INTERNATIONAL DIABETIC NEUROPATHY CONSORTIUM

ANNUAL REPORT 2015-2016

AARHUS UNIVERSITY

novo nordisk fonden

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"Diabetic neuropathy not only represents a high social and economic burden to society, but is also associated with increased morbidity and mortality."

PREFACE

The International Diabetic Neuropathy Consortium (IDNC) of researchers in Denmark, United Kingdom and the US was established in 2015 thanks to a generous six-year grant (2015-2021) of 60 million DKK (Euro 8.1 million) under the Novo Nordisk Foundation Challenge Program. The IDNC brings together internationally renowned researchers from Aarhus University, the University of Southern Denmark, the University of Oxford, United Kingdom, and the University of Michigan, Ann Arbor, USA to study the mechanisms giving rise to one of the main complications of diabetes: diabetic neuropathy.

Neuropathy is a highly neglected and understudied area. Diabetic neuropathy not only represents a high social and economic burden to society, but is also associated with increased morbidity and mortality. There is therefore a need to study the mechanisms giving rise to this debilitating condition in order to improve the diagnosis, treatment, and prevention of the condition. This is what the IDNC is about.

The IDNC represents an entirely new way of collaboration in which the host university - Aarhus University - expands its activities to mutual collaboration with elite universities.

By using expert knowledge at each of these institutions, it is hoped that we will be able to make a major step forward in understanding diabetic neuropathy. The 6-year program for the IDNC is ambitious with its focus on five major areas:

- Animal models of diabetic neuropathy
- Vascular changes in neuropathy
- Clinical profiling of diabetic neuropathy
- Epidemiological studies
- Metabolomics in diabetes and neuropathy

We are confident that it will be possible to make a major step forward in understanding the pathophysiology of neuropathy, which is crucial for a better treatment of the patients.

It has been a pleasure to experience how easy it has been to establish the collaboration between the four universities of the consortium. PhD students have been employed within all areas and studies are under way. Preliminary studies have already been presented and will soon be presented at different congresses.

In the year to come, the IDNC will strengthen its educational activities by participating in conferences and carrying out courses for both professionals and laypeople.

I would like to extend a particular thanks to the collaborators of the IDNC, to our international scientific advisory board and to Aarhus University for their support and help.

Troels Staehelin Jensen
Professor, Director of the IDNC

SCIENTIFIC BACKGROUND

The worldwide epidemic of diabetes affects 60 million Europeans and 29 million Americans, and its global incidence is increasing by 6% every year. The vast majority of diabetes is due to type 2 diabetes (T2D). In Denmark, 30,000 new cases of diabetes are diagnosed each year, and more than 320,000 people (~ 6% of the population) are currently living with diabetes. The most common complication of diabetes is diabetic neuropathy, which is defined as a symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and microvascular alterations as a result of chronic hyperglycemia exposure and cardiovascular risk covariates.

The prevalence of diabetic neuropathy ranges from 30 to 50%, and up to 25% of individuals with pre-diabetes also develop diabetic neuropathy. Diabetic neuropathy is characterized by progressive loss of peripheral nerve axons, resulting in decreased sensation, pain, and ultimately complete loss of sensation. It is the leading cause of diabetes-related hospital admissions and non-traumatic amputations in both Europe and the USA and incurs significant worldwide economic costs.

Currently there are no obvious disease-modifying treatments available for diabetic neuropathy other than glycemic control and symptomatic treatment of pain. Pain is a major problem in diabetic neuropathy, but current treatments are insufficient, with less than two-thirds of patients obtaining sufficient pain relief. Clearly there is a critical and unmet need to identify novel effective therapies for diabetic neuropathy.

Our limited understanding of the complex and different etiologies of diabetic neuropathy has hindered the development of targeted therapies for diabetic neuropathy. Clinical studies confirm that there are different mechanisms underlying diabetic neuropathy in type 1 and type 2 diabetes, including microvascular insufficiency, oxidative stress, dyslipidemia, insulin resistance, inflammation, and mitochondrial dysfunction.

