INTERNATIONAL DIABETIC NEUROPATHY CONSORTIUM

ANNUAL REPORT 2016-2017

AARHUS UNIVERSITY

SOLIDUM PETIT IN PROFUNDIS · UNIVERSITAS ARHUSIENSIS

novo nordisk fonden

The International Diabetic Neuropathy Consortium was awarded a grant of 60 million Danish Kroner for a 6-year period from the Novo Nordisk Foundation in December 2014.

Novo Nordisk Foundation: Grant number NNF14OC0011633

TABLE OF CONTENTS

PREFACE 5

IDNC AT A GLANCE 6

ORGANIZATION 10

RESEARCH GROUPS 12

WP1: ANIMAL MODELS OF DIABETIC NEUROPATHY 14

WP2: HYPOXIC NERVE DAMAGE 18

WP3: RISK FACTORS FOR TYPE 2 DIABETIC NEUROPATHY 20

WP4: CLINICAL PROFILE 24

WP5: METABOLOMICS AND LIPIDOMICS 34

EDUCATIONAL ACTIVITIES AND NETWORKING 38

PUBLICATIONS 42

> Diabetic neuropathy is a neglected complication of diabetes that needs to be detected before it is too late.

PREFACE

I am pleased to present the second annual report of the International Diabetic Neuropathy Consortium (IDNC). The IDNC was launched in 2015 as a joint collaboration between the universities of Aarhus (DK), Southern Denmark (DK), Michigan, Ann Arbor (US), and Oxford (UK) to study diabetic neuropathy.

There are several good reasons to study diabetic neuropathy. Neuropathy is one of the main complications of diabetes: it carries an increased morbidity and mortality and is associated with a high social and economic burden to society. In particular, type 2 diabetes represents a major challenge, and there is a clear need to study the mechanisms giving rise to diabetic neuropathy to better diagnose, treat, and hopefully prevent the condition.

The IDNC focus on five major areas in relation to diabetic neuropathy:

- Animal models
- Vascular changes
- Epidemiology
- Clinical profiling
- Metabolomics and lipidomics

Moreover, a research program coordinated by the research group at Oxford University has made it possible to include genetic aspects of diabetic neuropathy.

Members of the IDNC have actively participated at key national and international scientific meetings and two Denmark-based postdocs and a PhD student have had research stays at the University of Michigan, Ann Arbor. Furthermore, we have had several and further planned educational activities with healthcare providers in Denmark and abroad.

In the year to come, the IDNC will seek to strengthen its educational activities, including a specific educational/preventive program in association with Danish podiatrists.

Some of the research areas represent challenges, but we are confident that it will be possible to provide new insights into the pathophysiology of diabetic neuropathy by this new way of international collaboration.

I would like to give thanks to all collaborators of the IDNC for their enthusiasm, and to our international scientific advisory board and Aarhus University for their ongoing support and help.

Troels Staehelin Jensen
Professor, Director of the IDNC

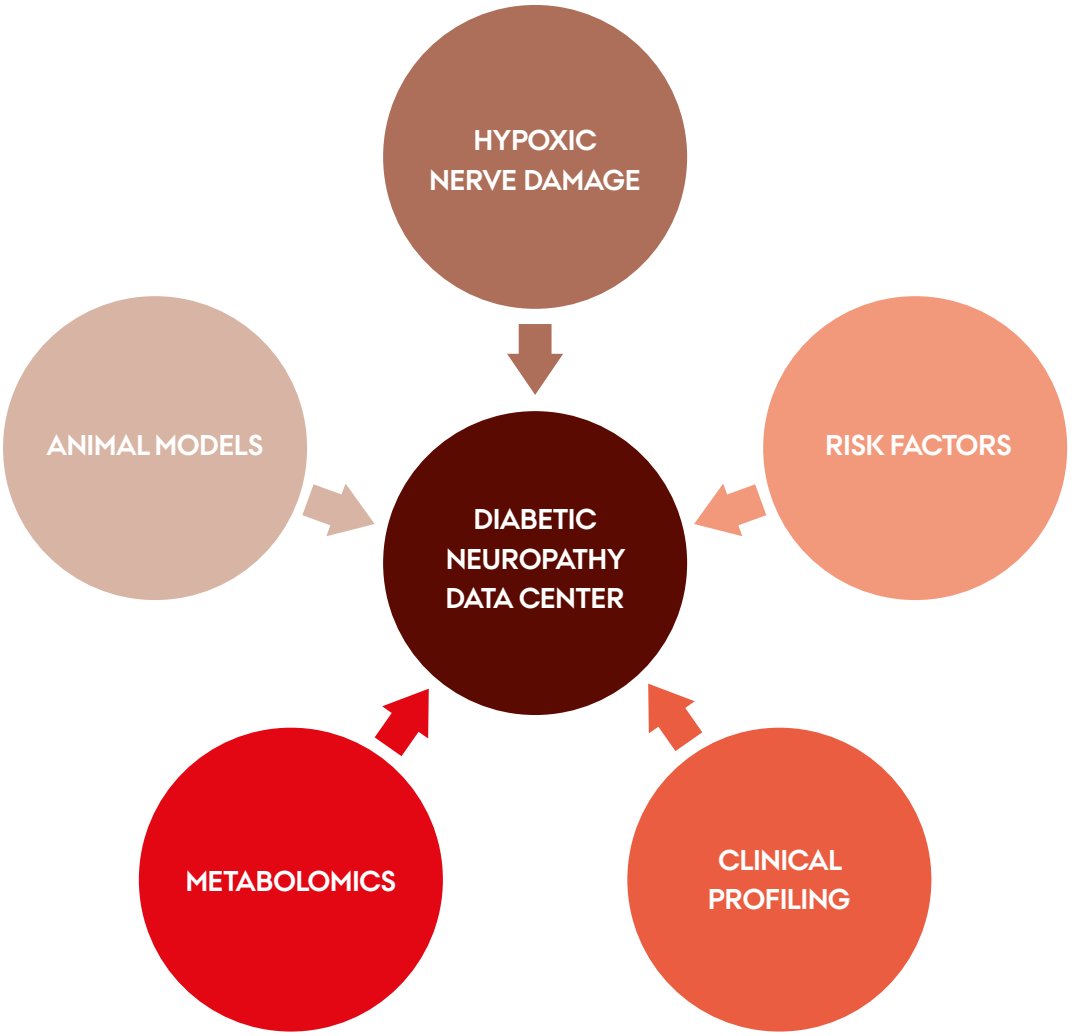
IDNC AT A GLANCE

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

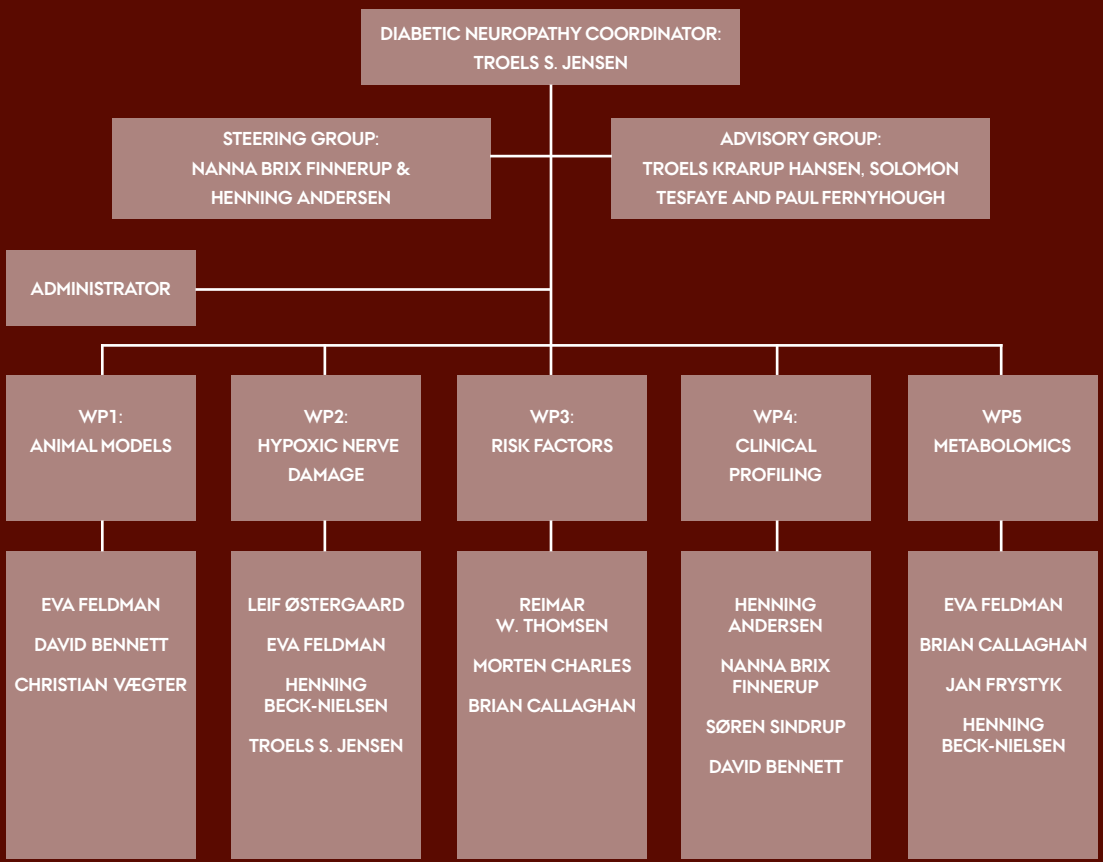
Mission: To study and unravel pathophysiological mechanisms of diabetic neuropathy, contribute to early identification, improve, and develop a uniform international classification in order to better treat and prevent the detrimental consequences of diabetic neuropathy. The IDNC does so by bringing researchers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

IDNC Summer Meeting 2016, Helnan Marselis Hotel, 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.

DIRECTOR

PROFESSOR TROELS STAEHELIN JENSEN
Director of the INDC,
Aarhus University Hospital,
DK.

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Aarhus University, DK.

