy are multiple, including unclarified pathophysiology, lack of consensus on diagnostic criteria, insufficient management and unknown risk factors. These topics are addressed by postdocs and PhD students in the IDNC, and the present report illustrates last year's progress

Among the many successful events last year, we had a 2-day research meeting attended by approx. 100 participants and with several international speakers. We are currently pursuing efforts to implement new examination methods for detecting and following up signs of neuropathy in order to better treat and prevent diabetic neuropathy. We still need to unravel the pathophysiology of diabetic neuropathy, but the present report shows that the brick-by-brick approach of the IDNC is increasing our knowledge of the mechanisms giving rise to this disorder.

Let me thank all participants in the IDNC for their commitment and hard work to fulfill the vision and mission of the consortium.

Troels Staehelin Jensen
Director of the IDNC

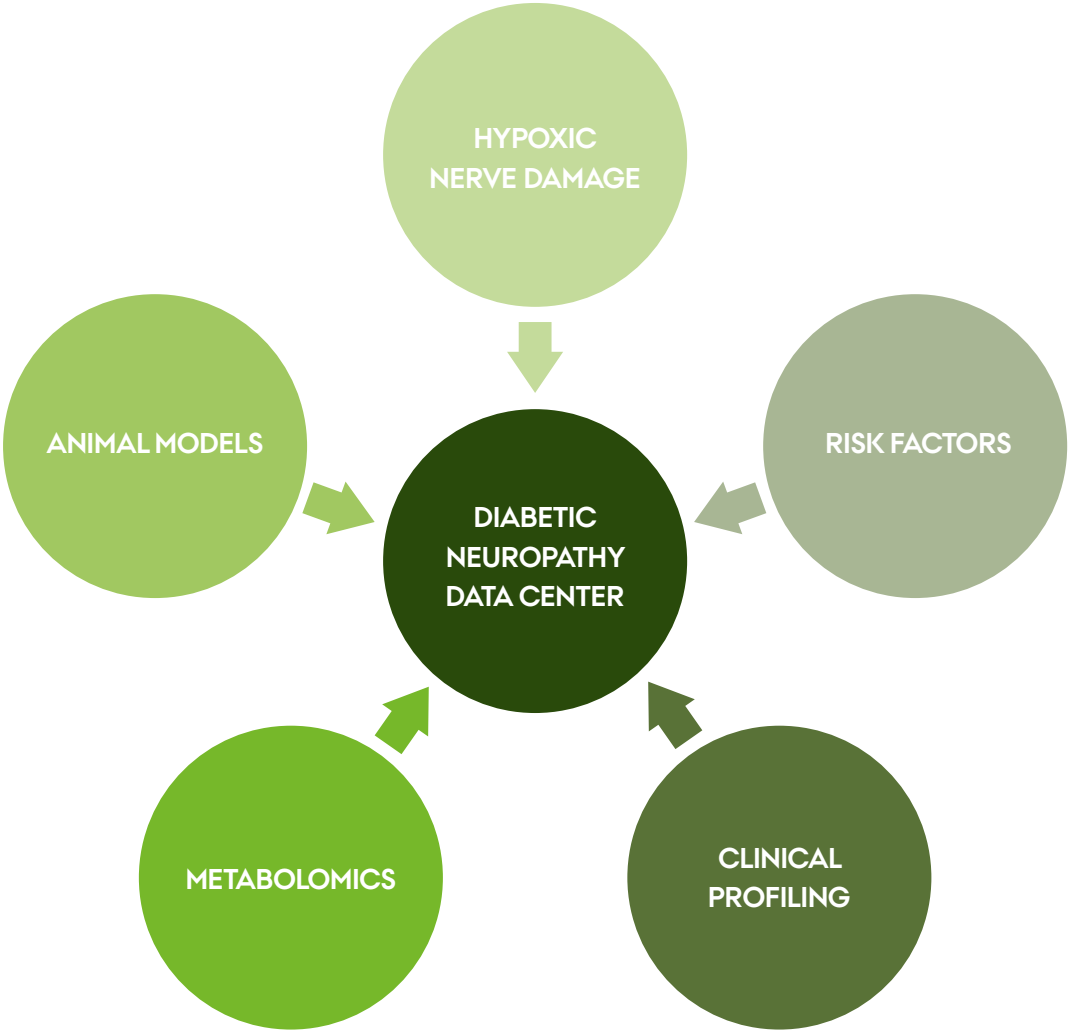
IDNC AT A GLANCE

Vision: To be a leading research group on diabetic neuropathy in Denmark with an outreach to the world.

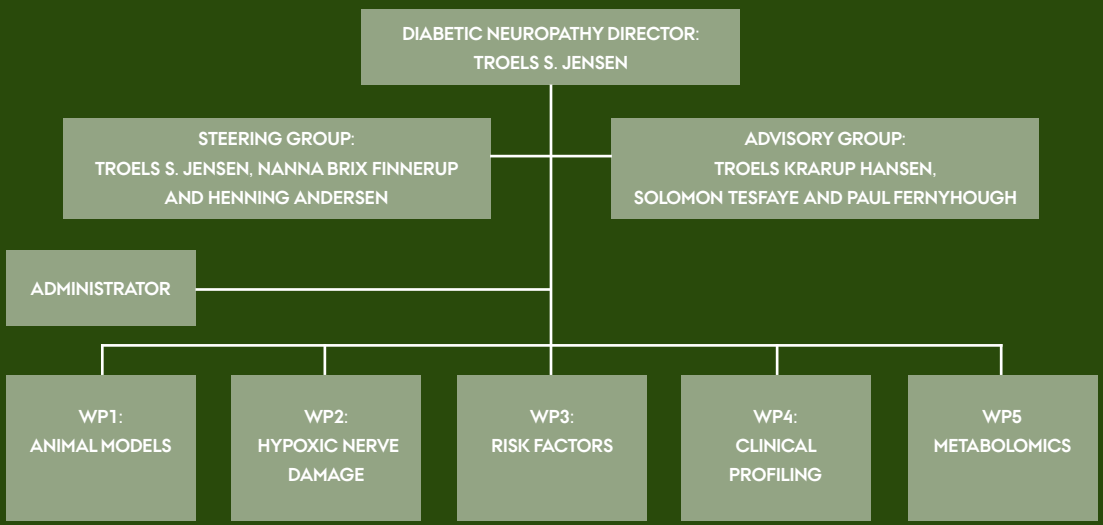
Mission: To study and unravel pathophysiological mechanisms of diabetic neuropathy, contribute to early identification, improve and develop a uniform international classification in order to better treat and prevent the detrimental consequences of diabetic neuropathy. The IDNC does so by bringing researchers and clinicians together in a stimulating and multidisciplinary environment in order to integrate and facilitate translational aspects of diabetic neuropathy.

Structure: A series of interlinked work packages devoted to explore the mechanisms, risks, prognostic factors and clinical profiles of diabetic patients with and without neuropathy.

Funding: A 6-year Novo Nordisk Foundation Challenge Program grant (Grant number NNF14OC0011633).


HYPOXIC
NERVE DAMAGE
ANIMAL MODELS
RISK FACTORS
DIABETIC
NEUROPATHY
DATA CENTER
METABOLOMICS
CLINICAL
PROFILING

The IDNC Annual Meeting 2018, Aarhus DK



ORGANIZATION

The management structure of the IDNC consists of the director, the steering group and the scientific advisory board. The steering group helps to identify important research initiatives and implement them in the IDNC. The internationally renowned scientific advisory board informs and is informed by the research studies and helps identify research questions critical to improving our understanding of diabetic neuropathy.

Aarhus University, Health hosts and supports the administration of the IDNC. The Danish Pain Research Center at Aarhus University Hospital houses the IDNC management.

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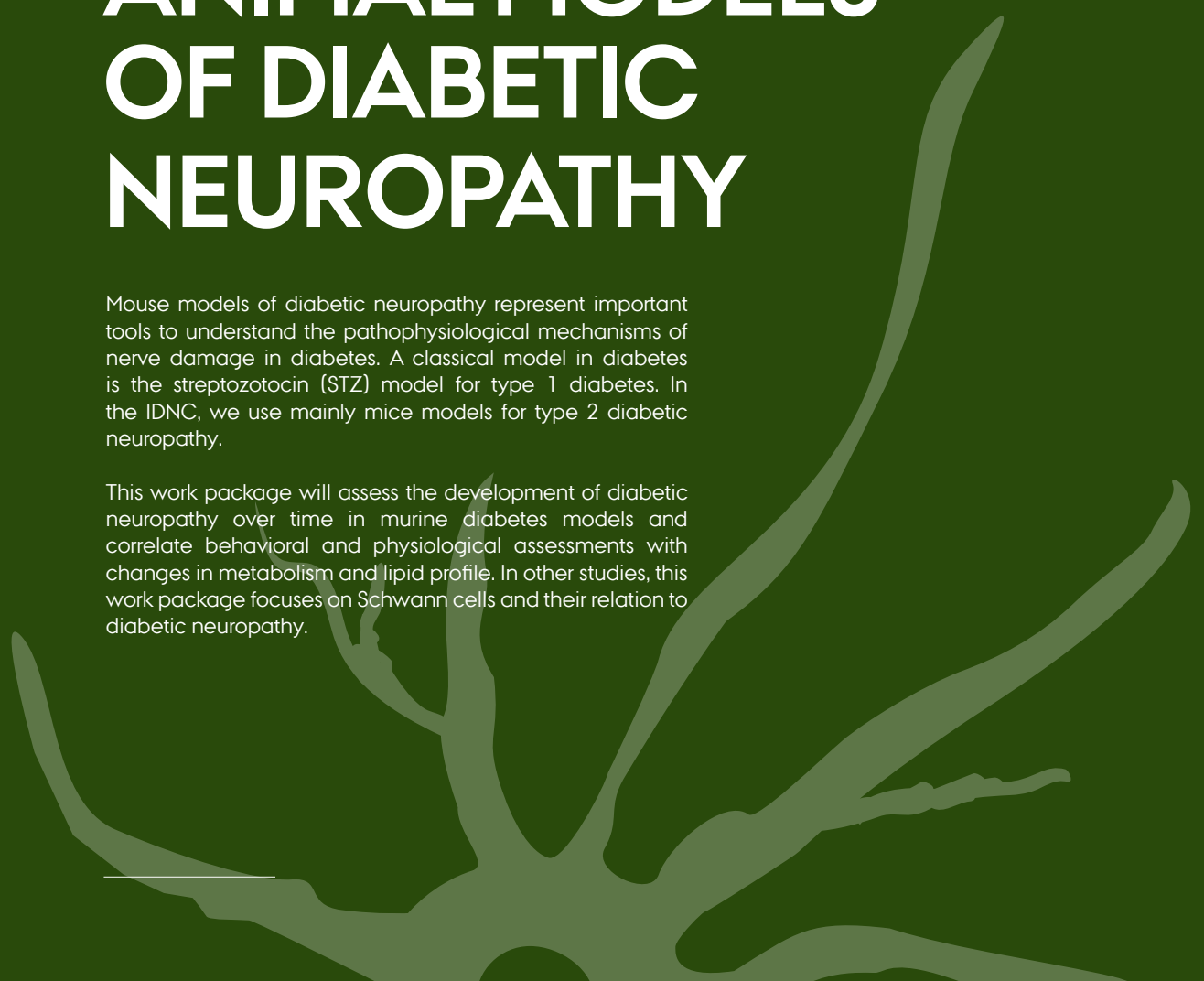
WP1: ANIMAL MODELS OF DIABETIC NEUROPATHY

Mouse models of diabetic neuropathy represent important tools to understand the pathophysiological mechanisms of nerve damage in diabetes. A classical model in diabetes is the streptozotocin (STZ) model for type 1 diabetes. In the IDNC, we use mainly mice models for type 2 diabetic neuropathy.