Diabetic neuropathy represents a severe, disabling – and until recently – largely neglected problem. There is therefore an urgent need to address this global problem and to carry out a multinational interdisciplinary project on fundamental aspects related to diabetic neuropathy. The IDNC is prepared to take up this challenge in the years to come.

RESEARCH AIMS

The aims of the International Diabetic Neuropathy Consortium are:

01 **To increase our understanding of the basic mechanisms and risk factors in diabetic neuropathy**

02 **To improve the detection of nerve damage and determine its clinical course**

03 **To contribute to the prevention and treatment of diabetic neuropathy**

The four centers in the consortium complement each other by combining existing clinical and biobank data sources in Denmark with new prospectively collected data to identify risk factors for neuropathy and its complications. By integrating metabolomics done in the USA with carefully clinically phenotyped patients in the UK and Denmark, we can identify potential neuropathy targets and also engage in biomarker development.

IDNC Summer Meeting 2016, Helnan Marselis Hotel, Aarhus, DK

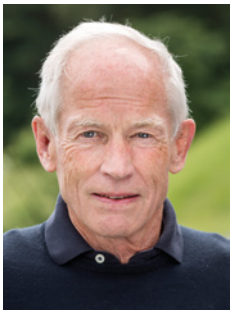
ORGANIZATION

The IDNC is hosted by the Faculty of Health and the Department of Clinical Medicine, Aarhus University, and housed at the Danish Pain Research Center, Aarhus University Hospital. The IDNC is organized with a management structure consisting of a Director, a Steering Group, and a Scientific Advisory board. In addition, the activities are supported by the IDNC administration and the administration at Aarhus University.

The Director of IDNC is Professor Troels Staehelin Jensen who together with Professors Henning Andersen and Nanna Brix Finnerup represents the Steering Group. The Steering Group is responsible for helping to identify important research initiatives and implement them in the IDNC. The members of the Steering Group are experienced researchers and clinicians with different backgrounds and strengths and have expertise in peripheral neuropathy, and diabetic neuropathy in particular, neurophysiology, neuropharmacology, and mechanisms and treatment of neuropathic pain.

The Scientific Advisory Board informs and is informed by the research studies and helps identify research questions critical to improving our understanding of diabetic neuropathy. The members of the Board are internationally renowned with expertise in research related to diabetes and diabetic neuropathy.

DIRECTOR



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MAIN RESEARCH AREAS

The IDNC addresses three fundamental and critical areas related to diabetic neuropathy where our present knowledge is insufficient and where unsolved problems block the development of new therapies:

01 **Pathophysiology of diabetic neuropathy**
Vascular studies, metabolomics, and lipidomics in rodents and humans

02 **Risk factors of painful and non-painful diabetic neuropathy**
Role of lifestyle, biomarkers, metabolic factors, drug use, and comorbidities

03 **Clinical phenotype**
Valid and reproducible methods for identifying painful and non-painful diabetic neuropathy

OUTLINE OF THE RESEARCH

The strategy of this translational initiative is to establish a series of interlinked work packages (WPs) devoted to explore the mechanisms, risks, prognostic factors, and clinical profiles of diabetic patients with and without neuropathy.

Each WP will in itself represent collaboration between the research groups where different techniques and expertise are utilized. By bringing these four multidisciplinary groups together, it is possible to address the pertinent questions related to diabetic neuropathy. The multi- and interdisciplinary structure as well as the collaboration within and between WPs ensures a strong synergy and dynamic of the consortium.

WP1: ANIMAL MODELS OF DN

Large clinical studies in humans have revealed different risk factors for diabetic neuropathy in type 1 and type 2 diabetes, leading to the hypothesis that the pathogenetic mechanisms underlying the two types of diabetes are different. Mouse models of diabetic neuropathy represent important tools to understand the pathophysiological mechanisms of nerve damage in diabetes and to determine differences and similarities between type 1 and type 2 diabetes.