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Aarhus University Hospital,
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SCIENTIFIC ADVISORY BOARD

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Chairman
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PROFESSOR PAUL FERNYHOUGH
University of Manitoba,
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HEALTH SYSTEM
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UNIVERSITY OF MICHIGAN

Department of Neurology
Eva L. Feldman
Brian Callaghan

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Aarhus University Hospital

AARHUS UNIVERSITY AND AARHUS UNIVERSITY HOSPITAL

Department of Public Health
Morten Charles
Signe Toft Andersen

Department of Clinical Epidemiology
Reimar Thomsen
Diana Hedevang Christensen

Center of Functionally Integrative Neuroscience and MINDLab
Leif Østergaard
Anete Dudele

Department of Neurophysiology
Hatice Tankisi
Alexander Gramm Kristensen

Department of Biomedicine
Christian Vægter
Nadia Pereira Goncalves

Department of Neurology
Karolina Snopek
Anders Stouge

Comparative Medicine Lab
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Martin Nors Skov

Danish Pain Research Center
Pall Karlsson
Sandra Sif Gylfadottir
Astrid J. Terkelsen

IDNC Administration and Technical Staff at Danish Pain Research Center
Helle O. Andersen
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UNIVERSITY OF OXFORD

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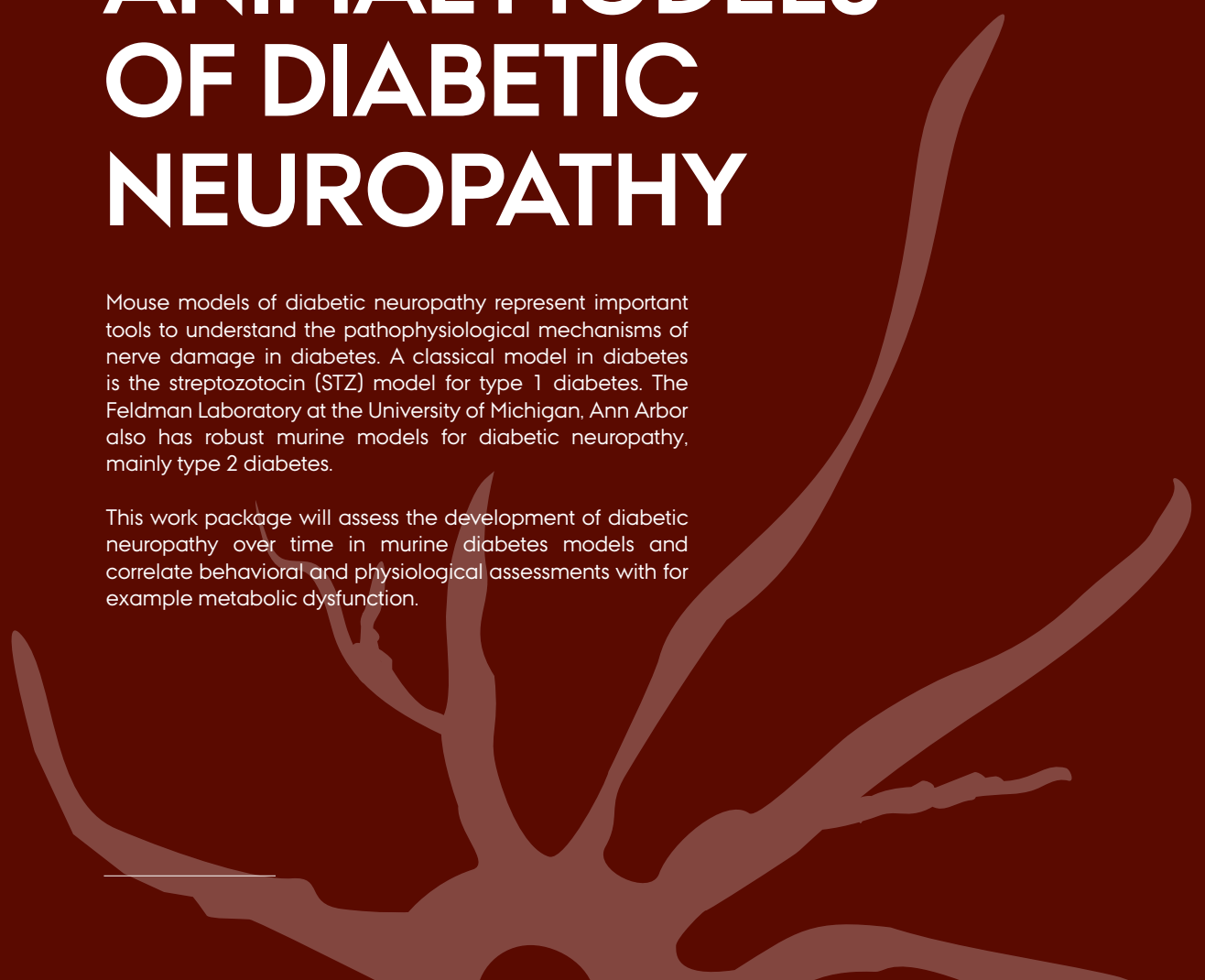
Department of Endocrinology
Henning Beck-Nielsen
Jan Frystyk

Department of Neurology
Søren Sindrup
Mustapha Itani
Thomas Krøigaard

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. The Feldman Laboratory at the University of Michigan, Ann Arbor also has robust murine models for diabetic neuropathy, mainly type 2 diabetes.

This work package will assess the development of diabetic neuropathy over time in murine diabetes models and correlate behavioral and physiological assessments with for example metabolic dysfunction.



WP1: SCHWANN CELLS AND THEIR ROLE IN DIABETIC NEUROPATHY

Nadia Goncalves *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).*

There is a huge gab in our understanding of how glial cells of the peripheral nervous system respond to diabetic conditions. We propose Schwannopathy as an integral factor in the pathogenesis of diabetic neuropathy (See Goncalves et al. Nat Rev Neurol 2017).

Utilization of a Schwann cell specific p75NTR knock out mouse model in combination with induced type 2 diabetes allows us to investigate how the neurotrophin receptor p75NTR in Schwann cells affects the cells under diabetic conditions and how this affects the progression of diabetic neuropathy. 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 diabetic neuropathy mechanisms and novel approaches to target reversal of diabetic neuropathy symptoms.

The role of p75NTR receptor signaling in neuron-Schwann cell communication and mye myelination under in vitro diabetic conditions are investigated with primary Schwann cell-neuron co-cultures, following the protocol learned by Nadia Goncalves during a research visit to the University of Melbourne. The methodology was established and preliminary results highlight a compromised ability of Schwann cells to myelinate axons under hyperglycemic conditions *(Fig. 1)*. A next step will be to understand whether p75NTR expressed in Schwann cells can modulate the production of myelin in conditions resembling diabetes, and whether demyelination caused by exposure to high levels of glucose can be prevented or rescued by neurotrophic factors.

For the in vivo work package associated with this project, Nadia Goncalves visited Professor Eva Feldman's lab at the University of Michigan, Ann Arbor for two weeks to learn how to characterize diabetic neuropathy mouse models. Wild-type and p75NTR Schwann cell conditional knock out mice were fed with a high fat diet containing 60% lard for 24 weeks to induce type 2 diabetes. Testing of the wild-type animals revealed that mice fed with the high fat diet develop hyperglycemia, mechanical allodynia and present decreased sensory and motor conduction velocity as compared with mice fed with a control diet, together indicating the presence of diabetic neuropathy. p75NTR transgenic mice will now be characterized and at a later stage nerve morphometric and transcriptomic analysis will be performed to unravel novel molecular pathways of disease.

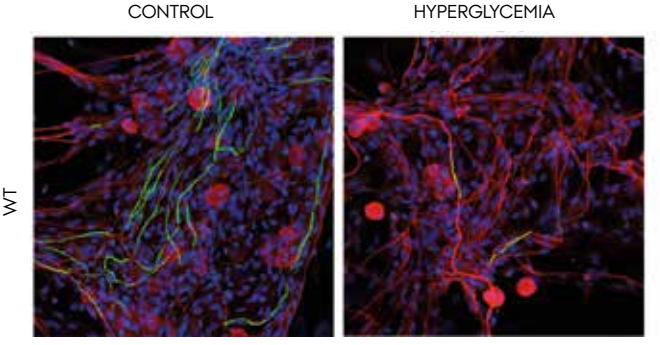


Figure 1. Compromised ability for Schwann cells to myelinate when exposed to high levels of glucose (75 mM). Myelin segments are labeled in green and axons and neurons in red.

WP1: BASIC NEUROPHYSIOLOGICAL STUDY

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

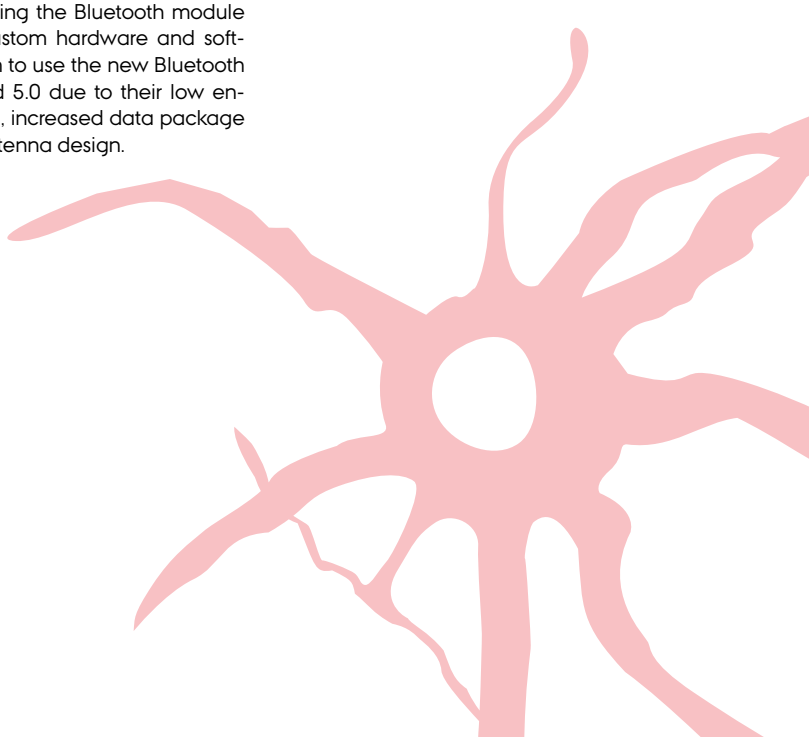
In his new project entitled: Novel telemetric approach to assess the impact of diabetes on the peripheral nervous system, Martin Nors Skov will investigate the early development of autonomic and sensory diabetic neuropathy using longitudinal electrophysiological measurements of both the sciatic sensory and renal sympathetic nerves. For the diabetic model, he will use the streptozotocindiabetic rat model. A research visit to Professor Stephen McMahon's Lab in London (UK) is planned to learn techniques for the project.