This work package will assess the development of diabetic neuropathy over time in murine diabetes models and correlate behavioral and physiological assessments with changes in metabolism and lipid profile. In other studies, this work package focuses on Schwann cells and their relation to diabetic neuropathy.



WP1: SCHWANN CELLS AND THEIR ROLE IN DIABETIC NEUROPATHY

Nadia Gonçalves *addresses the role of Schwann cells in diabetic neuropathy in her postdoc project. Associate Professor Christian Bjerregaard Vægter leads the research (Department of Biomedicine, Aarhus University, Denmark).*

BACKGROUND

In this work package we propose Schwannopathy as an integral factor in the pathogenesis of diabetic neuropathy and with the current project it is our main goal to expand this concept and evaluate how disruption of the interactions between Schwann cells and axons contribute to the pathogenesis of type 2 diabetic neuropathy.

Using both in vitro and in vivo models, we are focusing on how Schwann cells react to diabetic conditions and how this affects their communication with the closely attached sensory neurons. Utilization of a Schwann cell specific p75 neurotrophin receptor (p75NTR) knock out mouse model in combination with induced type 2 diabetes allow us to investigate how the expression of this receptor in Schwann cells impact the progression of DN. In vitro models, mimicking aspects of diabetes, will focus on the interplay between Schwann cells and neurons in relation to axonal myelination. The current project will provide valuable new understanding of DN mechanisms and novel approaches to target reversal of symptoms.

STATUS AND FUTURE PERSPECTIVES

The role of p75NTR receptor signaling in neuron-Schwann cell communication and myelination under in vitro diabetic conditions is being investigated with primary Schwann cell-neuron co-cultures. Results show a compromised ability of Schwann cells to myelinate axons under hyperglycemic conditions. In the absence of p75NTR, myelination is compromised in both naïve and hyperglycemic conditions, being slightly worse with hyperglycemia.

For the in vivo work Nadia Gonçalves uses diabetic neuropathy mouse models. Wildtype and p75NTR Schwann cell conditional KO mice are fed either a high fat diet or a control diet for 24 weeks to induce type 2 diabetes. Mice fed with the HFD develop mechanical allodynia and present decreased sensory and motor nerve conduction velocities as compared with mice fed with the control diet, indicating the presence of neuropathy. After the behavior testing, animals were euthanized and sciatic nerves dissected and processed for both morphometric analysis and RNA sequencing.

Electron microscopy and morphometry showed segmental demyelination and axonal atrophy in diabetic SC-p75NTR-KO mice (Fig. 1) as compared with controls and wildtype. Furthermore, a significant decrease of C-fiber density was observed in diabetic SC-p75NTR-KO along with several bands of Büngner. The studies by Nadia Gonçalves have been done in collaboration with researchers at the University of Melbourne, Australia; the University of Michigan, USA and the University of Linköping, Sweden.

In signaling studies, we found some common activation in diabetic neuropathy between strains, such as PPAR signaling pathway, genes involved in the glycerolipid metabolism or defense response, while genes associated with peroxisomes, lysosomes and phagosomes were mainly detected in the diabetic SC-p75NTR-KO mice nerves, suggesting more pronounced oxidative stress. We are now in the process of writing the manuscript that we plan to submit for publication by the end of 2018.

In the RNA sequencing, we found several genes associated with exosomes both in the wildtype and p75NTR KO situations with diabetes. This finding is of outmost importance since exosomes released by the Schwann cells can then be internalized by neurons or axons and function as a mechanism of cell to cell communication.

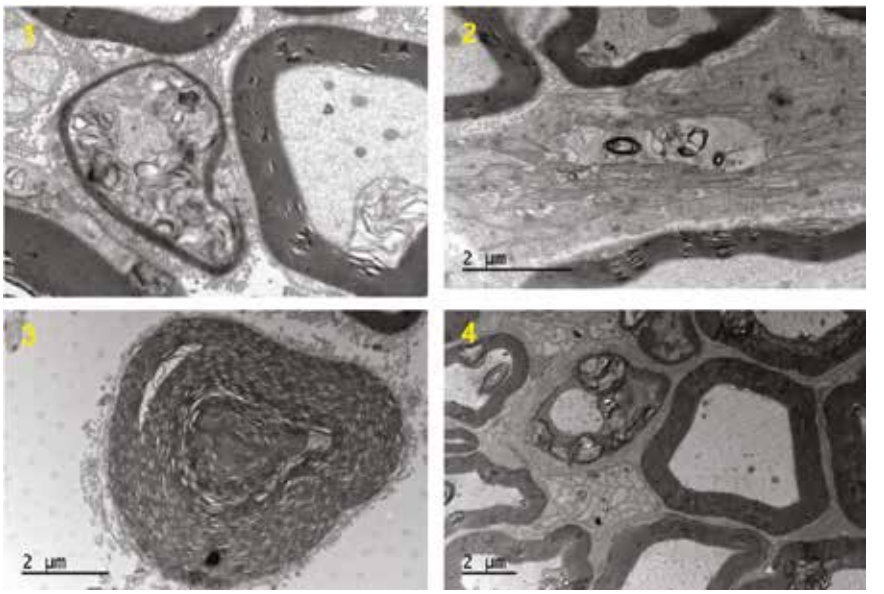


Figure 1. Morphological abnormalities found in nerve samples from SC-p75NTR-KO mice include segmental demyelination (1), presence of extensive bands of Büngner (2), axonal atrophy (3) and numerous lysosomes and phagosomes in remaining C fibers (4).

WP1: BASIC NEUROPHYSIOLOGICAL STUDY

Martin Nors Skov *is PhD student at the Comparative Medicine Lab at Aarhus University (DK).*

Professor Michael Pedersen leads the research.

The aim of this project is to develop telemetric implants to investigate the degenerative nature of diabetic neuropathy. It has two elements: 1) an implant to measure threshold and 2) an implant to collect data from autonomic nerves.

The primary investigator is Martin Nors Skov. In association with the project, Yrsa Larsen wrote her master thesis. In addition, a couple of engineering trainees assist with the project.

As part of master thesis study by Yrsa Larsen, we established Qtrac protocols for the investigation in ulnar, sciatic and tail nerves in rats and looked at the different responses between them. Only small differences between the nerves were observed. The best nerves to study with Qtrac in rats is the tail and sciatic nerves.

Currently, we are establishing benchmarks for endpoints in diabetic neuropathy in rats. The rats undergo a streptozotocin (STZ) protocol and are then left for 2 and 3 weeks. At the end of the period, the rats are anesthetized and go through the established Qtrac protocol. At the same time, we look at light evoked responses. Preliminary data of the Qtrac protocol show that there was no significant difference between the control group and the 2 and 3 weeks' diabetic groups, but the light evoked responses showed a significant reduction in amplitude in the diabetic groups.

The Qtrac implant development is still in progress and is in its third iteration. There are still some issues with the signaltonoise ratio, but we are optimistic that a working prototype is to be tested in the Fall.

A research visits to Professor Stephen McMahon's lab at Kings College London is planned to further develop the experimental setup.

The plan for Fall 2018 is to write two articles and get the implant to work for use in the spring 2019 in a chronic diabetic neuropathy study. The study intends to investigate the longitudinal development of diabetic neuropathy in rats.

The preliminary conclusions from this study suggest that the duration of diabetes may have been too short to give rise to degeneration of nerves, so longitudinal studies are need to pursue this aspect.

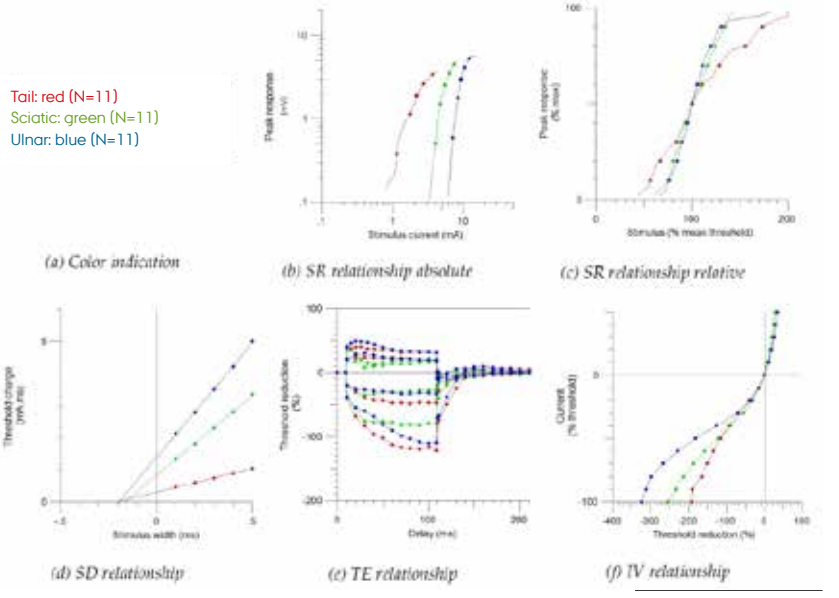


Figure 1. Visual representation of mean results from SNAP nerve excitability testing on caudal, sural ulnar nerves of healthy Sprague Dawley rats under anesthesia.