STATUS 2016

In this work package we take advantage of the animal models developed in the lab of Professor Eva Feldman, e.g. the db/db mouse and the high-fat diet diabetes model in mice. Postdoc Nadia Goncalves will be visiting the lab of Professor Feldman in 2016 to perform the behavioral and electrophysiological studies characterizing these animals (including measurement of heat/pain thresholds and nerve conduction velocities in the tail nerves). The fact that these animals go through different temporal phases permits the assessment of both hypersensitivity and hyposensitivity. Specifically, the role of neurotrophin signaling between sensory neurons and glia cells will be assessed. Neurotrophins are essential for the integrity and functionality of peripheral nerves and here we are examining the role of the p75NTR neurotrophin receptor in Schwann cells from the high-fat diabetes model (type 2 diabetes).

WP2: HYPOXIC NERVE DAMAGE

We propose that control of capillary flow is lost in diabetes due to endothelial/glycocalyx damage, loss of pericytes, thickening of capillary basement membranes, and elevated blood viscosity. We have shown that the resulting disturbance of capillary flow patterns causes "physiological shunting" of oxygen and glucose through tissue and propose that this capillary dysfunction contributes to endoneurial hypoxia, oxidative stress, metabolic changes, and eventually nerve damage in diabetic neuropathy.

Hyperglycemia is associated with damage to the capillary lining and pericyte apoptosis, and we expect the resulting loss of capillary flow control to be a dominating source of capillary dysfunction in type 1 diabetes. In type 2 diabetes, obesity, hypertension, and hyperlipidemia cause systemic inflammation and oxidative stress that expose endoneurial capillaries to activated blood cells and oxidized lipoproteins. Unlike what is seen in type 1 diabetes, hyperglycemia may therefore be only one of several sources of nerve damage in type 2 diabetes.

STATUS 2016

In this work package we have already established the two-photon microscopy and the optical coherence tomography in order to measure capillary transit time heterogeneity in the sciatic nerve of anesthetized mice. Additional studies will be carried out in normal mice before the findings can be extended to diabetic mice. Postdoc Anete Dudele is planning a visit to the Feldman lab in the fall of 2016 to learn their diabetic neuropathy models. At a later stage, it is planned to extend these studies to humans. Here assessment of capillary transit time heterogeneity will be done with tracer kinetic models for indirect, non-invasive quantification of capillary transit time heterogeneity.

The optical coherence tomography lab

WP3: EPIDEMIOLOGY: RISK FACTORS OF DIABETIC NEUROPATHY IN TYPE 2 DIABETES

The overall hypothesis in this work package is that factors other than hyperglycemia represent important, potentially modifiable, risk and prognostic factors for diabetic neuropathy in type 2 diabetes.

STATUS 2016

In an epidemiological study PhD student Diana Hedevang Christensen is studying risk factors for the development of diabetic neuropathy and related conditions in type 2 diabetic patients and the role of existing pharmacological therapy, including statins, fibrates and incretin-based therapy, in the prevention of diabetic neuropathy and related conditions. Following a validation study of diabetic neuropathy, foot ulcer, and lower leg amputation in diabetes, she will determine the effectiveness of lipid-lowering and antihyperglycemic therapy on the risk of diabetic neuropathy and related diabetic foot disease in type 2 diabetes based on a series of data sources available in Denmark. This will be followed by a study of the association between the metabolic syndrome and diabetic neuropathy in type 2 diabetes.

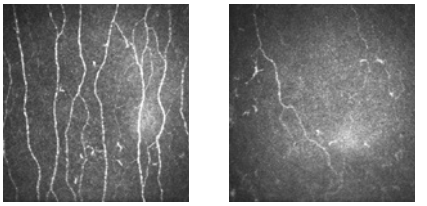
The ADDITION project is a cohort and biobank study of 1533 patients with type 2 diabetes who were examined in 2001. A 10-year follow-up study of surviving patients that were screened for diabetic neuropathy in 2006 is carried out by PhD student Signe Toft Andersen. At this follow up, patients are examined in different cities in Denmark with a series of screening tests for diabetic neuropathy. The course and development of diabetic neuropathy will also be determined in this group of patients. The first part of the study with examination of 200 patients in Aarhus is completed and data will be presented at congresses in the fall of 2016.