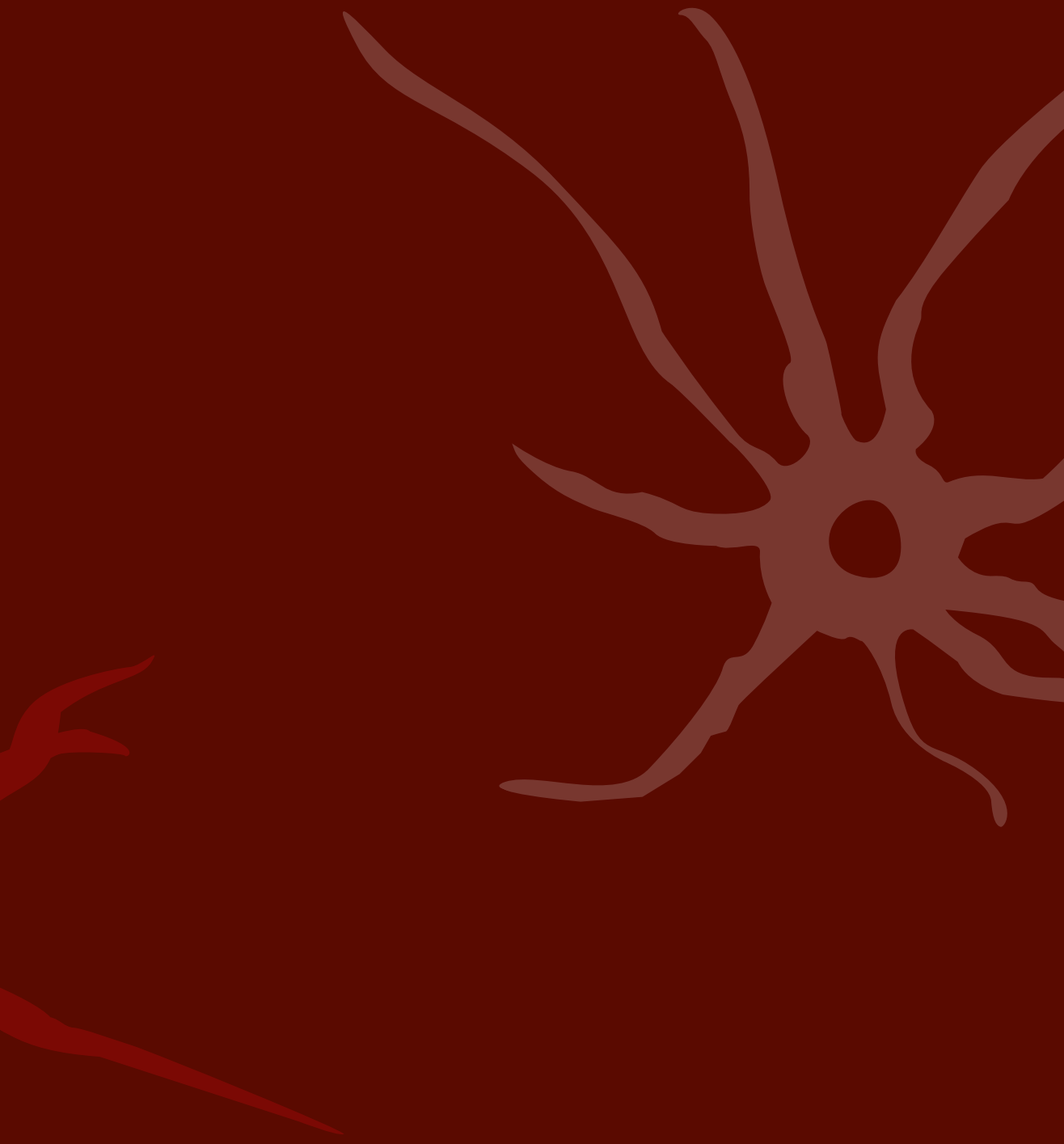
Diabetic neuropathy is a common and a debilitating condition that affects both large and small fibers and cause sensory and motor disturbances. It may also affect the autonomic nervous system, and this effect is important because it is associated with increased mortality in diabetic patients.

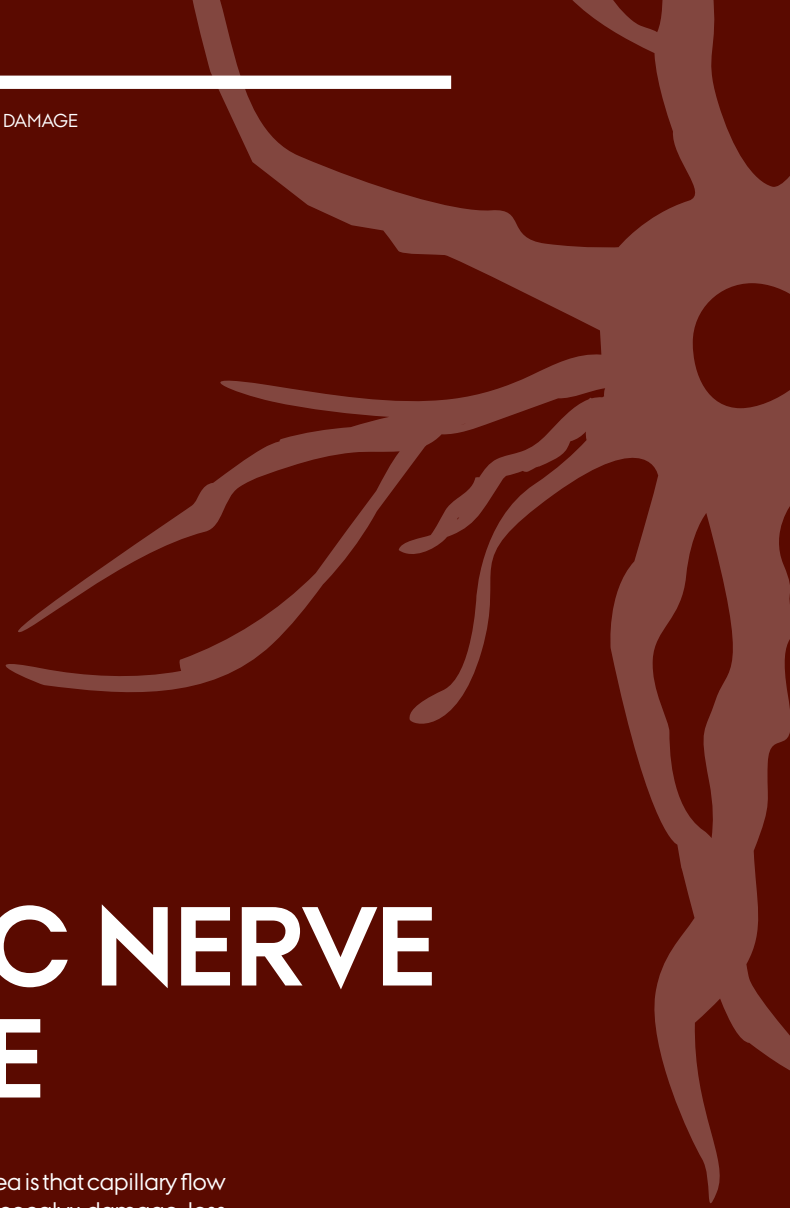
To investigate the early onset and progression of diabetic neuropathy, the first part of the project will be the development of a telemetric implant. To do this, he is in the progress of adapting the Bluetooth module "PSOC 4" with custom hardware and software. It is the plan to use the new Bluetooth standards 4.2 and 5.0 due to their low energy consumption, increased data package size, and small antenna design.

The implant will be placed in the abdominal cavity, and wires with cuff electrodes will be put on the investigated nerves. To use as little power as possible, only the most necessary data processing will be done on the chip, including analog to digital conversion and filtering. Instead, the signal will be transferred to a dedicated computer, which will do the advanced signal and data processing (with custom software). The implant is designed to work in the rat for about 9-12 weeks, with recordings taken for 30 min. twice a week.

This new project will hopefully reveal new insight to both the use of telemetric implants and the early longitudinal nerve degeneration associated with diabetic neuropathy.







WP2: HYPOXIC NERVE DAMAGE

In this challenging 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 (streptozotocin model) and type 2 models for diabetic neuropathy (db/db model and the "fat diet model") are studied using two-photon microscopy combined with optical coherence tomography (OCT). With these methods, we can test the hypothesis that elevated capillary transit time heterogeneity and reduced oxygen tension are in fact early features of diabetic neuropathy in mice.

WP2: ARE DIABETIC NERVES SUFFOCATING?

Anete Dudele

Blood serves an instrumental transport function in our bodies, carrying oxygen and nutrients to the metabolically active tissue and removing metabolic waste products. Gas and nutrient exchange between blood and tissue takes place at the level of capillaries – the smallest vessels of the body. If capillaries are damaged or do not function normally, then tissue oxygenation and nutrient exchange can be limited, which may lead to tissue damage.

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 and MINDLab, Aarhus University (DK) leads the research.

The two-photon microscopy and optical coherence tomography methods are used to visualize and quantify capillary blood flow in peripheral nerves in health and disease in real time, and thus assess the contribution of capillary dysfunction to diabetic neuropathy *(Figs. 1 and 2)*.

Preliminary work in control animals shows promising results of blood flow measures in the perineurium. To better understand the pathophysiology of diabetic neuropathy, Anete Dudele will study capillary function in nerves of type 1 and 2 diabetic animals at different time points of disease. If the capillaries are dysfunctional and contribute to initiation and progression of diabetic neuropathy, then it should be possible to proceed to investigate interventions aimed at improving capillary function.

In preliminary studies in the fat mouse model of type 2 diabetes, it has been technically difficult to get a satisfactory overview to make quantifiable measures of blood flow. The coming experiments will therefore focus on the streptozotocin model. At a later stage, these findings – if successful – will be extended to human studies in patients with type 1 and 2 diabetic neuropathy.