WP2: HYPOXIC NERVE DAMAGE

In this work package, the idea is that capillary flow is lost in diabetes due to endothelial glycocalyx damage, loss of pericytes, thickening of capillary basement membranes and elevated blood viscosity. Capillary flow in sural nerves of both type 1 and type 2 models for diabetic neuropathy are studied using two-photon microscopy combined with optical coherence tomography (OCT). With these methods, we test the hypothesis that elevated capillary transit time heterogeneity and reduced oxygen tension are involved in diabetic neuropathy in mice.

WP2: ARE DIABETIC NERVES SUFFOCATING?

Anete Dudele
Postdoc
Anete Dudele hypothesizes in her studies that diabetes reduces capillary function in peripheral nerves and that this contributes to nerve damage and the development of diabetic neuropathy through limited oxygen and nutrient delivery to the nervous tissue. Professor Leif Østergaard, Center of Functionally Integrative neuroscience (CFIN), Aarhus University (DK) leads the research.

Using state-of-the-art two-photon in vivo microscopy, for the first time we have measured partial pressure of oxygen in sural nerve vasculature of mice (Fig. 1). To understand the role of hypoxic nerve damage plays in the onset and development of diabetic neuropathy, it is important to investigate the normal levels of tissue oxygenation in healthy animals, which we have now done.

Figure 1 shows an arterial (depicted in orange and red) and venous (blue and green) vasculature surrounding murine sural nerve. This sensory nerve is the target of investigation in our studies of diabetic neuropathy.

Endoneureal hypoxia can be a result of capillary dysfunction, characterized by an uneven distribution of capillary blood flow to the nervous tissue. To investigate how capillary function is affected by diabetes and if it contributes to development of diabetic neuropathy, we have established and verified an optical method that allows us to track appearance and distribution of a bolus of a contrast agent in murine nerve vasculature. This method allows us to evaluate capillary function in this vascular bed (Fig. 2). We have now assessed the normal sural nerve capillary function in animal models and have tested how different experimental parameters affect it. By doing this we have built a solid foundation for acquiring information on how diabetes in animal models changes capillary function and flow patterns.

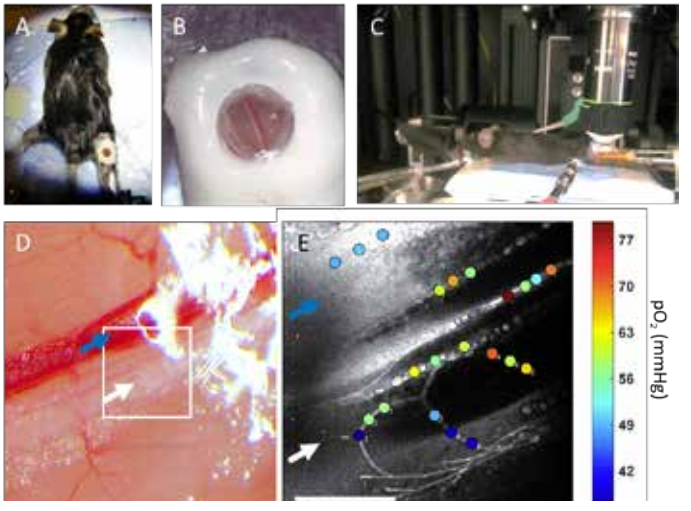


Figure 1. PO2 measurement in murine peripheral nerves.

A & B - Sural nerve is exposed and fixed in an anesthetized mouse.
C - Mouse is moved into two-photon microscopy facility where throughout imaging process vital signs (blood pressure, end tidal CO2, ventilation rate, core and leg temperature) are monitored and adjusted as needed.
D – sural nerve with the adjacent vein. The white frame indicates imaging location. The white arrow indicates sural nerve and the blue arrow the large vein (both in D and E)
E - Intravenous injection of contrast agent allows for visualization of blood vessels (depicted in white color), and nervous blood supply can be identified. After injecting O2 sensitive dye, blood PO2 can be measured in the points of interest (here at the location of vessels supplying blood to nerve, and the adjacent vein as a reference value for venous blood). Scale bar = 200µm.

WP3: RISK FACTORS FOR TYPE 2 DIABETIC NEUROPATHY

The ADDITION cohort and the DD2 cohort are the basis of this work package where we are studying: 1) the metabolic risk factors for diabetic neuropathy, 2) the effect of therapy on diabetic neuropathy and 3) the determinants for the clinical course of diabetic neuropathy and its prognosis.

The ADDITION Study (Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen Detected Diabetes in Primary Care) is a study on early detection and intensive treatment of type 2 diabetes in primary care, where patients have been followed since their screen-detected diagnosis of type 2 diabetes 14 years ago.

The prospective Danish Center for Strategic Research in Type 2 Diabetes (DD2) cohort and biobank continuously enroll newly diagnosed type 2 diabetes patients throughout Denmark. The DD2 database was started in 2010 and currently holds approx. 10,000 individuals.

The DD2 cohort is essential in the IDNC, both for epidemiological studies and as a resource for clinical and genetic studies. The DD2 cohort represents a unique sample of type 2 diabetes patients to determine not only the prevalence and clinical profile of diabetic neuropathy in early type 2 diabetes, but various risk factors for the development of diabetic neuropathy over time.

WP3: DIABETIC NEUROPATHY IN A COHORT WITH SCREEN-DETECTED TYPE 2 DIABETES: ADDITION-DENMARK

***Signe Toft Andersen** carries out her PhD project based on the ADDITION Denmark study. Senior researcher Morten Charles, Department of Public Health, Aarhus University (DK) leads the research. screen-detected type 2 diabetes: ADDITION-Denmark.*

This PhD project assessed the development and the presence and progression of diabetic polyneuropathy (DPN) and cardiovascular autonomic neuropathy (CAN) in the Danish arm of the ADDITION study and estimated effects of potential risk factors for DPN and CAN. In summary, the project showed a much lower prevalence and progression of neuropathy than seen in previous cohorts of people with type 2 diabetes and it confirmed that hyperglycemia, obesity and dyslipidemia are risk factors for DPN and CAN.

The first project assessed incident DPN by the Michigan Neuropathy Screening Instrument questionnaire prospectively during the first 13 years after a screening-based diagnosis of type 2 diabetes (n=1256) and showed a cumulative incidence of DPN of 10%. This project identified older age, obesity, low HDL cholesterol and higher levels of methylglyoxal, a marker of dicarbonyl stress, as potential causal risk factors for the development of DPN.

The second project (n=452) determined retrospective cardiometabolic risk-factor trajectories (in terms of levels and changes over time) preceding a clinical diagnosis of DPN 13 years after a screening-based diagnosis of type 2 diabetes. A prevalence of DPN of 27% was seen after 13 years of diabetes. This study replicated the finding of obesity being a risk factor for DPN. Furthermore, this study showed, that the rate of HbA1c increase affects the development of DPN over and above the effect of HbA1c levels, notably in a cohort with overall good glycemic control (Fig. 1).

The third project (n=777, n=443) examined the course of CAN and related cardiometabolic risk factors at the 6-year and 13-year

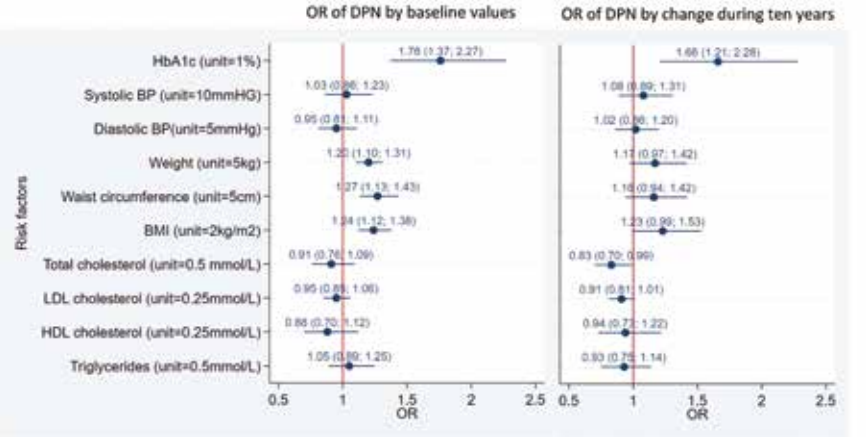


Figure 1. Risk of DPN after 13 years of screen-detected diabetes per clinically relevant differences in modeled baseline levels (intercepts) and changes during ten years (slopes) of risk factors by multivariate logistic regression models, ADDITION-Denmark. Andersen ST et al. Diabetes Care 2018; 41: 1955-62.

follow-up examinations in ADDITION-Denmark. It showed a progressive yet heterogeneous course of CAN between the 6- and the 13-year follow-up (Fig. 2). The prevalence of CAN increased from 9% to 15% between the 6- and the 13-year follow-up. Hyperglycemia, obesity and hypertriglyceridemia were negatively related to indices of CAN, although these effects diminished over time. The observed heterogeneous course of CAN challenges the present clinical approach of using categorical classification of CARTs for diagnosing CAN or the notion of CAN irreversibility.

Figure 2. The flow of participants between clinical groups of CAN: ADDITION-Denmark.
Number of participants and changes in their CAN status among defined categories; No CAN (green), early CAN (yellow) or manifest CAN (red) from the 6- to the 13-year follow-up examination in ADDITION Denmark. Andersen ST et al. Diabetes Care 2018 (in press)

WP3: DD2 COHORT AND REGISTRIES

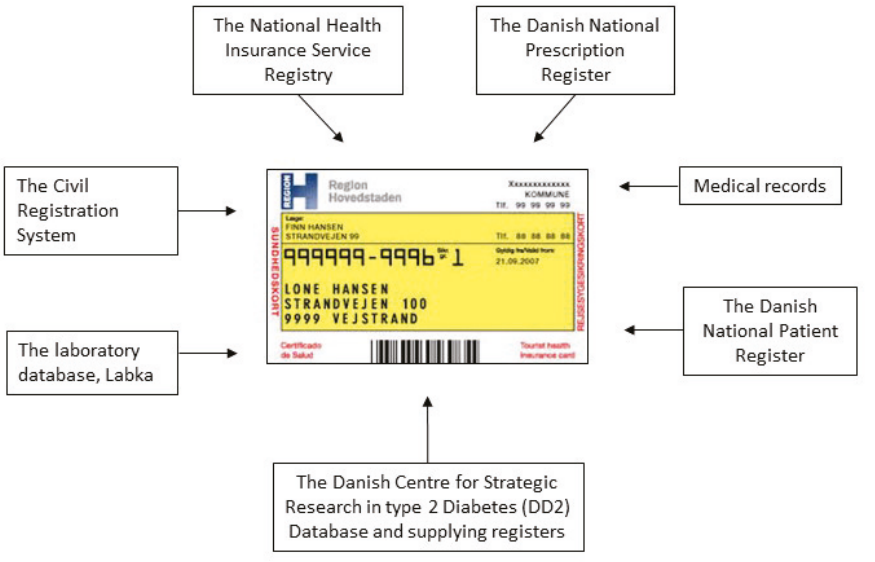
Diana Hedevang Christensen *hypothesizes in her epidemiological PhD study that risk factors other than hyperglycemia play a key role for the development of diabetic neuropathy in type 2 diabetes. Associate Professor Reimar W. Thomsen, Department of Clinical Epidemiology, Aarhus University Hospital (DK) supervises the research.*

Danish routine care registries represent a goldmine for studying diabetic neuropathy if diagnosis codes are valid. Diana Hedevang Christensen has validated different types of ICD-10 codes for neuropathy against medical records (Fig. 1). She found a positive predictive value of 71% (95% confidence interval [CI]: 49-87%) of an algorithm based on diabetes and polyneuropathy ICD-10 G-chapter codes in Danish registers. The positive predictive value further increased to 86% (95% CI: 70-95%) when prescription codes for neuropathic pain medication were added. These algorithms will form the basis for registry-based epidemiological studies on diabetic polyneuropathy in future IDNC studies.