WP4: CLINICAL PROFILE

This work package will determine the presence of pain and sensory abnormalities in the ADDITION and DD2 cohorts and in a cohort of patients from Oxford, UK, using multiple techniques to achieve a singular goal: increasing our understanding of the pathophysiology of pain in type 1 and type 2 diabetic neuropathy.

STATUS 2016

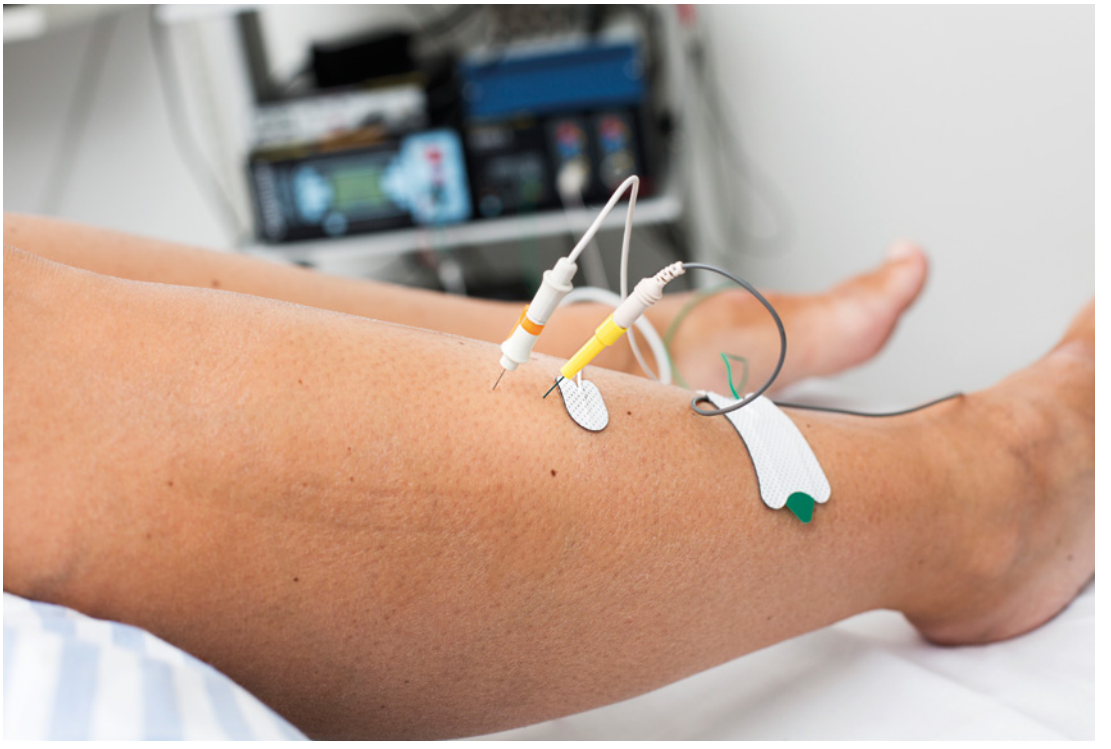
This work package involves clinical studies at three sites of the consortium: Aarhus University, the University of Southern Denmark, and the University of Oxford. Patients will be examined using the same protocol and examination procedures. Three hundred patients have already been examined in Oxford. This part is carried out by Postdoc Andreas Themistocleous under the supervision of Professor David Bennett. Using the same examination protocol, we will examine the frequency of neuropathy in patients from the Danish DD2 database of diagnosed type 2 diabetic patients with approx. 7000 patients. The Michigan Neuropathy Screening Instrument will be used to select patients with and without signs of neuropathy. Data from this sample have already been received and from September 2016, following approval from the relevant authorities, we will start recruiting patients to the Aarhus and Odense sites for detailed examination, approx. 300 patients for each site. A large battery of tests will be carried out and allow us to determine essential facts about clinical neuropathy. The studies will be carried out by PhD student Thomas Krøigård at the University of Southern Denmark and PhD student Sif Gylfadottir at Aarhus University. In addition, PhD student Alexander Gramm Kristensen will study the role of ion channels in neuropathy and other neurophysiological measures for the development of neuropathy and pain in a separate project at Aarhus University.