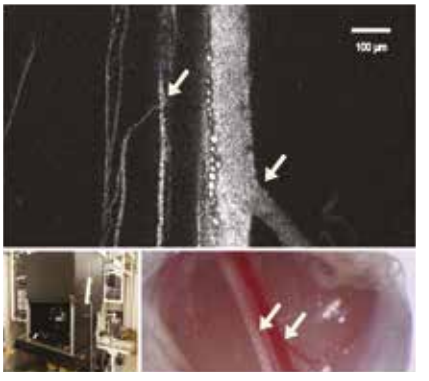


Figure 1. In vivo two photon microscopy. Sural nerve vasculature visualized in an anesthetized adult male mouse. Arrows indicate recognizable vessel branching points.

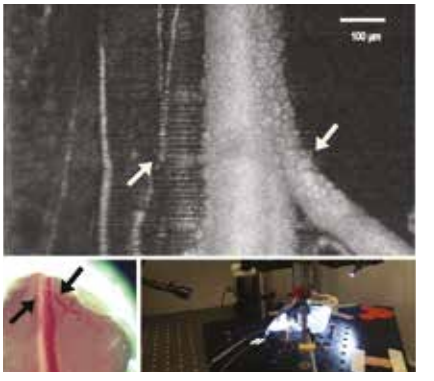


Figure 2. In vivo optical coherence tomography. Sural nerve vasculature visualized in an anesthetized C57 adult male mouse with optical coherence tomography.

WP3: RISK FACTORS FOR TYPE 2 DIABETIC NEUROPATHY

Two large cohorts: the ADDITION cohort and the DD2 cohort form 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.

The prospective Danish Centre 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. 8,000 individuals.

The DD2 cohort is pivotal in the IDNC, both for epidemiological studies and as a patient recruitment source 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 also genetic and other risk factors for the development of diabetic neuropathy and longterm follow-up.

WP3: THE ADDITION STUDY

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.*

Using the Michigan Neuropathy Screening Instrument (MNSI) questionnaire, Signe Toft Andersen has observed a low cumulative incidence of diabetic neuropathy of 10% following 13 years of type 2 diabetes. Risk factors for diabetic neuropathy included obesity, low HDL and LDL cholesterol, and oxidative stress as measured by higher levels of methylglyoxal.

Using accepted clinical diagnostic criteria, around 35% of this cohort had a probable diagnosis of diabetic neuropathy, and more than 70% had a possible diagnosis of diabetic neuropathy at the 10-year examination. Confirmative examinations of diabetic neuropathy by nerve conduction studies have been carried out in a subset of the cohort (Aarhus). These examinations show that nearly 20% of this subcohort have a confirmed diagnosis of diabetic neuropathy *(Fig. 1)*.

Data from corneal confocal microscopy (CCM) are analyzed in a subset of the ADDITION cohort in collaboration with other IDNC researchers and experts in the field of CCM *(Fig. 2)*.

As part of her IDNC program, Signe Toft Andersen has just finished a 3-month research stay at Professor Eva Feldman's Laboratory to study metabolomics and lipidomics in a subgroup of the ADDITION cohort to understand the underlying pathogenesis of diabetic neuropathy.

Future plans focus on the prevalence of diabetic neuropathy using other criteria and on the possible relationship between symptoms and signs of diabetic neuropathy.

The knowledge generated in this PhD project will provide insight into the epidemiology, risk factors, and clinical delineation of diabetic neuropathy.

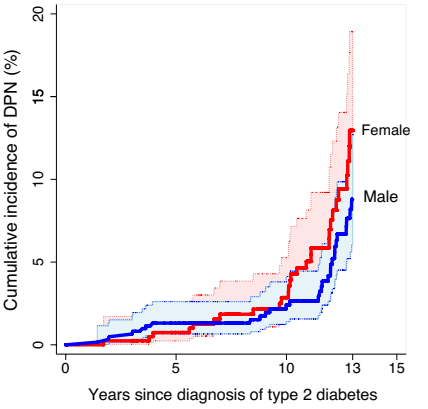


Figure 1. Cumulative incidence of diabetic neuropathy during 13 years of type 2 diabetes. Diabetic neuropathy assessed by the MNSI questionnaire in the ADDITION Denmark study.

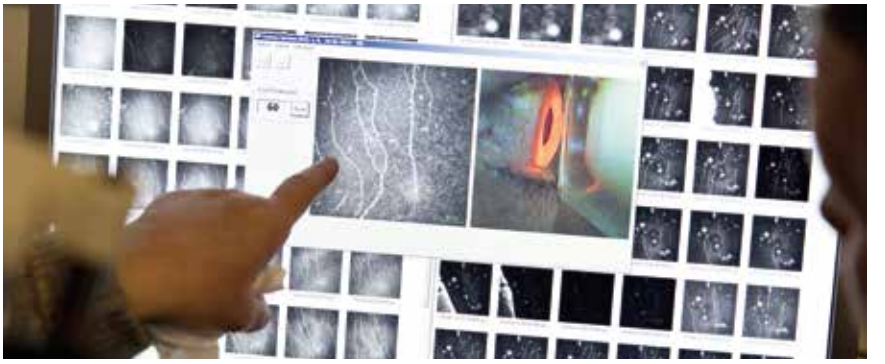


Figure 2. Cornea sections derived from corneal confocal microscopy

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.*

Diana Hedevang Christensen will explore the role of risk factors in four studies:

1) examine the association of painful/non-painful neuropathy and metabolic factors like hypertension, dyslipidemia, obesity etc;
2) generate and validate algorithms to identify diabetic neuropathy and diabetic foot ulcers in the Danish registers;
3) study the effect of lipid-lowering therapy on the risk of diabetic neuropathy and related diabetic foot disease;
4) examine the impact of glucose-lowering agents on the risk of diabetic neuropathy and related foot disease.

Study 1 is primarily based on the DD2 cohort and studies 2-4 are primarily based on Danish health care register data.

During 2016, Diana Hedevang Christensen and biostatistician Sia Kromann Nicolaisen conducted a large questionnaire survey concerning painful and non-painful neuropathy in the DD2 cohort. Thus, in June 2016, a detailed questionnaire on neuropathy and pain was sent to all enrolled DD2 patients at that time (N=6726).

A total of 85% of DD2 cohort members have provided self-reported data on neuropathy, and these data now also form the basis of ongoing recruitment of patients with neuropathy symptoms for the clinical PhD studies in WP4 of the IDNC.

Diana Hedevang Christensen has started to examine the validity of diabetic neuropathy-related outcomes in the Danish National Patient Register and the National Health Service Prescription Database: 300 random patients with coded neuropathy have been retrieved, and medical record validation at the departments is ongoing.

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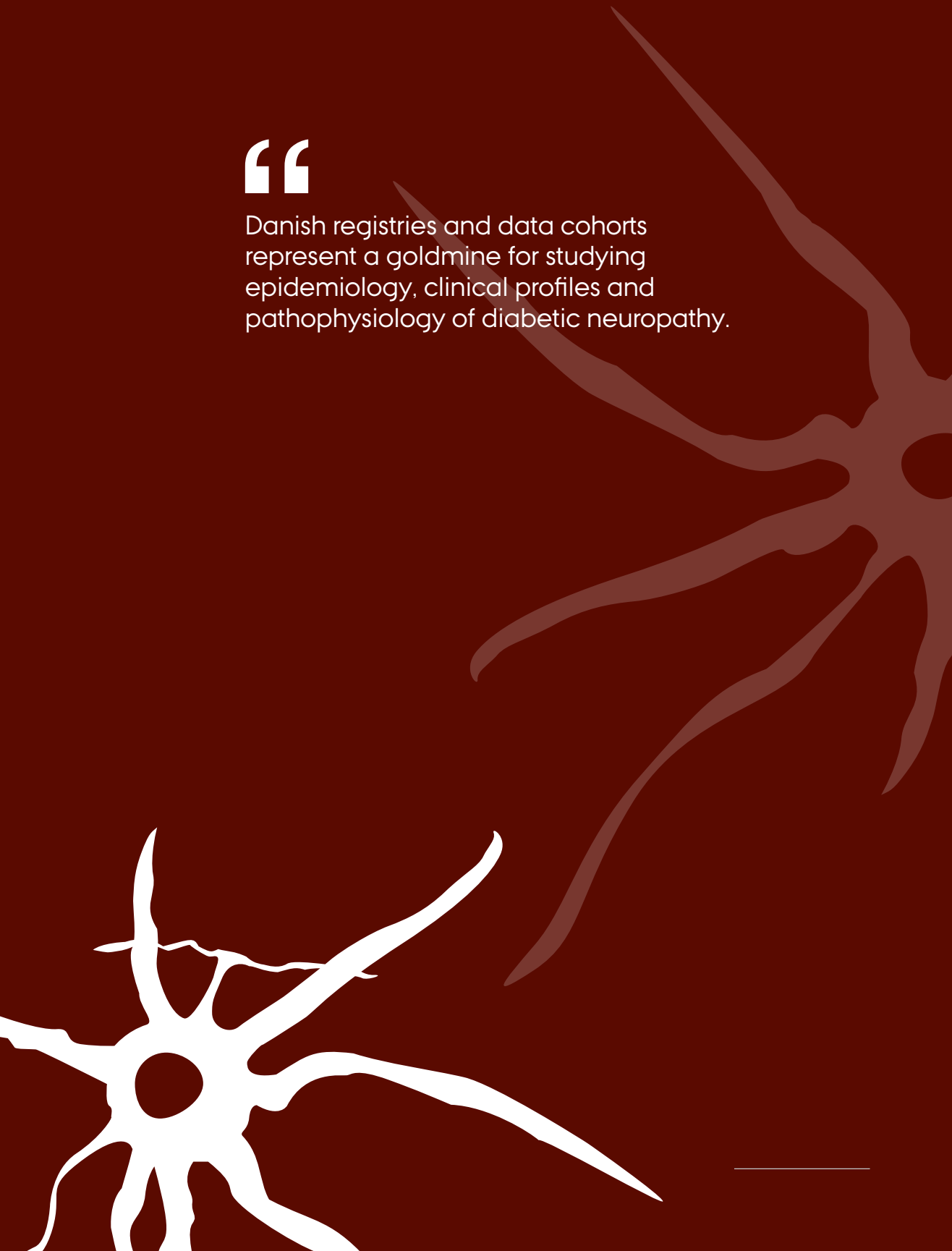
Danish registries and data cohorts represent a goldmine for studying epidemiology, clinical profiles and pathophysiology of diabetic neuropathy.