Newer antihyperglycemic drugs may – besides their glucose-lowering effects – have neuroprotective properties. A study by Diana Hedevang Christensen on nationwide time trends in the utilization of all glucose-lowering drugs in Denmark showed that by 2014, 4 per 1,000 Danish inhabitants used a glucagon-like peptide 1-receptor agonist (GLP1-RA), 3 per 1,000 used a combination pill of metformin and dipeptidylpeptidase-4 inhibitors (DPP4i) and 3 per 1,000 used a single DPP4i.

Updated prescription data on these newer drugs (including 2017) will be available in the fall of 2018. Diana Hedevang Christensen has initiated a large pharmacoepidemiological study, using the new validated diabetic polyneuropathy algorithm to study the possible impact of GLP1-RAs and DPP4i on decreased risk of developing diabetic polyneuropathy.

Using the IDNC-DD2 questionnaire dataset on self-reported neuropathy together with DD2 data, Diana Hedevang Christensen currently evaluates how different obesity measures and weight changes in type 2 diabetes patients are associated with the prevalence of painful and non-painful diabetic neuropathy.



WP4: CLINICAL PROFILE

In this large work package, IDNC is determining the presence of pain and sensory abnormalities in type 2 diabetes. The hypothesis is that based on history, and in particular clinical examination and detailed sensory profiling, it will be possible to find distinguishing characteristics in in patients with type 2 diabetes, diabetic neuropathy and painful diabetic neuropathy.

The clinical profiling involves work done at the University of Oxford (UK), University of Southern Denmark and Aarhus University (DK). Clinical profiling in Denmark is carried out on the basis of the DD2 cohort. The examinations and profiling in the three study sites are similar to those done in the major multicenter project DOLORisk, which aims to understand risk factors and determinants for neuropathic pain (dolorisk.eu). DOLORisk is funded by the European Commission Horizon 2020 and is coordinated by IDNC affiliated researcher Professor David Bennett, Oxford University with Professor Nanna Brix Finnerup, Aarhus University as deputy project coordinator.

WP4: SOMATOSENSORY PHENOTYPING, THRESHOLD TRACKING AND GENETICS IN DIABETIC NEUROPATHY

The Oxford group explores the possible drivers of neuropathic pain in diabetic neuropathy and how patients with neuropathic pain can be better stratified.

Our first study was a deep phenotyping study that used a multidisciplinary approach to phenotype a large cohort of patients with painful and painless diabetic neuropathy (DPN). Our participants with diabetic neuropathy underwent neurological examination, quantitative sensory testing, nerve conduction studies, skin biopsy for intraepidermal nerve fiber density assessment and a set of questionnaires to assess the presence of pain, pain intensity, pain distribution and the psychological and functional impact of pain. The sensory profile of patients with painful DPN was distinct from those patients with painless DPN showing greater hyposensitivity to sensory stimuli across a range of sensory modalities. This study provided a firm basis to rationalize further phenotyping of painful DPN and has served as a basis for all our subsequent work.

We then went on to explore the contribution of genetic variability in neuropathic pain and examined the relationship between variants in the voltage-gated sodium channel Nav1.7 and Neuropathic pain. The rationale for studying Nav1.7 is that this voltage gated sodium channel is expressed in nociceptors and amplifies subthreshold stimuli. It is therefore a key determinant of nociceptor excitability and selective blockers of Nav1.7 are under active development as novel analgesics. Nav1.7 has been shown to be important in pathological pains states in humans. Although no rare variants were found in participants with painless DPN, we identified twelve rare Nav1.7 variants in ten study participants with painful DPN. Five of these variants had previously been described in the context of other Neuropathic pain disorders and seven have not previously been linked to neuropathic pain. Those patients with rare variants reported more severe pain and greater sensitivity to pressure stimuli on quantitative sensory testing. In vitro electrophysiological characterization of two of the novel variants demonstrated gain of function changes as a consequence of markedly impaired channel fast inactivation. Our observations suggest that rare Nav1.7 variants contribute to the development of neuropathic pain in patients with DPN. Their identification will aid in the understanding of sensory phenotype, patient stratification, and help target treatments more effectively. For example, in collaboration with Dr Sulayman Dib-Hajj and Professor Stephen Waxman, we were able to use structural modelling to predict pharmacoresponsiveness for a variant identified in NaV1.8. The variant was identified

in one of our patients with painful DPN. In vitro experiments showed that pretreatment with a clinically achievable concentration of carbamazepine corrected activation of the variant NaV1.8 channels and reduced DRG neuron excitability as predicted from the pharmacogenomic model. A genetic approach offers a promising approach to gain insight into mechanisms underlying painful DPN. We are continuing to recruit participants as part of the DOLORisk consortium to explore the genetic landscape. A variety of genetic approaches will be used. A genome wide association study will explore associations from a large cohort of patients primarily phenotyped from questionnaires. Exome sequencing will be applied to subset of patients that have been deeply phenotyped, in line with our original phenotyping from 2016.

We are continuing our work on using electrophysiological tools to discriminate between patients with painful and painless DPN. Threshold tracking is a neurophysiological tool that assesses large nerve fiber axonal excitability. It is an indirect measure of the ion channel excitability within myelinated nerve fibers. We have recorded from 151 participants. Preliminary data analysis of four outcomes does not show differences between participants with painful and painless DPN. We will continue to explore the data to determine whether symptoms or other clinical variables are related to our four key outcomes.

Our deeply phenotyped cohort of patients has provided a powerful platform to explore underlying mechanism for chronic neuropathic pain. We used a multimodal clinical neuroimaging approach to interrogate whether the sensory phenotype of painful DPN involves altered function of the ventrolateral periaqueductal grey—a key node of the descending pain modulatory system. We found that ventrolateral periaqueductal grey functional connectivity is altered in study participants suffering from painful DPN; the magnitude of which is correlated to their spontaneous and allodynic pain as well as the magnitude of the cortical response elicited by an experimental tonic heat paradigm. We therefore conclude that ventrolateral periaqueductal grey-mediated descending pain modulatory system dysfunction may reflect a brain-based pain facilitation mechanism contributing to painful DPN.

David Bennett moderating session at the IDNC Annual Meeting 2018

David Bennett discussing with Eva Feldman and Brian Callaghan at the IDNC Annual Meeting 2018

WP4: CLINICAL PROFILING OF DIABETIC NEUROPATHY

Mustapha Itani

Professor Søren Sindrup and PhD student Mustapha Itani are engaged in clinical profiling of people with type 2 diabetes from the DD2 cohort with respect to neuropathy. The research is carried out at the IDNC site at the Department of Neurology, Odense University Hospital (Clinical Research, Neurology, University of Southern Denmark).

The main studies in this project are:

1) To describe the pattern of peripheral nerve affection in non-selected type 2 diabetic patients with and without symptomatic polyneuropathy and search for overall and neuropathy specific risk factors.

2) To evaluate if small fiber neuropathy is a specific category of neuropathy in type 2 diabetic patients or if it is merely a precursor of generalized peripheral nerve affection.

3) To compare the pattern of peripheral polyneuropathy found in type 2 diabetic patients to the pattern found in patients with idiopathic polyneuropathy.

Examples of sensory testing in patients suspected of neuropathy

WP4: CLINICAL PROFILING OF PAINFUL DIABETIC NEUROPATHY

***Sandra Sif Gylfadottir** is focusing on painful diabetic neuropathy in patients derived from the DD2 questionnaire study. Professor Nanna Brix Finnerup, Danish Pain Research Center, Aarhus University (DK) supervises the research.*

The aims of the three studies in this project are:

1) To estimate the prevalence of painful diabetic neuropathy in Danish type 2 diabetic patients.

2) To characterize the pain in DPN and identify subgroups of patients with specific clusters of symptoms and signs and to compare DPN patients with and without pain to identify abnormalities specific to pain.

3) To examine morphological chances in intraepidermal nerve fibers and differentiate between patients with and without pain.

We invited participants from the nationwide Danish Centre for Strategic Research in type 2 Diabetes (DD2) cohort to participate in a clinical examination at two centers in Denmark, i.e. Aarhus and Odense. We both selected a random sample and a stratified sample based on questionnaire responses.

The inclusion started in November 2016 and until now, we have examined 165 patients and 35 healthy controls in Aarhus. The inclusion will end 1 October 2018 in both centers (Fig. 1).

The examinations consist of neurological evaluation, standardized questionnaires and clinical scoring systems, skin biopsies, blood samples, quantitative sensory testing, confocal corneal microscopy and nerve conduction studies.

Impact of the study:
It is important to estimate the prevalence, risk factors and underlying mechanisms of painful diabetic neuropathy and it will hopefully contribute to significant improvements in the treatment and prevention of the disease.