Sub-basal nerve images acquired with Corneal Confocal Microscopy showing C-fiber innervation in the central cornea. Left: Healthy individual with normal nerve fiber density. Right: Diabetic polyneuropathy patient with significant loss of fibers.

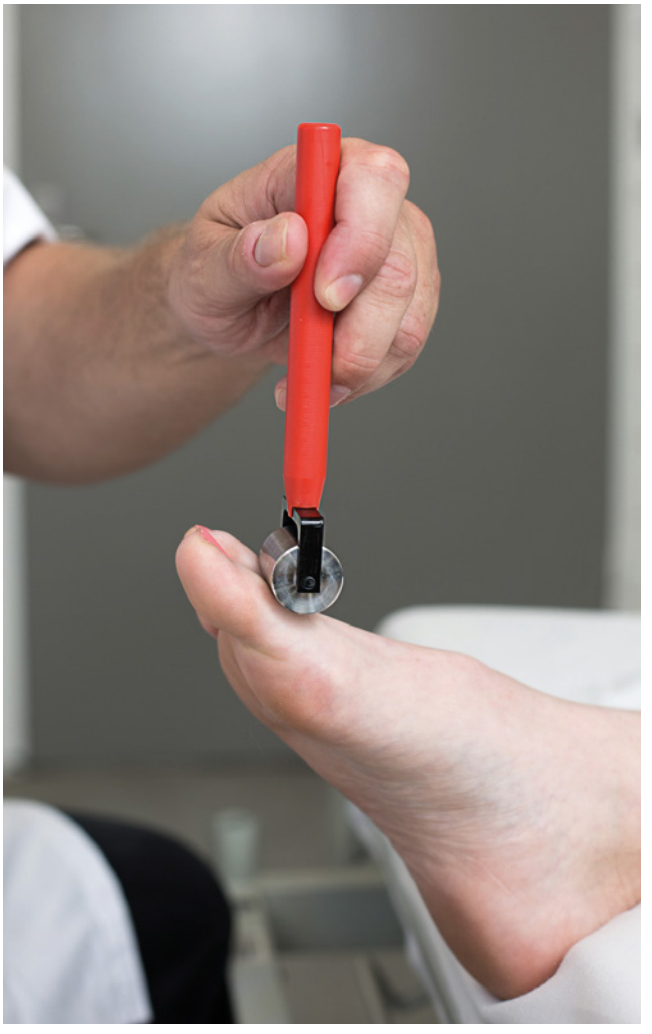
Corneal confocal microscopy

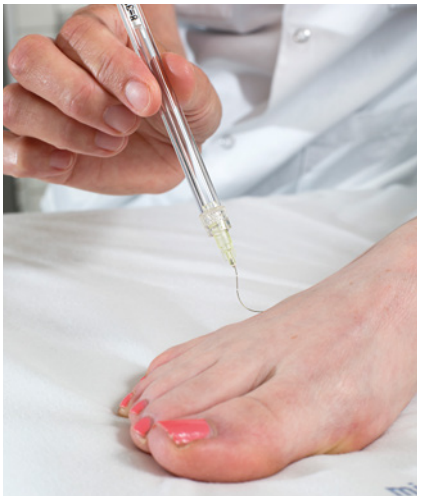
"The Danish DD2 cohort of type 2 diabetes is a goldmine for generating novel epidemiological, clinical, and pathophysiological information about diabetic neuropathy."