WP4: CLINICAL PROFILE

In this large work package, we will determine 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 patients with type 2 diabetes, diabetic neuropathy, and painful diabetic neuropathy. The clinical profiling involves work done at the University of Oxford (UK), South Danish University, and Aarhus University (DK). Clinical profiling in Denmark is carried out on the basis of the DD2 cohort. The examinations and profiling carried out at these 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-PHC-2014 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

Detailed sensory phenotyping and threshold tracking (as a measure of excitability) are done in patients with diabetic neuropathy at the IDNC site in Oxford. Professor David Bennett and his team at the Nuffield Department of Clinical Neurosciences, Oxford University (UK), lead the research. In the initial analysis looking at the sensory phenotype in painful and painless diabetic neuropathy, they have found a positive correlation between neuropathic pain and neuropathy severity as well as glycemic control.

As a next phase, Bennett's group is now enhancing recruitment in a manner fully aligned with Aarhus in order to reach the numbers required for a detailed genetic analysis in order to determine the role of genetics in the development of neuropathic pain. Now that they have the first data arising on neuropathic pain questionnaires from the DD2 cohort, we are making firm plans to coordinate this genetic project within both IDNC and DOLORisk. The Oxford group have already completed their first analysis showing that there is an association between rare variants in the sodium ion channel Nav1.7 and the development of neuropathic pain *(Fig. 1)*. David Bennett and his colleagues Andreas Themistocleous and Julie Blesneac have demonstrated that some of these variants display a gain of function. This work was presented at the 2017 PNS meeting for which Andreas Themistocleous won the Peter James Dyck Award.

Bennett's group has also used a cohort of patients with detailed somatosensory phenotyping as a resource to stratify patients in collaboration with other research projects. They have here collaborated with Professor Irene Tracey in Oxford to study the functional brain connectivity of the descending pain modulatory system in patients with painful versus painless diabetic neuropathy and show here altered connectivity of the ventrolateral periaqueductal gray (PAG).

Finally, Bennett's group is undertaking stereological analysis of skin biopsy samples from patients with diabetes, particularly focusing on swellings in sensory fibers in relation to sensory symptoms. This research is carried out in collaboration with Assistant Professor Pall Karlsson at the Danish Pain Research Center, Aarhus University. In summary, there have been no significant deviations from the work plan.

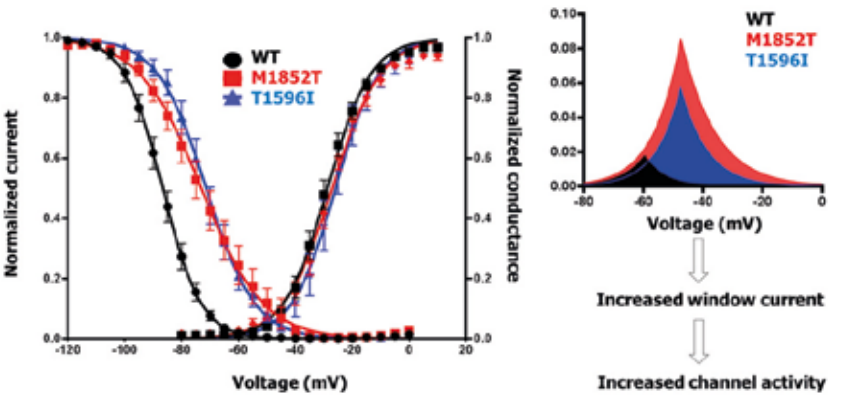


Figure 1. Electrophysiological characterization of two new genetic variants M1852T and T1596I in participants with painful diabetic neuropathy.

WP4: CLINICAL PROFILING OF DIABETIC NEUROPATHY

Mustapha Itani

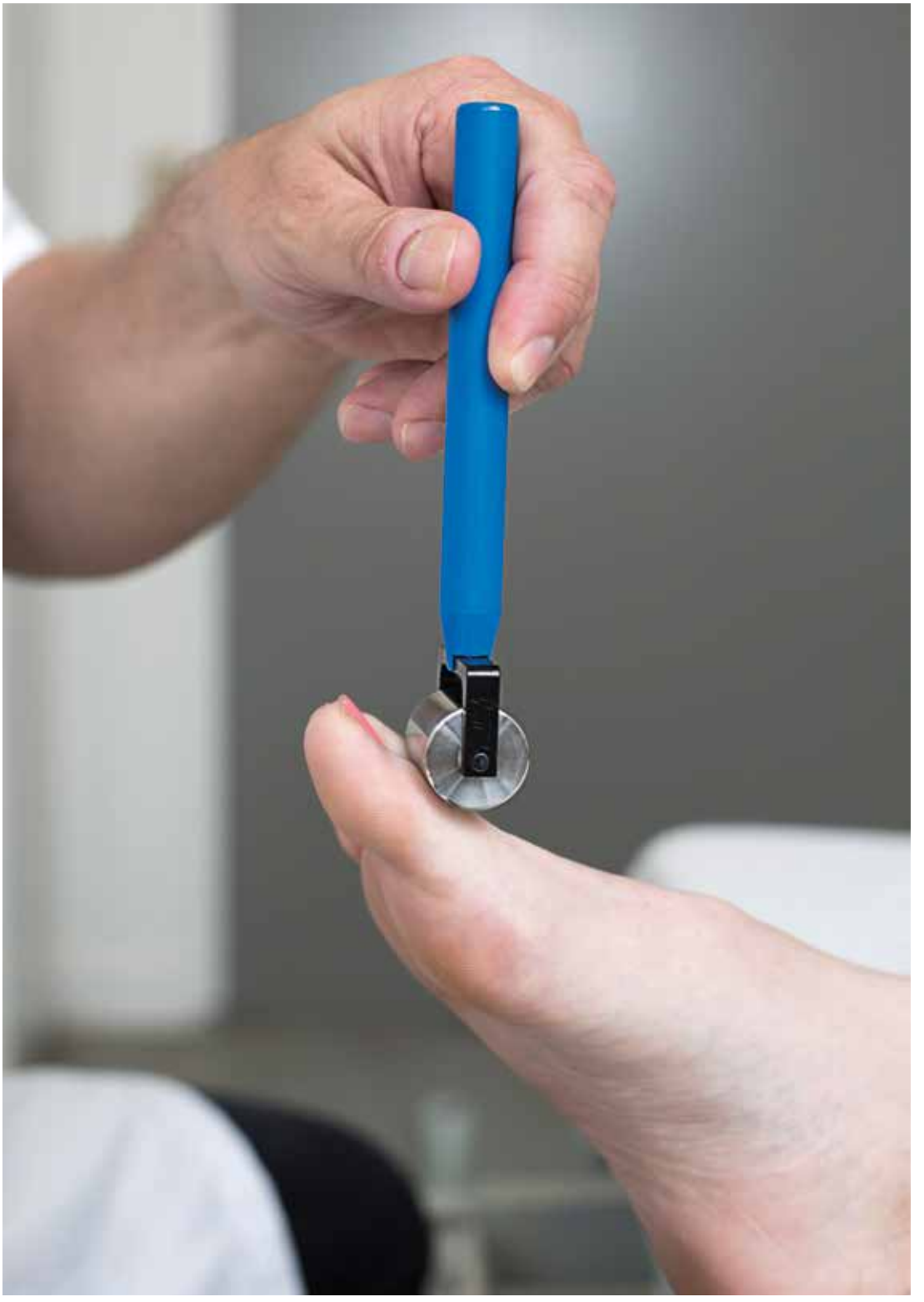
Professor Søren Sindrup and PhD student Mustapha Itani are engaged in clinical profiling of people with type 2 diabetes from the DD2 cohort with respect to neuropathy. The research is carried out at the IDNC site at the Department of Neurology, Odense University Hospital (Clinical Research, Neurology,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

As of June 2017, 70 patients with diabetes and 12 control subjects had been through the extensive examination program. During the fall of 2017, the Odense site plans to see 7-8 patients each week.

This part of the profiling focusing on distinguishing features between patients with and without neuropathy in type 2 diabetes is identical to the clinical profiling in Aarhus, where the focus is on differences and similarities between painful and painless diabetic neuropathy.

In Odense, they are also compiling comprehensive data on non-selected patients evaluated for peripheral neuropathy. A subgroup of this cohort of patients will serve as a control or comparison group for one of the clinical profiling studies in DD2 patients. This cohort presently comprises more than 300 patients, of which 120 have been evaluated and 63 have a confirmed neuropathy diagnosis.

Figure: Example of measuring touch (left) and cold (right) sensation on first toe



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.*

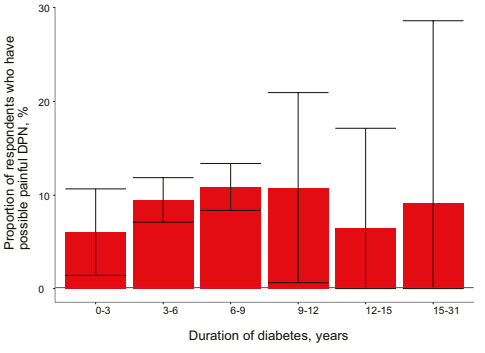
The main goal of Sandra Sif Gylfadottir's project is to:

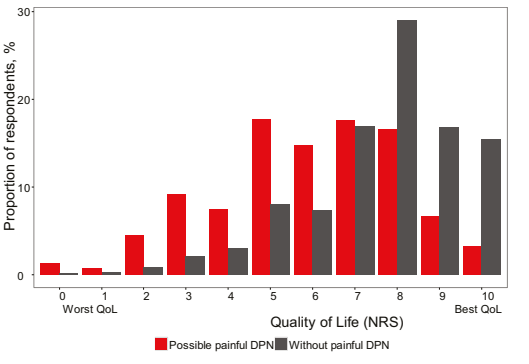
1) estimate the prevalence of painful diabetic neuropathy in Danish type 2 diabetic patients;
2) characterize the pain in diabetic neuropathy and identify subgroups of patients with specific clusters of symptoms and signs and to compare diabetic neuropathy patients with and without pain to identify abnormalities specific to pain;
3) examine morphological changes in intraepidermal nerve fibers and differentiate between patients with and without pain.