Examples of information collected in the clinical part of the DD2 study

Questionnaires,clinical examinations and laboratory tests	Background information
• Standard neurological examination • Questionnaires on neuropathy and neuropathic pain (DN4, NPSI, MNSI, PROMIS, COMPASS-31) • Toronto Clinical Scoring System • Utah Early Neuropathy Scale • Quantitative sensory testing • Corneal confocal microscopy • Skin biopsies • Nerve conduction studies • Assessment of cardiovascular autonomic neuropathy • Blood samples (levels of HbA1c and cholesterol, Methylglyoxal and markers of inflammation)	• Sex • Age • Smoking habits • Alcohol consumption • Educational level • Physical activity • Duration of diabetes • Medical history and comorbidity • Overall quality of life • Symptoms of depression and anxiety, sleep disturbance and fatigue • BMI

WP4: MOTOR DYSFUNCTION IN DIABETIC NEUROPATHY

Karolina Snopek *carries out the PhD project entitled: Resistance training in patients with diabetic neuropathy. The project consists of four substudies and is supervised by Professor Henning Andersen, Department of Neurology, Aarhus University Hospital (DK).*

Muscle weakness and unstable gait are debilitating complications of diabetes mellitus. In later stages, diabetic neuropathy (DN) can lead to severe neurogenic muscle impairment with atrophy and weakness. Furthermore, diabetes may also impair striated muscles directly resulting in a diabetic myopathy. Thus, diabetic patients with motor dysfunction may suffer from gait instability, muscle weakness and decreased mobility of joints that limits their independency in daily living. Motor dysfunction may be alleviated by physical training including resistance and gait training; however, it is unknown whether patients with neuropathy and myopathy can regain muscle strength and if they can improve their gait stability. Furthermore the molecular basis for the neurogenic atrophy and myopathy remains unknown.

The PhD project consists of four main studies.

1) The first part of this study aims to describe the relationship between falls, fractures related to falling, muscle strength and signs of neuropathy in patients with type 2 diabetes by evaluating 45 patients with a history of falling. Until Now, we have included 28 patients that had suffered at least one fall over the past year.

2) In the second part, we aim to include 20 patients with diabetic neuropathy and substantial muscle weakness based on muscle strength measures from the biodex. Patients will undergo resistance training for a period of 12 weeks. By using a run-in period, patients will serve as their own control and will be examined three times over a period of 6 months. Until now, we have included 8 patients for this study.

3) Study 3 is a randomized single-blinded clinical training trial where we want to examine the effects of 12 weeks of resistance training in individuals with DN versus non-DN and healthy control subjects. All participants are randomized to high-intensity resistance training or no training and are evaluated at baseline and after a period of 12 weeks training (Fig. 1).

4) Study 4 includes all participants from study 2 and 3 with examination of their muscle biopsies (gastrocnemius muscle). A muscle biopsy is performed before and after 12 weeks of training. Using rt-PCR the expression of the muscle growth factors (IGF-1, myostatin) and neurotrophic factors (NT-3, NT-4 and BDNF) are determined.

Figure 1.

Examples of resistance training used in the randomized training trials 2 and 3

WP4: CLINICAL NEUROPHYSIOLOGICAL MEASURES IN DIABETIC NEUROPATHY

Alexander Gramm Kristensen *attempts to obtain the optimal strategy for diagnosing diabetic neuropathy using neurophysiology and to examine the mechanisms behind diabetic neuropathy. Associate Professor Hatice Tankisi, Department of Neurophysiology, Aarhus University Hospital (DK) supervises the research.*

In the past year, Alexander has continued to include participants for his studies, now with over 200 included. Alexander is close to finishing his study on motor unit number estimation using MScanFit MUNE, as a measure of early motor involvement in diabetic polyneuropathy (DPN). The preliminary results show that diabetics with neuropathy in the lower extremities have fewer and larger motor units in the abductor pollicis brevis muscle, prior to nerve conduction study abnormalities (Figs. 1-2). A preliminary analysis of our groups with ROC curves also showed a slightly higher area under the curve for MScan-Fit MUNE, compared to peak. We hope that our study will provide new insight into the development of DPN any may be a new method of diagnosing and tracking progression of DPN in patients.

With the inclusion of participants concluding, Alexander will begin to analyze data for a study on nerve excitability and a study on nerve conduction on the distal sural nerve. The aim of the excitability study is to compare sensory and motor nerve excitability changes in patients with DPN as well as correlate these changes to clinical measures. The goal for the distal sural nerve study is to examine the utility of this new method in diagnosis of DPN.

Alexander is currently working on a method of nerve excitability testing on a-delta fibers in cooperation with associate professor Carsten Dahl Mørch and Professor Ole Kæseler Andersen at Aalborg University. This method may provide great insight into the changes in excitability of the specific nerves of interest to DPN.

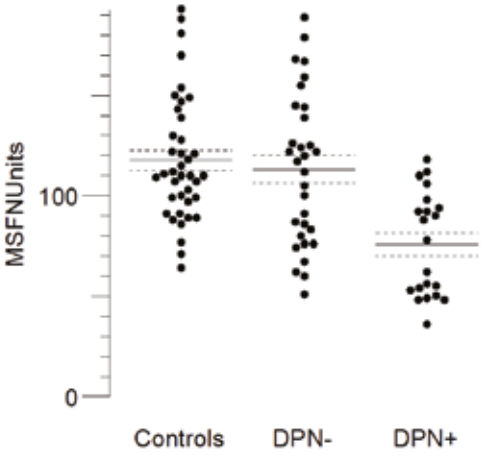


Figure 1. Dot plot comparing the estimated number of motor units between controls, diabetics without neuropathy (DPN-) and diabetics with neuropathy (DPN+).

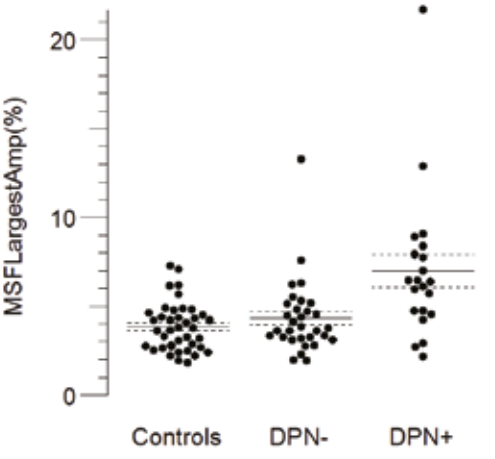


Figure 2: Dot plot comparing the size of the largest motor unit between the three groups.

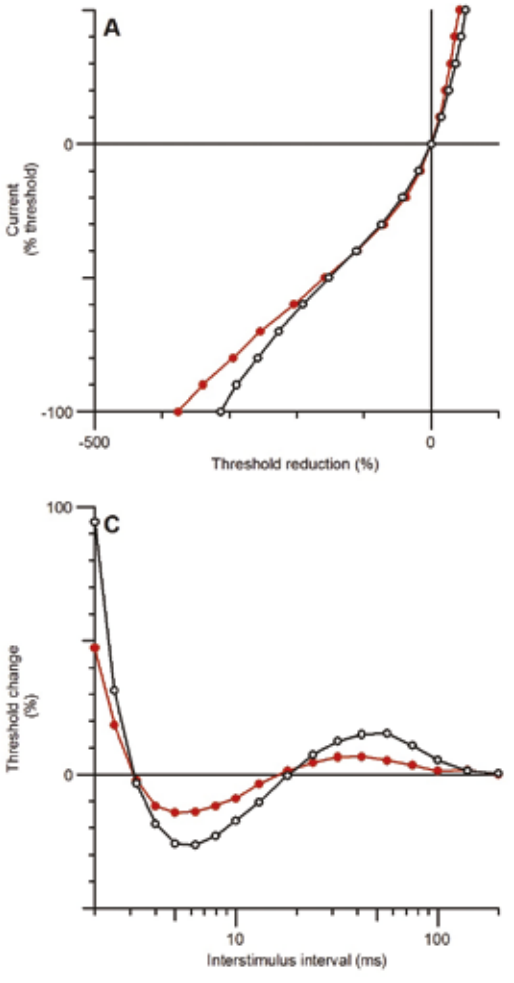


Figure 3: An example of nerve excitability measures comparing a diabetic with neuropathy with laboratory controls. Black line mean of controls. Red line type 2 diabetic with neuropathy.

Fig 3.A is the current/voltage relationship, Fig 3.B is threshold electrotonus, Fig. 3.C is the recovery cycle

WP4: AUTONOMIC NEUROPATHY IN DIABETES

Astrid J. Terkelsen

Small nerve fibers of A and C type are essential for the perception of heat, cold and pain and for regulating heart and endo- and exocrine gland functions such as sweat and smooth muscles. The autonomic small fibers control cardiovascular system, renal, bowel, bladder and sexual functions. Following injury to the autonomic system, such as in diabetes, severe changes may occur.

Small nerve fiber damage resulting in neurogenic autonomic dysfunction and small fiber polyneuropathy is common but overseen in diabetes and associated with reduced quality of life, increased morbidity and sudden death. Patients with autonomic involvement may present with orthostatic hypotension or syncope, enteric dysfunction, sexual impotence, anhidrosis and urinary retention or incontinence. Based on new and quantitative methods it is now possible to diagnose neurogenic autonomic dysfunction (tilt table test, Valsalva maneuver (Fig. 1), deep respiration (Fig. 2) and 24-hour blood pressure) and small fiber polyneuropathy (skin biopsy and sweat test) very early before major nerve damage. However, despite these accurate methods patients may progress to moderate to severe autonomic dysfunction with supine hypertension, orthostatic hypotension and syncope..

At present, PhD student Thorsten Rasmussen and Associate Professor Astrid Juhl Terkelsen focus on the adrenergic (sympathetic) dysfunction and thus abnormal blood pressure regulation and changes in local sweat.

The aims are to

- Detect diabetes patients with moderate to severe adrenergic dysfunction
- Improve 24-hour blood pressure measurements by using both pulse transit time and oscillometric blood pressure measurements
- Improve the diagnostic methods for small fiber neuropathy, the intraepidermal nerve fiber density by adding the information about innervation and structure of the adrenergic innervated sweat glands and vessels
- Based on a database containing small fiber polyneuropathy in patients with diabetes and other etiologies different small fiber phenotypes are characterized.