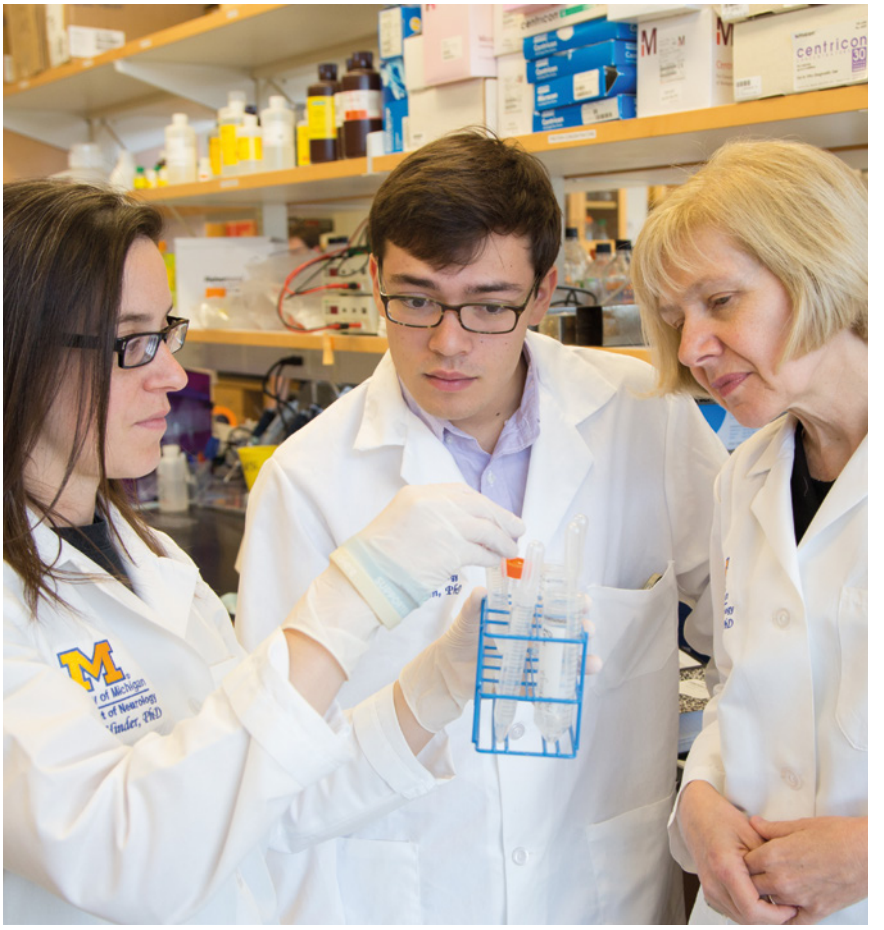
Examples of examinations in profiling patients with diabetic neuropathy


Digitimer
DS5





WP5: METABOLOMICS



In this work package, which is carried out at the Ann Arbor site of the IDNC, we are examining metabolomics, which is a field of life science research that uses high throughput technologies to characterize all the small molecules or metabolites in a given cell, tissue, or organism (i.e. the metabolome). In the IDNC study, particular focus will be on lipidomics in animal models of type 2 diabetes as well as in patients with type 2 diabetes. Eva Feldman's group is currently doing targeted lipidomics in mice with type 2 diabetes. Samples from the clinical ADDITION cohort will be shipped to Ann Arbor in the fall of 2016 for analysis.