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

Inclusion started in November 2016 and so far, 82 patients and 13 healthy controls have been examined in Aarhus.

Using the DD2 questionnaire dataset, Sandra Sif Gylfadottir estimated the prevalence of possible painful diabetic neuropathy, defined as pain in both feet and a score ≥3 on the Douleur Neuropathique (DN4) questionnaire. This analysis revealed that 9.8% of DD2 questionnaire respondents had possible painful diabetic neuropathy. There was no difference in the prevalence of painful diabetic neuropathy by duration of diabetes, but patients with possible painful diabetic neuropathy had lower quality of life (See Figs.).





Sandra Sif Gylfadottir collecting samples for genetic analysis at Oxford University (top) and performing sensory examination (right) (Photos: Martin Dam Kristensen)

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).*

Diabetic patients with neuropathy may suffer from incapacitating symptoms such as muscle weakness, impaired balance, unstable gait, and falls. It is unknown whether exercise should be recommended to avoid such falls.

Karolina Snopek's studies aim to:

- describe the relationship between falls, muscle strength and signs of neuropathy in patients with type 2 diabetes;
- assess the effects of resistance training on neuropathy symptoms, muscle strength, and muscle structure;
- examine the effect of resistance training on small and large nerve fiber function, muscle strength, and muscle structure in patients with type 2 diabetes and diabetic neuropathy;
- evaluate if resistance training has an effect on the expression of nerve and muscle regenerative factors.

The inclusion of participants will start August 2017.

Using the DD2 questionnaire dataset, Karolina Snopek has looked at the association between reported neuropathy symptoms and falls *(Fig. 1)*. Thus, patients who complained of neuropathy symptoms had significantly higher odds for falls than patients without symptoms of neuropathy. Out of 5,315 DD2 questionnaire responders, 896 (17%) reported 1 or more falls over the past year. Patients with type 2 diabetes and a MNSI questionnaire score >3 had a 3- to 4-fold higher risk of falls, after adjustment for alcohol consumption, smoking, physical activity, BMI, gender, and age.

Findings from the present studies may lead to recommendations for resistance training for patients with diabetic neuropathy to improve muscle strength, postural stability, and quality of life and to alleviate symptoms of neuropathy.

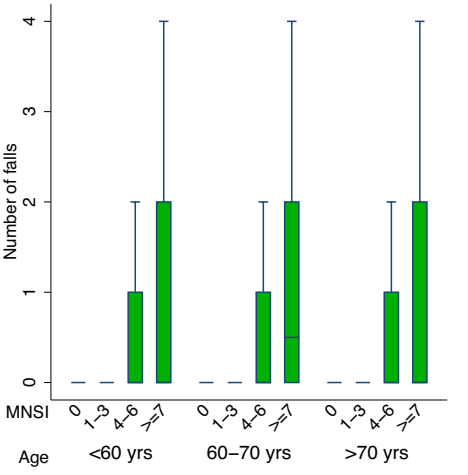


Figure 1. Graph illustrates the tendency of a higher MNSI questionnaire score if the patient had suffered from a fall in all age groups.

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.*

The aim of Alexander Gramm Kristensen's project is to:

1) find the optimal diagnostic criteria for large fiber dysfunction in diabetic neuropathy comparing comprehensive routine nerve conduction study (NCS) examinations with clinical examination;
2) understand the mechanisms of diabetic neuropathy by use of novel electrophysiological techniques such as threshold tracking as well as motor unit number estimation (MUNE) tests.

In 2016-2017, more than 100 patients have been included. Alexander Gramm Kristensen has performed routine NCS examinations and nerve excitability testing and the novel MUNE method MScanFit MUNE.

The preliminary results show that examining the distal segments of the lower extremity sensory nerves increases the sensitivity of electrophysiological diagnosis of diabetic neuropathy. However, MUNE examination in the nerves of the hand did not increase the sensitivity, probably due to the length dependent feature of diabetic neuropathy.

Alexander has ongoing collaboration with Associate Professor Carsten Dahl Mørch and Professor Ole Kæseler Andersen from the Integrative Neuroscience Group at Aalborg University (DK) to introduce a new method of nerve excitability testing suitable for examining the small nerve fibers in the upper extremity.

Evaluation of a nerve excitability assessment

WP4: AUTONOMIC NEUROPATHY IN DIABETES

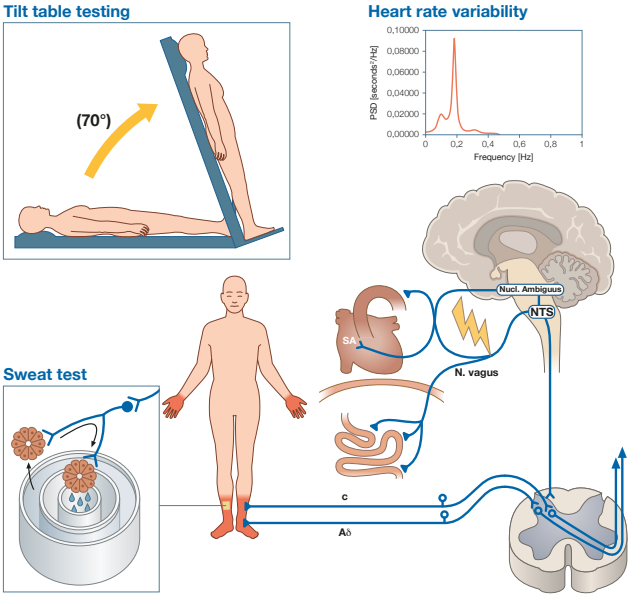
Astrid J. Terkelsen

Small nerve fibers of Aδ 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.

In the Autonomic Lab at Aarhus University Hospital headed by senior researcher Astrid J. Terkelsen, Department of Neurology, Aarhus University Hospital (DK), autonomic functions are assessed by questionnaires, e.g. COMPASS31. Small fibers are tested by sweat testing at the forearm, leg (postganglionic cholinergic sudomotor function), and quantitative sensory test *(Figure).* The generalized autonomic dysfunction is evaluated by measuring blood pressure and heart rate during different maneuvers. Blood pressure changes during 70 degrees tilting to upright position and during the Valsalva maneuver evaluate the cardiovascular adrenergic function. The cardiovagal function is tested with heart rate variability, deep respiration, and the Valsalva maneuver.

In a new study supervised by Astrid J. Terkelsen, autonomic functions in patients with type 2 diabetes, diabetic neuropathy, and painful diabetic neuropathy from the DD2 cohort will be assessed.

Diabetes and autonomic neuropathy

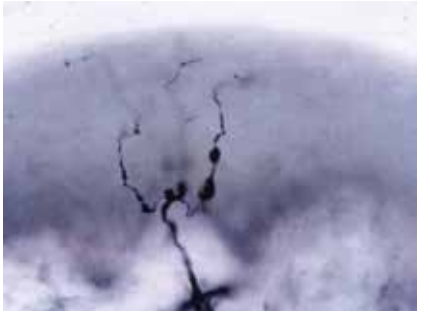


WP4: STRUCTURAL AND FUNCTIONAL ASSESSMENT IN DIABETIC NEUROPATHY

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 biopsies represent an important diagnostic tool in diabetic neuropathy, and a hallmark of diabetic neuropathy is a significant loss of these fibers *(Fig. 1)*. However, the quantification of the nerve fibers does not explain the different symptoms reported by the patients. Patients with and without painful symptoms have the same low nerve fiber density. An important step towards a better understanding of pain pathophysiology and treatment is to extract more information from the skin biopsies: to look beyond the nerve fiber density and identify biomarkers that are more useful.

Pall Karlsson recently completed a study with the overall aim to perform a detailed structural and functional analysis of the nerve fibers. The following assessments were performed to evaluate the regeneration rate of the nerve fibers: nerve conduction studies, quantitative sensory testing, a 3-mm punch skin biopsy at the distal leg, a 30-minute 10% capsaicin application at the distal leg to chemically deplete the nerve fibers, and a second and a third biopsy 24 hours and 90 days after the capsaicin application. Profound functional and structural changes were found in diabetic neuropathy patients with type 1 diabetes. In an upcoming study, Pall Karlsson will use a large panel of antibodies to identify different subtypes of nerve fibers in patients with type 1 and 2 diabetes.



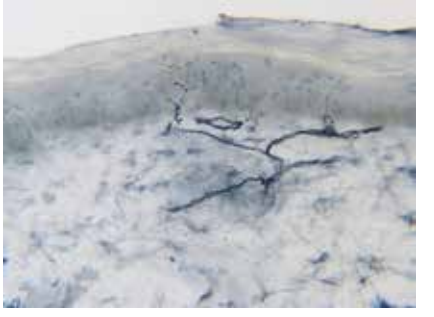


Figure 1. Top left: examples of axonal swellings. Bottom left: Representative stained biopsy section from a patient with diabetic neuropathy with almost a complete loss of nerve fibers. Right: representative stained biopsy section from a healthy individual with normal intraepidermal nerve fiber density (IENFD).

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 lead by Professor Eva Feldman, Department of Neurology, University of Michigan, Ann Arbor (US).

WP5: METABOLOMICS AND LIPIDOMICS

Eva Feldman's Laboratory continues 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.

ACCOMPLISHMENTS

As an overall theme, Eva Feldman's lab use 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. Gene-level analysis identified low levels of concordant gene expression in diabetic neuropathy (DN, 54% of 1,558 genes), between type 1 and type 2 diabetes. These results suggested that distinct pathogenic mechanisms exist in DN across diabetes type. When they next assessed DN in control and T2D mice with or without pioglitazone treatment, they discovered differential drug-mediated regulation of critical inflammatory pathways. In the same study, self-organizing map analysis revealed that mitochondrial dysfunction was normalized in the nerve by treatment; however, conserved pathways were opposite in their directionality of regulation. We piloted matrix-assisted laser desorption/ionization mass spectroscopy imaging (MALDI-MSI) and found that diabetic nerve had a significant increase in ATP/AMP ratio. We discovered decreased glucose and fatty acid metabolism in the nerve of the T2D mouse model.