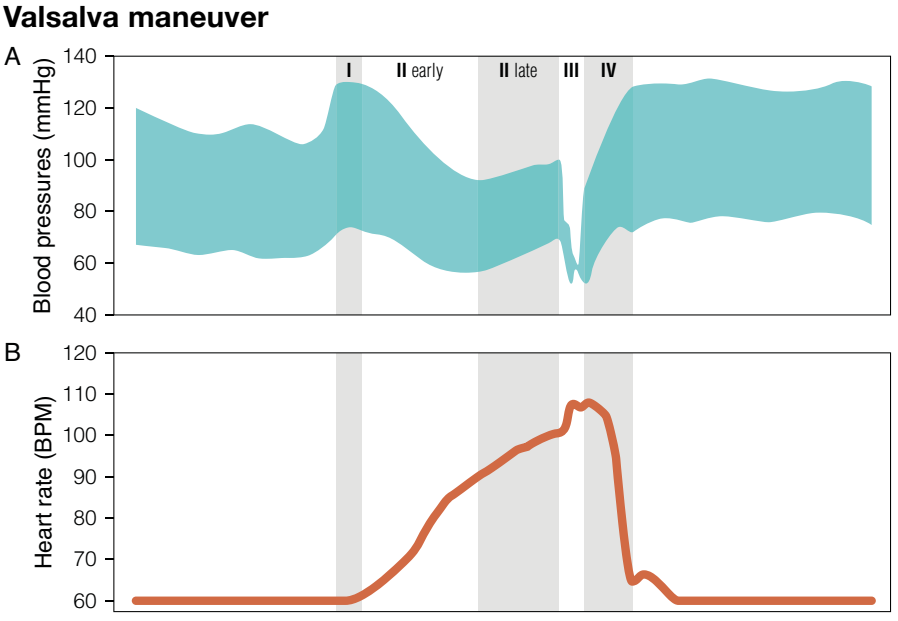


Figure 1. Blood pressure (A) and heart rate (B) responses to the Valsalva maneuver. The presence of late phase II and fast recovery phase IV in the continuously measured blood pressure indicates a normal cardiovascular adrenergic function. The heart rate response with a significant Valsalva ratio indicates a normal cardiovagal and cardiovascular adrenergic function.

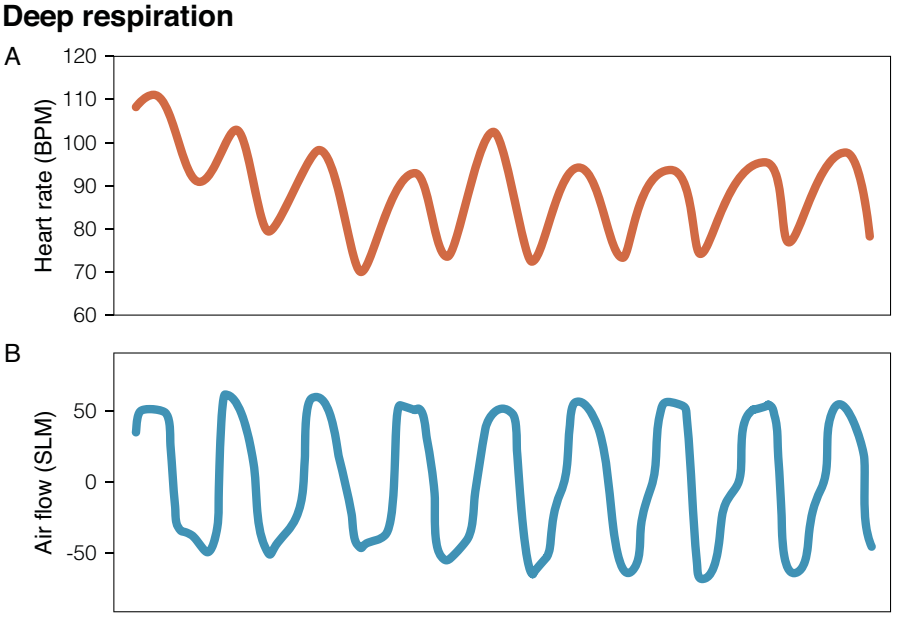


Figure 2. Heart rate variability (A) to deep respiration (B). The significant variation in heart rate indicates a preserved cardiovagal activity.

WP4: DETAILED SKIN INNERVATION ANALYSIS IN PAINLESS AND PAINFUL DIABETIC POLYNEUROPATHY

Pall Karlsson, *Assistant Professor Pall Karlsson, Danish Pain Research Center and Core Center for Molecular Morphology, Section for Stereology and Microscopy, Department of Clinical Medicine, Aarhus University (DK) has active collaboration with leading experts on skin biopsies, e.g. Guiseppe Lauria in Milan (Italy), Michael Polydefkis in Baltimore (US), and more recently with Maria Nolano in Naples (Italy).*

Skin biopsy enables quantification of small nerve fibers penetrating the epidermaldermal basement membrane. In DPN, there is a decreased density of intraepidermal nerve fibers (IENFD) in 85% of the cases. However, that alone does not explain functional deficits of the small fibers. Identification of a neuropathic pain biomarker that differentiates between DPN patients with and without neuropathic pain is therefore essential.

Peptidergic nerve fibers that express neuropeptides are important in nociception, including substance P (SP) and calcitonin gene related peptide (CGRP). In an ongoing study, we performed skin biopsies in three groups of DPN patients: a) with painless DPN and normal nerve conduction study (NCS), b) with painful DPN and normal NCS and c) with painful DPN and abnormal NCS and compared them with healthy controls.

A total of 94 patients were analyzed, and the results showed that diabetic patients with mixed fiber neuropathy (abnormal NCS and abnormal IENFD) had higher SP and CGRP density in the dermis compared with the other groups, even if IENFD was lowest in patients with mixed fiber neuropathy. Additionally, when counting total peptidergic fibers (the sum of SP- and CGRP fibers), we found a significantly higher density in patients with pain compared with patients without pain and healthy controls. Lastly, there was a significant correlation between IENFD and QST results. These findings indicate that patients with pain have higher dermal peptidergic fibers but lower IENFD compared with patients without pain, and may therefore be an important additional measure in skin biopsies. A manuscript based on these findings is under preparation.

Another important aspect is to identify subtle changes to the small nerve fibers in diabetic patients as early as possible. Axonal swellings are degenerative changes that contain watery axoplasm, dilated vesicular profiles, granular debris, abnormal mitochondria and a paucity of particulate organelles such as neurofilaments. In an ongoing study we investigate whether axonal swellings are related to somatosensory profile in a large well-defined diabetic cohort that has undergone detained and rigorous sensory phenotyping.

Corneal confocal microscopy as a tool for detecting diabetic polyneuropathy in a cohort with screen-detected type 2 diabetes

Corneal confocal microscopy (CCM) is a non-invasive ophthalmic technique that allows quantification of corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD) and corneal nerve fiber length (CNFL). Patients with DPN have lower CCM measures compared with healthy controls. However, the majority of previously published studies have reported unadjusted analyses of the associations between CCM measurements and DPN, and few studies have adjusted for age.

It is currently unknown if CCM is a useful tool for detecting DPN in populations with type 2 diabetes followed in general practice. In a new study, we explored CCM as a potential tool for detecting DPN defined by the Toronto consensus criteria in a cohort with screen-detected type 2 diabetes, when controlling for the effect of sex and age.

We found that CNFD was lower both in participants with confirmed DPN (n=27) and in participants without confirmed DPN (n=117) compared with controls. No differences were observed for CNFD between participants with and without DPN. Out study suggests that CCM cannot distinguish between patients with and without neuropathy, but CNFD is lower in patients with type 2 diabetes compared to controls.

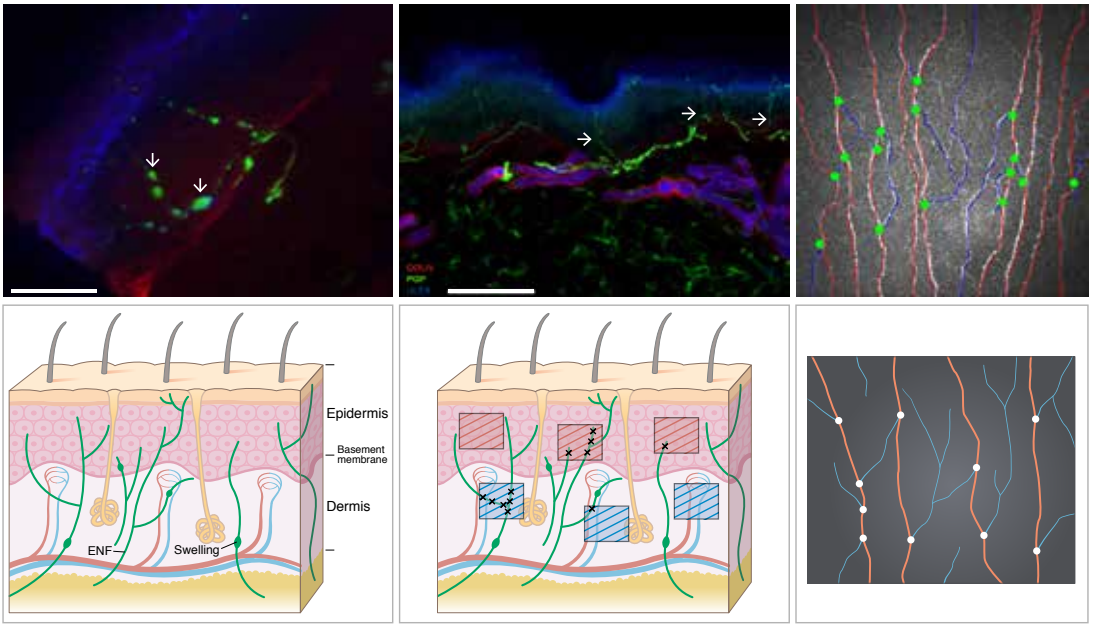


Figure 1.
Morphological assessment of key small fiber parameters on skin biopsy and CCM. Upper panel: Skin biopsy and CCM analysis in a diabetic patient. Left: Arrows: axonal swellings (>1.5µm). Scale bar = 30 µm. Middle: IENFs stained with PGP 9.5 (green), Collagen IV and ULEX (blood vessels). Arrows: Nerve fibers in dermis and epidermis. Scale bar = 100 µm. Right: example of a CCM image. Red traced nerve fibers: CNFD (main nerve fibers); blue traced fibers: CNFL (branched nerve fibers); green dots: branch points. CCM image is 400 x 400 µm. Lower panel: Schematic representations of the main structures in the upper skin and of a typical CCM image. Left: visualization of how IENFs are quantified. The nerve fibers (green) must be visible at both upper dermis and epidermis and therefore intersect the basement membrane. Axonal swellings are represented on some of the IENFs (definition of axonal swellings varies among studies, e.g. enlargements exceeding 1.5µm in diameter, or enlargements at least three times the diameter of the afferent nerve fiber). Middle: visualization of how epidermal NFLD (red boxes) and dermal NFLD (blue boxes) is quantified using stereology. Right: schematic representation of a CCM image, showing main nerve fibers (red), branched nerve fibers (blue) and branch points (white dots). From P. Karlsson et al., Pain 2018, epub ahead of print (reproduced with permission from Wolters Kluwer Health, Inc).