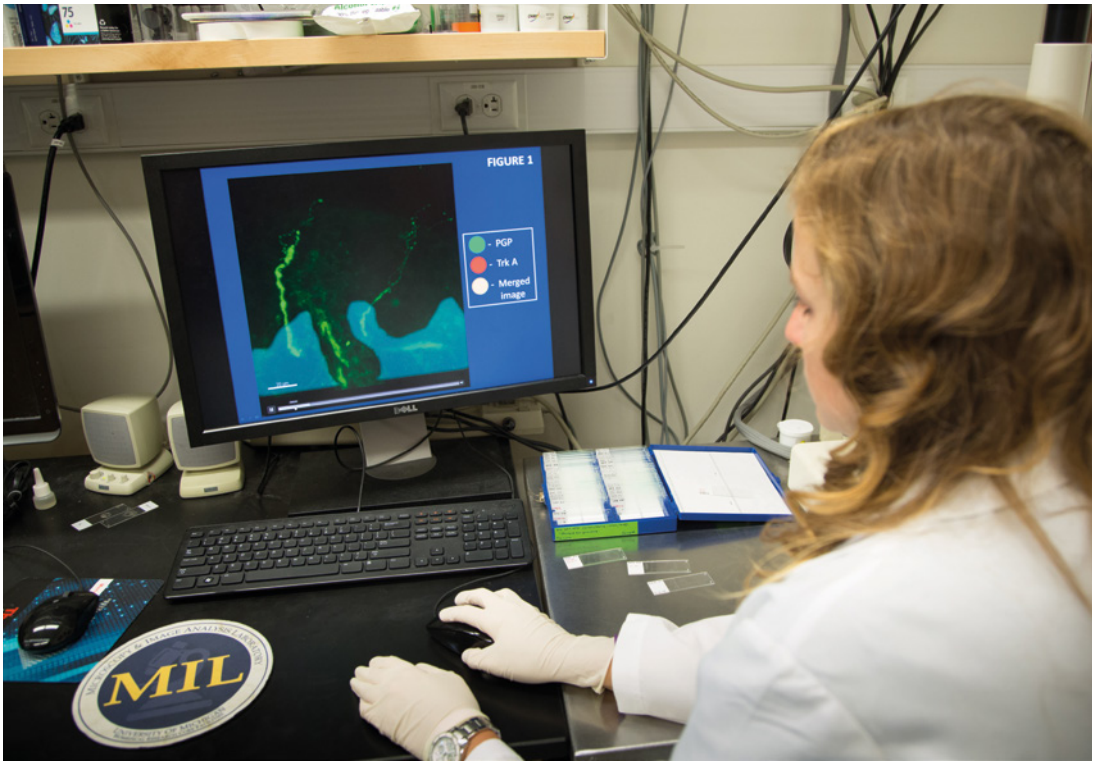


FIGURE 1
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EDUCATIONAL ACTIVITIES AND PUBLICATIONS

Annual research meetings, international visiting scientists, and other educational activities constitute important elements of the IDNC. The IDNC sponsor research presentations, symposia, workshops, and other meetings to encourage constructive dialogue and collaboration between scientists interested in neuropathy and diabetes. Researchers at IDNC participate in educational activities at different levels ranging from seminars for lay people to specialized research meetings.

MONTHLY IDNC PRESENTATIONS
The monthly IDNC presentation series bring together researchers, postdoctoral fellows, PhDs, and research year students based in Denmark to network and discuss their latest research and showcase cutting edge research from multiple disciplines that directly informs IDNC researchers and affiliates.

IDNC KEYNOTE LECTURES
IDNC keynote lectures bring together researchers in a multi disciplinary 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 diabetic neuropathy. So far, Professor Rayaz A. Malik, Weill Cornell Medical College (Doha & New York) and University of Manchester and Professor Hugh Bostock, Institute of Neurology, University College London – both internationally renowned keynote lecturers – have engaged in educational and scientific activities related to diabetic neuropathy in Denmark.

Professor Malik visited the IDNC and helped establish confocal corneal microscopy (CCM) at the Danish Pain Research Center for future IDNC studies.

Similarly, Professor Bostock delivered expertise, intellectual input, and training of the IDNC staff in the use of threshold tracking as a tool to investigate nerve physiology and neuropathies. Both professors gave IDNC keynote lectures during their stay in Denmark.