The Feldman Lab have extended their studies to humans and analyzed diabetic kidney transcriptomic data and urinary metabolites from a cohort of PIMA Indians and discovered specific urine but not plasma metabolites predicted progression of diabetic kidney disease, and in this same cohort reported DN in over 80% of the subjects. So far, their published preliminary data highlight tissue-specific changes in both transcriptomics and metabolism in complication-prone tissues in diabetes and support our contention that distinct nerve-specific metabolic reprogramming in the nerve plays a critical role in DN pathogenesis. Their progress further confirms our integrated multidisciplinary approach to diabetic microvascular complications with our emphasis on DN. These data also highlight the potential dangers of a 'one size fits all' approach to T1D and T2D therapeutics.

Danish IDNC postdocs Nadia Goncalves (leftmost) and Anete Dudele (bottom left) together with members of Eva Feldman's Lab at the 2017 PNS meeting in Sitges.

WP5: PROGRESS REPORT FOR WORK PLANS

To identify dysregulated lipid metabolism in diabetic complication tissue, shotgun lipidomics using LC/MS was used to determine differences in complex lipids present in plasma and sciatic nerve, as well as kidney and retina from db/db T2D diabetic mice and db/+ normoglycemic littermate controls. Over 500 unique lipid features were detected in each sample matrix, with 364 lipid features present in plasma and all 3 tissues *(Fig. 1A)*.

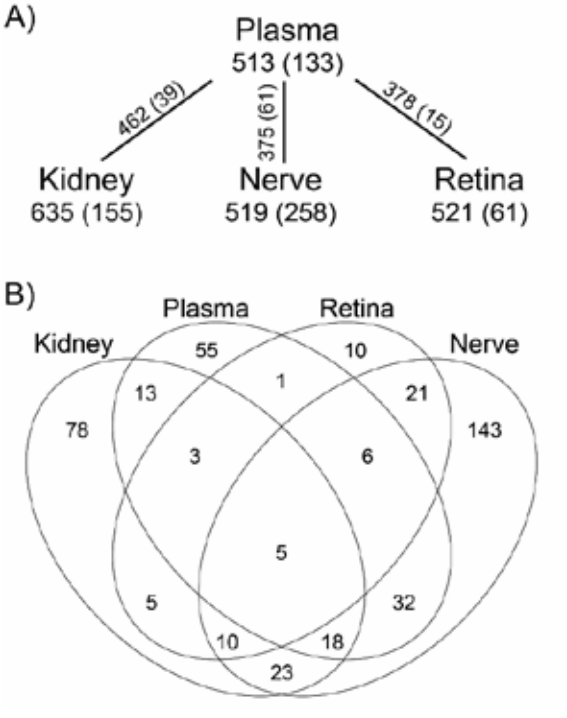


Figure 1: Overlap of differential lipid changes in diabetic mouse plasma, kidney, nerve and retina. (A) Shotgun lipidomics identified over 500 lipids in each plasma, kidney cortex, sciatic nerve and retina from 24-week-old diabetic versus control mice. Shown are the number of lipids identified in each tissue and the number (in parentheses) that differed between control and diabetic conditions. The numbers of unique features shared between plasma and each tissue are shown on the connecting line, followed by those (in parentheses) that were significantly different between control and diabetic conditions in both plasma and each tissue. (B) Venn diagram of significantly altered lipid features between control and diabetic mice in plasma, kidney cortex, sciatic nerve and retina. P < 0.01 based on a two-sample t-test with FDR correction, n = 10/group.

Of the 364 unique lipid features present in every sample matrix, only 15 were significantly different between control and diabetic mice in all 3 complications-prone tissues, and only 5 were significantly changed in plasma, kidney, nerve, and retina. The direction of change in lipid levels within the majority of classes varied by tissue type, further supporting that diabetes alters the lipidome in a tissue-specific manner. We also assessed the relationship between plasma lipid levels and lipid levels in each of the 3 tissues. There were no commonly co-regulated lipid subclasses between diabetic plasma and all three diabetic tissues, and most commonly co-regulated subclasses were between plasma and just one of the tissues examined. Diacylglycerols (DAGs) were commonly co-regulated between plasma and kidney in both control and diabetic mice, as correlation patterns between shorter DAGs with a low degree of saturation were similar between control and diabetic plasma and kidney. However, levels of the medium- and long-chain DAGs with a greater degree of saturation were only similarly regulated between diabetic plasma and kidney. The short-chain DAGs were also commonly co-regulated between diabetic plasma and diabetic retina, and the long-chain cardiolipins (CLs) were commonly co-regulated between diabetic plasma and both diabetic kidney and nerve. We are currently integrating the lipidomic data with transcriptomics, and have completed an initial nerve analyses showing robust correlation for glycerophospholipids.

THE GOAL IN THE NEXT FUNDING CYCLE IS TO:

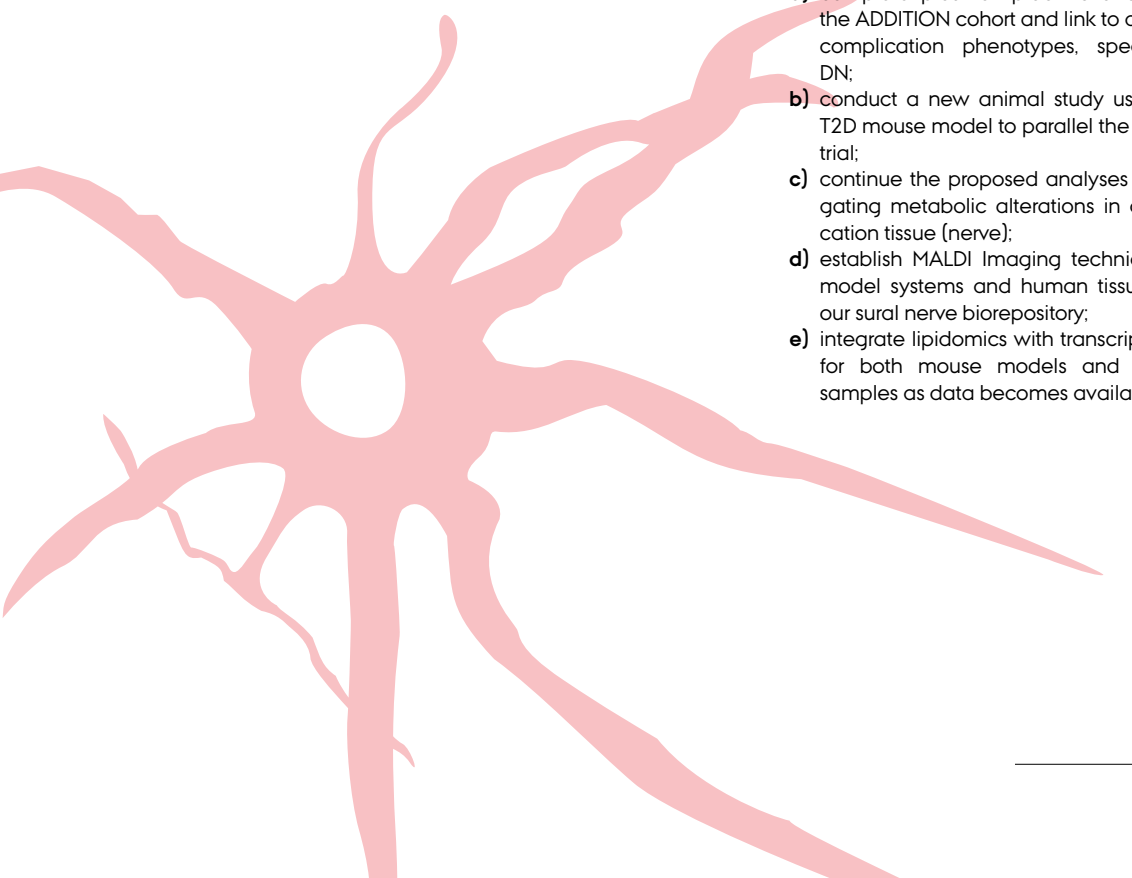
a) complete plasma lipidomic analysis in the ADDITION cohort and link to diabetic complication phenotypes, specifically DN;

b) conduct a new animal study using the T2D mouse model to parallel the human trial;

c) continue the proposed analyses investigating metabolic alterations in complication tissue (nerve);

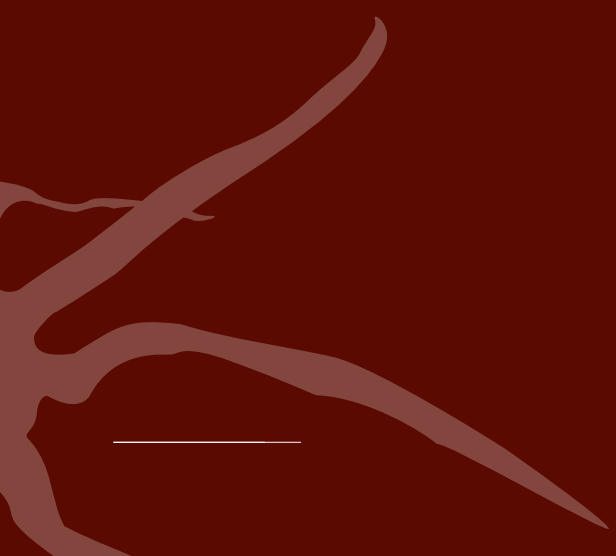
d) establish MALDI Imaging techniques in model systems and human tissue from our sural nerve biorepository;

e) integrate lipidomics with transcriptomics for both mouse models and human samples as data becomes available.



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 list, and are generally open to everyone interested – free of charge.



INTERNATIONAL SYMPOSIUM
7 October 2016
Aarhus University Hospital, Denmark

Mechanisms and pathophysiology of diabetic peripheral neuropathy
The Danish Diabetes Academy and the IDNC organized a joint symposium on diabetic neuropathy featuring presentations by an impressive line-up of 10 speakers, including 7 international experts. A total of 71 registered participants, mostly PhD students, attended the symposium. The symposium clearly strengthened the collaboration between IDNC members and international research groups.