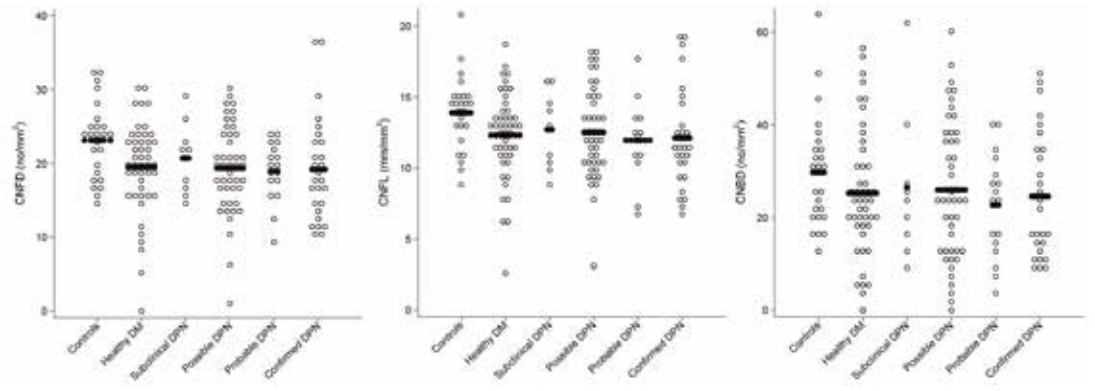


Figure 2. CNFD, CNFL and CNBD values in healthy controls and in subgroups of patients with diabetes with and without DPN.

WP5: METABOLOMICS AND LIPIDOMICS

In this work package, the focus is on oxidative stress pathways and lipid metabolites for the identification and quantitation of the proteins and metabolites that are predictive of diabetic neuropathy. This work package is led by Professor Eva Feldman, Department of Neurology, University of Michigan, Ann Arbor (US).

WP5: METABOLOMICS AND LIPIDOMICS

Eva Feldman's Laboratory at the University of Michigan, Ann Arbor uses a systems approach employing transcriptomics, metabolomics and metabolic flux analysis to identify nerve-specific differences in type 1 (T1D) and 2 diabetic (T2D) mice and man. Our progress in diabetic neuropathy can be divided into two parts: our clinical studies in man and our basic mechanistic work in multiple murine models of the disorder.

CLINICAL STUDIES IN MAN

In studies of 102 obese participants and 53 lean controls, the prevalence of neuropathy was 3.8% in lean controls (n=2), 11.1% in the obese participants with normoglycemia (n=5), 29% in the obese participants with prediabetes (n=9) and 34.6% in the obese participants with T2D (n=9) ($P<.01$ for trend). This study showed that the prevalence of neuropathy is high in obese individuals and that diabetes, prediabetes and obesity are the likely metabolic drivers of this neuropathy.

In a recent study to address the question of metabolic drives for neuropathy, we assessed diabetic neuropathy only (DPN) longitudinally in the Danish arm of the Anglo-Danish-Dutch study of Intensive Treatment of Diabetes in Primary Care (ADDITION). Of the total cohort of 1,533 people, 1,445 completed the MNSIQ at baseline, and 189 (13.1%) had DPN at baseline. The cumulative incidence of DPN was 10% during 13 years of diabetes. Age, weight , waist circumference, BMI , methylglyoxal, HDL cholesterol and LDL cholesterol at baseline were significantly associated with the risk of incident DPN. This study provides further epidemiological evidence for obesity and components of the metabolic syndrome as risk factors for DPN.

In summary, our clinical studies support our contention that drivers of neuropathy include not only glucose but obesity and associated components of the metabolic syndrome.

MURINE MODELS OF NEUROPATHY

Nerve Metabolism in Diabetes

In order to understand how hyperglycemia and components of the metabolic syndrome contribute to nerve injury in diabetes, we examined glucose and fatty acid metabolism in nerves from murine models of T2D with DPN. Metabolic flux studies revealed a ratelimiting reduction in glucose uptake or glycolytic enzyme activity in diabetic nerve, and fatty acid flux studies demonstrated an incomplete mitochondrial β-oxidation in the nerve in T2D. These data suggest an overall decrease in energy metabolism in diabetic nerve in T2D. This in turn results in insufficient energy production to maintain normal nerve function, with subsequent nerve injury and the signs and symptoms of DPN.

As a next step, we performed shotgun lipidomics to determine the differences in plasma and nerve lipid profiles in murine models of T2D and DPN compared to control animals. One hundred and thirty three (25.9%) of the plasma lipids were unique to diabetes and 258 (49.7%) were unique to diabetic nerves. These lipids were primarily long chain triacylglycerols, further supporting the concept that incomplete mitochondrial β-oxidation of fatty acids occurs in the nerve in response to T2D and likely mediates nerve injury.

In summary, these data suggest that T2D impairs nerve metabolism of both glucose and fatty acids, impairing both glycolysis and β-oxidation, leading to mitochondrial damage and insufficient energy for normal nerve function.

Mitochondrial function in DPN

To better understand mitochondrial function in T2D, we treated primary adult mouse dorsal root ganglion (DRG) neurons with physiologically relevant concentrations of the saturated fatty acid palmitate and glucose to evaluate the impact of hyperlipidemia and hyperglycemia on mitochondrial transport, depolarization and bio-energetics. Treatment with physiologically relevant concentrations of palmitate significantly reduced the number of motile mitochondria in DRG axons, while physiological concentrations of glucose did not impair mitochondrial trafficking dynamics. Palmitate-treated DRG neurons exhibiting a decrease in motile mitochondria also showed a reduction in mitochondrial velocity. Moreover, there

was decreased ATP production in palmitate treated DRG neurons along with mitochondrial depolarization. Collectively, these results suggest that saturated fatty acids induce DRG neuron mitochondrial depolarization, inhibiting axonal mitochondrial trafficking and decreasing ATP production.

In a second approach to understand mitochondrial function, we used the Seahorse Analyzer to assess changes in mitochondrial respiratory chain function in the DRG neurons (proximal) and sural nerve tissue (distal) of two mouse models of peripheral neuropathy: the 60% High Fat Diet (HFD) mouse model of obesity and prediabetes and the BLKS-db/db model of T2D. Resting mitochondrial oxidative metabolism was upregulated in DRG neurons from mice with neuropathy, with increased resting ATP production and maintained mitochondrial coupling. In contrast, resting ATP generation was decreased in sural nerve from mice with neuropathy, with decreased coupling efficiency. These data suggest a change in absolute number and function of sural nerve mitochondria and a conserved cross-model proximaldistal bioenergetic profile in peripheral neuropathy. We are currently exploring the relationship between these changes and neuropathy pathogenesis.

TRANSCRIPTOMICS IN DPN

In our transcriptomics studies, we found that multiple immune molecules were upregulated at both early and later stages of disease (PMC5955815).

Finally, we reanalyzed previously published DN-related microarray datasets from human and multiple murine models using a unified analysis pipeline. Eight microarray datasets on peripheral nerve samples from murine models of T1D (STZ-treated) and T2D (db/db and ob/ob) diabetes of various ages and human participants with non-progressive and progressive DPN were collected in effort to identify a possible disease mechanism conserved between various models and humans with DPN. Differentially expresses genes (DEGs) were identified between non-diabetic and diabetic samples in murine models and non-progressive and progressive human samples using a unified analysis pipeline. A transcriptional network for each DEG set was constructed based on literature-derived genegene interaction information. Seven pairwise human-vs-murine

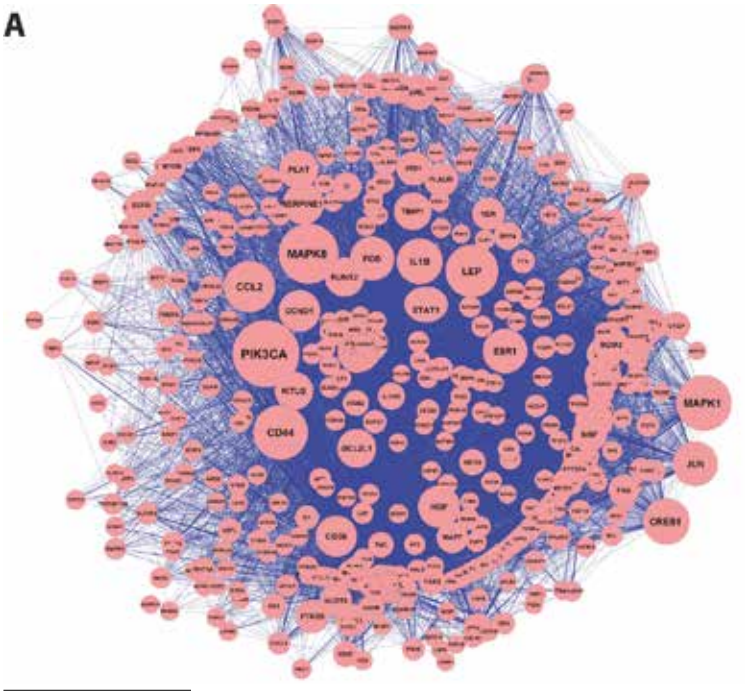
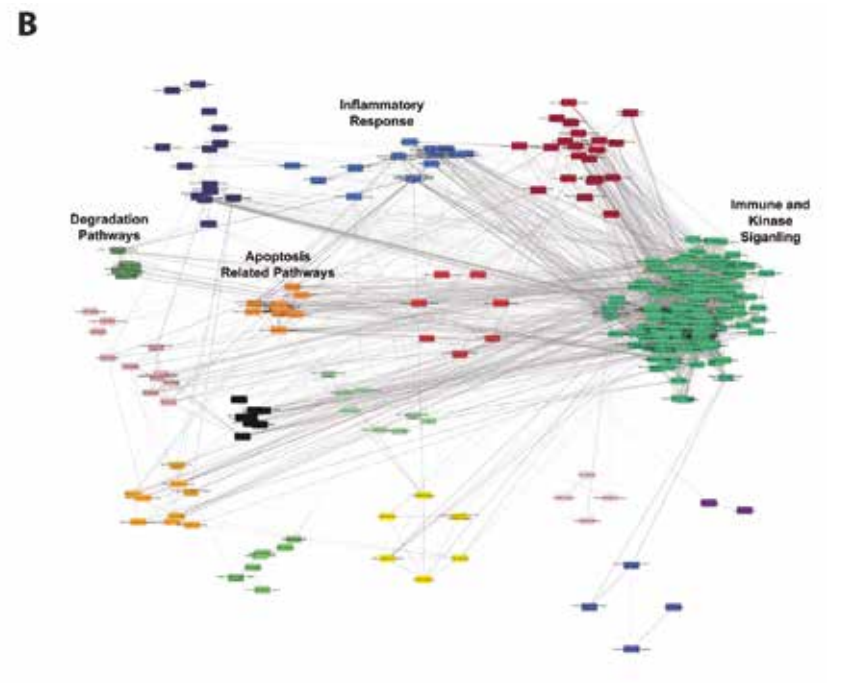


Figure 1. Conserved gene network and IPA enriched pathways. (A) A transcriptional network was generated combining the seven cross-species shared transcriptional networks. Size indicate the number of connected genes. (B) The most central 64 genes from (A) were subjected to functional enrichment analysis using IPA. The network illustrates the gene-content similarity of 272 significantly enriched canonical pathways of these central genes. The largest cluster of canonical pathways is associated with immune response and inflammation (in green). Colors of the node denote the clusters identified by InfoMap.

comparisons using a network-comparison program resulted in shared sub-networks including 46 to 396 genes, which were further merged into a single network of 688 genes (Fig. 1A). Pathway and centrality analyses revealed highly connected genes and pathways including LXR/RXR activation, adipogenesis, glucocorticoid receptor signaling and multiple cytokine and chemokine pathways. The top 50 genes ranked within our centrality measures of closeness, betweenness, degree and eigenvector totaled 64 genes, representing the most centrally connected genes within our network.