EXCHANGE VISITS
Exchange visits for PhD fellows, postdocs, and MDs in clinical training between the participating sites constitute an important element of the scientific and educational program of the consortium. The IDNC provides the optimal setting for exchange visits and training.

ENGAGEMENT ACTIVITIES

IDNC consortium members have attended and lectured at several scientific conferences and meetings, including:

- **American Diabetes Association**, 75th Scientific Session, Boston, Massachusetts, June 2015.
- **American Neurological Association**. Meeting Derek Denny-Brown Symposium to honor the recipients of ANA's Young Neurological Scholar Award in the areas of basic and clinical science, Chicago, Illinois, September 2015.
- **Annual Meeting of the Diabetic Neuropathy Study Group of the EASD (NeuroDiab 2015)**, Elsinore, Denmark, September 2015.
- **9th Congress of the European Pain Federation EFIC® (EFIC 2015)**, Vienna, Austria, September 2015.
- **Cambodia Pain Society**, Phnom Penh, Cambodia, November 2015.
- **Diabetes Update 2015**, Copenhagen, Denmark, November 2015.
- **Center for Brain Research**, University of Auckland, Auckland, New Zealand, December 2015.
- **American Diabetes Association**, 76th Scientific Session, New Orleans, Louisiana, USA, June 2016.

IDNC consortium members have presented posters at several scientific conferences and meetings, including:

- **Gordon conference**, Ventura, California, March 2015.
- **American Diabetes Association**, 75th Scientific Sessions, Boston, MA, June 2015.
- **Peripheral Nerve Society Meeting**, Quebec City, Quebec, Canada, June 2015.
- **American Neurological Association Meeting**, Chicago, Illinois, September 2015.
- **Annual Meeting of the Diabetic Neuropathy Study Group of the EASD (NeuroDiab 2015)**, Elsinore, Denmark, September 2015.
- **Keystone "Mitochondrial Dynamics" Symposium**, Steamboat Springs, Colorado, USA, April 2016.
- **American Diabetes Association**, 76th Scientific Session, New Orleans, Louisiana, USA, June 2016.
- **Annual Meeting of the Diabetic Neuropathy Study Group of the EASD (NeuroDiab 2016)**, Bucharest, Romania, September 2016.

PEER REVIEWED PUBLICATIONS (PUBLISHED)

1. **Hur J**, **O'Brien PD**, **Nair V**, **Hinder LM**, **McGregor BA**, **Jagadish V**, **Kretzler M**, **Brosius FC**, **Feldman EL**. Transcriptional networks of murine diabetic peripheral neuropathy and nephropathy: Common and distinct gene expression patterns. Diabetologia. 2016; 59(6),1297-306.

2. **O'Brien PD**, **Hur J**, **Robell NJ**, **et al.** Gender-specific differences in diabetic neuropathy in BTBR ob/ob mice. J Diabetes Complications 2016;30(1):30-7.

3. **Ostergaard L**, **Finnerup NB**, **Terkelsen AJ**, **et al.** The effects of capillary dysfunction on oxygen and glucose extraction in diabetic neuropathy. Diabetologia 2015;58(4):666-77.

4. **Themistocleous AC**, **Ramirez JD**, **Shillo PR**, **et al.** The Pain in Neuropathy Study (PiNS): a cross-sectional observational study determining the somatosensory phenotype of painful and painless diabetic neuropathy. Pain 2016;157(5):1132-45.

OTHER PUBLICATIONS

1. **Jensen TS**. Diabetisk neuropati: En overset følgesygdom. Behandlerbladet (Diabetesforeningen) 2015;(41), 20-22.

2. **Jensen TS.** Forskere jagter årsag til neuropati. Diabetes 2015;75(2), 48.

3. **Hinder LM**, **Sullivan KA**, **Sakowski SA**, **Feldman EL.** Development and Progression of Diabetic Polyneuropathy (Book Chapter). In Neurobiology of Disease, 2nd ed. , 2016 In press.

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