IDNC GUEST LECTURES
IDNC guest lectures continue to bring together researchers in a multidisciplinary international discussion. The topics of the international speakers differ and cover broad fields of interesting research and existing and emerging concepts and approaches in the field of pain and diabetic neuropathy.

11 October 2016
Aarhus University Hospital, Aarhus, Denmark
The human single axon as a window for understanding pathological repetitive activity
IDNC guest lecture by Joseph Bergmans, University of Louvain, Belgium.

1 December 2016
Aarhus University Hospital, Aarhus, Denmark
The LDIFlare technique: a simple and quick method to assess C-fiber function in diabetic neuropathy
IDNC guest lecture by Gerry Rayman, Ipswich Hospital, UK.

8 December 2016
Aarhus University Hospital, Aarhus, Denmark
Threshold tracking transcranial magnetic stimulation: A novel method in examining cortical excitability
IDNC guest lecture by Bülent Cengiz, Gazi University Hospital, Turkey.

29 June 2017
Scandic Aarhus City, Aarhus, Denmark
Small fiber neuropathies
IDNC guest lecture by Karin Faber Department of Neurology, MUMC+, Maastricht, The Netherlands.

29 June 2017
Scandic Aarhus City, Aarhus, Denmark
Visualizing nerve fibres and other structures from skin biopsies
IDNC guest lecture by Maria Nolano Neurology Department, 'Salvatore Maugeri' Foundation, IRCCS, Institute of Telese Terme, Telese Terme, Italy.

OTHER ACTIVITIES

4 October 2016

Webinar on diabetic neuropathy

In collaboration with the Danish Diabetes Academy (DDA), Páll Karlsson gave a 30-minute online introduction to diabetic neuropathy.

3 February 2017

Aarhus University Hospital, Denmark

Half-yearly progress meeting

IDNC affiliated researchers, PhD students, and postdocs based in Denmark were given the opportunity to give an update on their respective IDNC studies and get internal feedback.

29-30 June 2017

Scandic City, Aarhus, Denmark

IDNC summer meeting

The IDNC summer meeting produced constructive dialogue and collaboration between IDNC researchers with participation and feedback from the scientific advisory board. As part of this year's meeting, Karin Faber, MUMC+, Maastricht (The Netherlands) gave a talk on small fiber neuropathies and Maria Nolano, Institute of Telese Terme, Telese Terme (Italy) gave a talk on visualizing nerve fibers and other structures from skin biopsies. Both internationally renowned experts delivered additional expertise and intellectual input throughout the meeting.

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 2016/17, including the following:

- **NEURODIAB**
  Bucharest, Romania, 9-12 September 2016.
- **IASP World Congress on Pain**
  Yokohama, Japan, 26-30 September 2016.
- **ASEAPS**
  Yangon, Myanmar, 16-19 February 2017.
- **DDA Winter School**
  Malaga, Spain, 20-23 March 2017.
- **IX SIMPAR-ISURA Congress**
  Florence, Italy, 29 March to 3 April 2017.
- **SASP Annual Meeting**
  Aalborg, Denmark, 27-28 April 2017.
- **Pain in the Baltics**
  Kaunas, Lithuania, 28-29 April 2017.
- **NeuPSIG**
  Gothenburg, Sweden, 15-18 June 2017.
- **EAN**
  Amsterdam, the Netherlands, 24-27 June 2017.
- **PNS Annual Meeting**
  Sitges, Spain, 8-12 July 2017.

IDNC researchers at the IDNC Summer Meeting 2017 in Aarhus. Karin Faber (second from the left) and Maria Nolano (third from the left)

Podiatrists from the Southern part of Denmark visited the IDNC administration at Aarhus University in April 2017.

COLLABORATION WITH DANISH PODIATRISTS

In Denmark, all patients with type 1 or type 2 diabetes are offered an annual foot screen, which is done by educated and certified podiatrists throughout the country (currently >1000 certified podiatrists in Denmark). The annual screen involves a lengthy examination including examination for certain neurological deficits. The examination is supported by the Danish Regions, covering 50% of expenses.

As of October 2017, data will be stored in a national database. Via a collaboration with the IDNC and the national podiatrist organization, we will explore the possibility for early recognition of diabetic neuropathy as well as other signs of diabetic foot disease. This collaboration will hopefully also lead to future educational activities.

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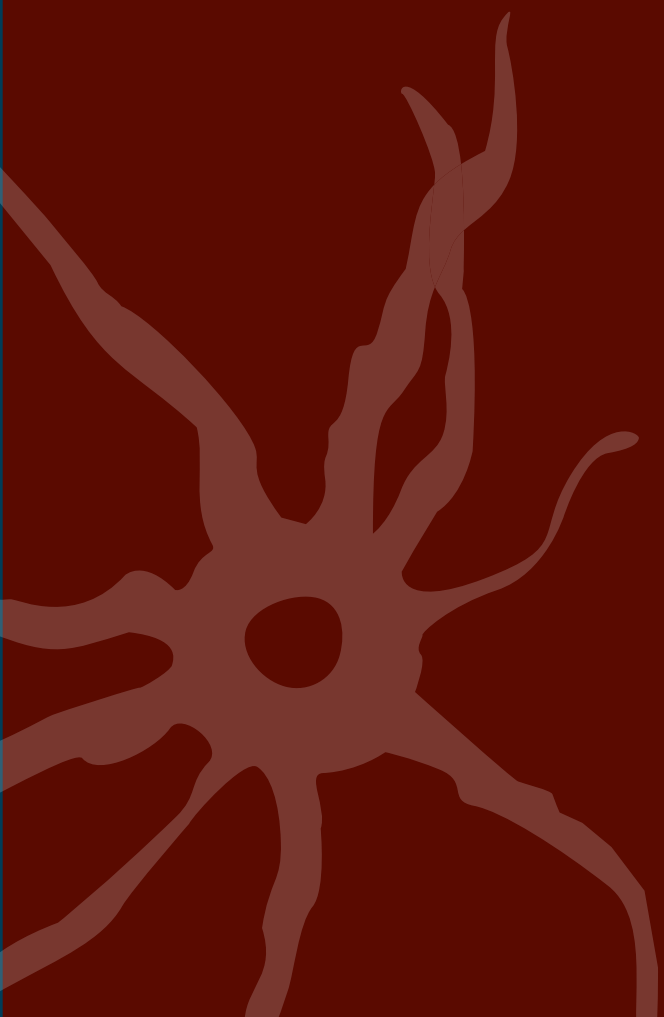

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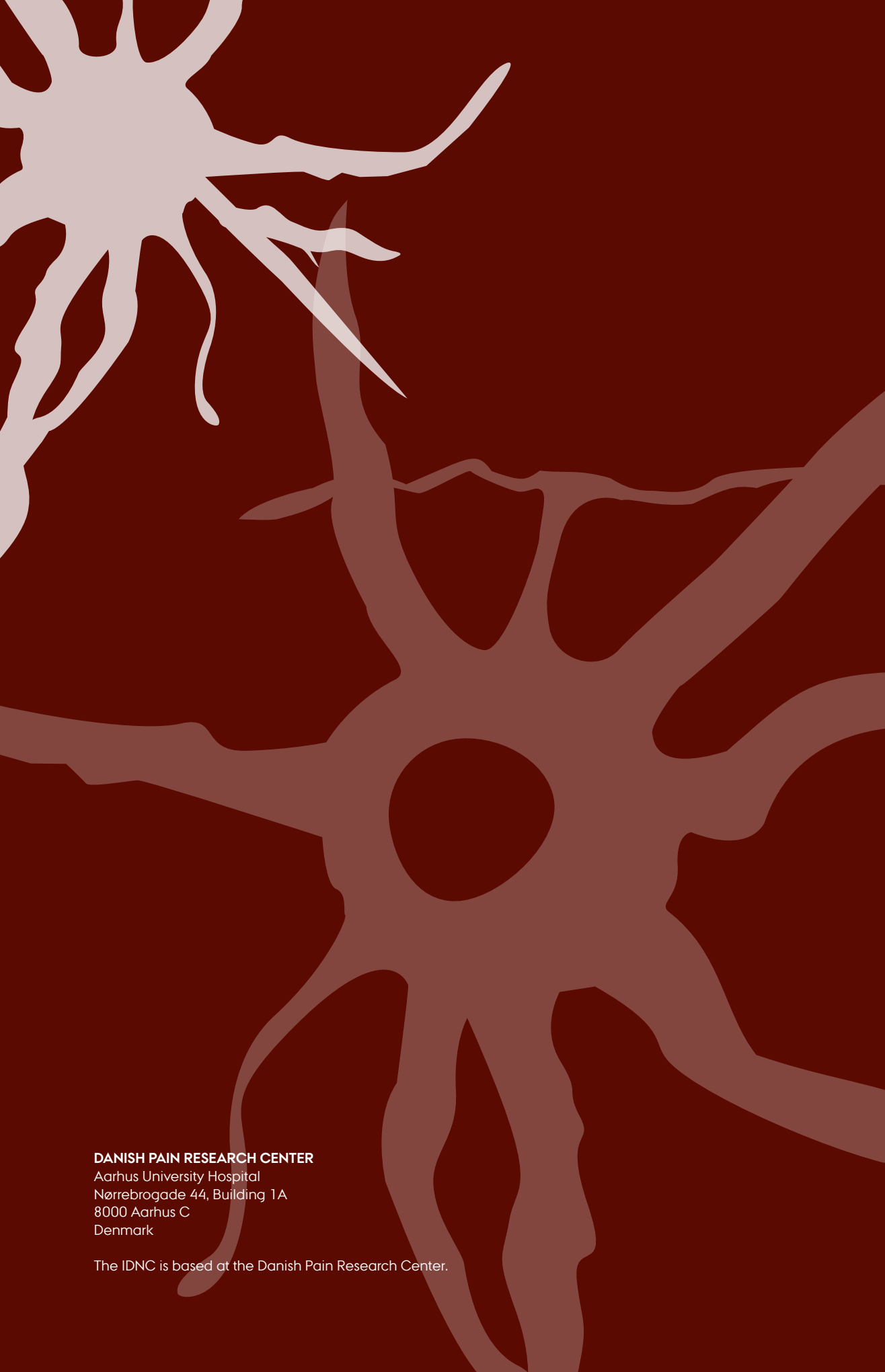
Printing:
vahle+nikolaisen

Print run: 150



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