Mitochondrial Function: Our transcriptomic data also lead us to examine whether mitochondrial uncoupling alters progression of DPN; we completed one large animal study examining the effect of the mitochondrial uncoupler, niclosamide ethanolamine (NEN), on DPN. Male BKLS-db/db and db/+ controls were fed control or NEN chow 6-12 wk and 6-24 wk. Metabolic and DPN phenotyping occurred at 12/24 wk. T2D was assessed via body weight, fasting blood glucose and glycated hemoglobin. Unexpectedly, NEN treatment did not lower body weight or hyperglycemia in db/db mice, and treatment did not prevent the development of DPN. Those data suggest that directly targeting mitochondrial function with uncoupling drugs is unlikely to provide therapeutic benefit for DN.

We continue to pursue the overarching hypothesis that fatty acid uptake, lipid oxidation and lipid biosynthetic pathways are dysregulated by diabetes in the peripheral nervous system and that elucidation of these altered pathways will provide new mechanism-based therapeutic targets for neuropathy prevention and treatment. During the first three years of IDNC funding, we completed clinical studies that support the idea that components of the metabolic syndrome converge with hyperglycemia to mediate nerve injury and DPN. In murine studies, we have used a combination of gene expression analysis, steady-state and dynamic fluxomics and lipidomics and have discovered a down-regulation of energy metabolism in the peripheral nerve in mice with diabetes and DPN. Specifically we have discovered a functional downregulation of glucose and lipid metabolism, leading to energy failure as the likely underlying pathogenesis of DPN.



EDUCATIONAL ACTIVITIES AND NETWORKING

Educational activities and networking continue to constitute important elements of the IDNC. IDNC events are announced on the consortium's webpage (www.idnc.au.dk), university websites and mailing lists and are generally open to everyone interested – free of charge.

INTERNATIONAL SYMPOSIUM
7-8 June 2018 Aarhus Denmark
IDNC Annual Meeting 2018
Diabetic neuropathy and the way forward
The IDNC Annual Meeting 2018 took place in Aarhus on 7-8 June 2018 with an attendance of 90 national and international researchers, including a large group of young participants.

GUEST LECTURES

The role of fat in diabetic neuropathy
Brian Callaghan, University of Michigan, Ann Arbor (USA)

Lipid changes in diabetic neuropathy
Eva Feldman, University of Michigan, Ann Arbor (USA)

New molecular mechanisms in diabetic neuropathy
Douglas Zochodne, University of Alberta (Canada)

Are Schwann cells critical for diabetic neuropathy?
Nadia Gonçalves, Aarhus University (DK)

Antiinflammatory effects of exercise in diabetes
Bente Klarlund Pedersen, Rigshospitalet (DK)

Exercise as therapy for diabetic neuropathy
John R. Singleton, University of Utah (USA)

Effect of exercise in neurological disease
Ulrik Dalgas, Aarhus University (DK)

Diabetic neuropathy staging
A. Gordon Smith, Virginia Commenhealth Universty (USA)

The diabetic foot
Solomon Tesfaye, Sheffield Teaching Hospitals (UK)

Imaging in peripheral diabetic neuropathy
Henning Andersen, Aarhus University (DK)

The PiNS Study
Andreas Themistocleous, University of Oxford (UK)

Clinical and experimental assessment of autonomic neuropathy
Roy Freeman, Harvard Medical School (USA)

What is known/unknown about cardiac autonomic neuropathy?
Rodica Pop-Busui, University of Michigan (USA)

Heart rate variability in diabetes
Jesper Fleischer, Aarhus University (DK)

Peripheral and central autonomic disturbances
Astrid Juhl Terkelsen, Aarhus University Hospital (DK)

The philosophy behind Steno Diabetes Centers
Jannik Hilsted, Novo Nordisk Foundation (DK)

The Steno Diabetes Centers in Denmark: From traditional structures to cross-sectorial collaboration
Allan Flyvbjerg, Steno Diabetes Center Copenhagen (DK)

Steno Diabetes Center Aarhus: Current status
Troels Krarup-Hansen, Steno Diabetes Center Aarhus (DK)

The IDNC Annual Meeting 2018, Aarhus DK


IDNC
TYPE 2 DIABETES:


Radisson blu
SCANDINAVIA HOTEL
AARHUS

GUEST VISITS

Robert Helme, Honorary, Medicine
Royal Melbourne Hospital
July-August 2017

Pain Specialist Danita Weeracharoenkul
Associate Professor Supranee Niruthisard
Faculty of Medicine Chulalongkorn University King Chulalongkorn Memorial Hospital Bangkok 10330, Thailand
June 2018

RESEARCH STAYS ABROAD

Pall Karlsson
Skin Biopsy Lab, Telese Terme IRCCS, Telese Terme, Italy
September 2017 and June 2018

Sif Gylfadottir
deCODE genetics, Reykjavik, Iceland
May and June 2018

AWARDS AND PRIZES

Andreas Themistocleous
NIAA Research Award, The National Institute of Academic Anaesthesia, 2018.

Troels Staehelin Jensen
Honorary Member of the International Association for the Study of Pain.
September 2018

Anders Stouge
Best oral presentation at Neurodiab 2018.

KEY NOTE LECTURES

Troels Staehelin Jensen
Diabetic neuropathy: diagnosis and staging
Indonesia Neurological Society, Pekanbaru, Sumatra, Indonesia, 12-14 October 2017

Painful neuropathy diagnosis and treatment
Faculty of Medicine Chulalongkorn University King Chulalongkorn Memorial Hospital Bangkok, Thailand, 24 January 2018

Neuropathic pain and diabetes
Department of Anesthesiology, University of Santo Tomas, Manila, Philippines, 21-24 March 2018

SCIENTIFIC MEETINGS AND TEACHING ACTIVITIES

Members of the IDNC gave lectures and poster presentations at numerous key national and international scientific meetings and courses on pain and diabetes in 2017/2018, including the following:

- **American Diabetes Association's 77th Scientific Sessions**
  San Diego, California, USA, 9-13 June 2017
- **16th European Congress of Clinical Neurophysiology**
  Budapest, Hungary, 30 August to 2 September 2017
- **EFIC Pain Congress**
  Copenhagen, Denmark, 6-9 September 2017
- **Neurodiab 2017**
  Coimbra, Portugal, 9-11 September 2017 TSJ key note speaker
- **53rd EASD Annual Meeting of the European Association for the Study of Diabetes**
  Lisbon, Portugal, 11-15 September 2017
- **1st Annual Research Meeting, Department of Clinical Medicine**
  Aarhus University, 14 November 2017
- **9th Nordic EMBL Partnership Meeting**
  Oslo, Norway, 11-14 September, 2018
- **31st International Congress of Clinical Neurophysiology**
  Washington DC USA, 1-6 May 2018
- **Neurodiab 2018**
  Rome, Italy, 4-7 September 2018
- **IASP World Congress on Pain**
  Boston, USA,12-16 September 2018

PUBLICATIONS

PEER-REVIEWED PUBLICATIONS

1. Andersen ST, Witte DR, Andersen H, Bjerg L, Bruun NH, Jørgensen ME, Finnerup NB, Lauritzen T, Jensen TS, Tankisi H, Charles M. Risk-factor trajectories preceding diabetic polyneuropathy: ADDITION-Denmark. Diabetes Care 2018; 41: 1955-1962.

2. Andersen ST, Witte DR, Dalsgaard EM, Andersen H, Nawroth P, Fleming T, Jensen TM, Finnerup NB, Jensen TS, Lauritzen T, Feldman EL, Callaghan BC, Charles M. Risk Factors for Incident Diabetic Polyneuropathy in a Cohort With Screen-Detected Type 2 Diabetes Followed for 13 Years: ADDITION-Denmark. Diabetes Care 2018; 41: 1068-1075.

3. Andersen ST , Grosen K, Tankisi H, Charles M, Andersen NT, Andersen H, Petropoulos IN, Malik R, Jensen TS, Karlsson P. Corneal confocal microscopy as a tool for detecting diabetic polyneuropathy in a cohort with screen-detected type 2 diabetes: ADDITION-Denmark. J Diabetes Complications 2018. (accepted)

4. Blesneac I, Themistocleous AC, Fratter C, Conrad LJ, Ramirez JD, Cox JJ, Shillo PR, Tesfaye S, Rice ASC, Tucker SJ, Bennett DLH. Rare Nav1.7 variants associated with painful diabetic peripheral neuropathy. PAIN 2018: 159 : 469-480.

5. Callaghan BC, Burke JF, Kerber KA, Albers JW, Feldman EL. Electrodiagnostic tests are unlikely to change management in those with a known cause of typical distal symmetric polyneuropathy. Muscle Nerve 2017; 56: E25.

6. Galosi E, La Cesa S, Di Stefano G, Karlsson P, Fasolino A, Leone C, Biasiotta A, Cruccu G, Truini A. A pain in the skin. Regenerating nerve sprouts are distinctly associated with ongoing burning pain in patients with diabetes. Eur J Pain 2018 Jun 8. [Epub ahead of print]

7. Gonçalves NP, Vægter CB, Pallesen LT. Peripheral Glial Cells in the Development of Diabetic Neuropathy. Review. Front Neurol 2018; 9: 268.

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