Diabetic polyneuropathy in type 2 diabetes

Prevalence, risk factors, mental health, and diagnostic validity

PhD dissertation

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Dedication

I dedicate this thesis to all the patients who participate in the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project. Their willingness to participate in the DD2 project are of major importance for type 2 diabetes research.
In continuation of the neuropathy questionnaire survey conducted in the DD2 cohort during 2016, I received many phone calls from DD2 participants, who wanted to add a comment or ask a question about diabetic polyneuropathy.
Below are listed a few of these comments. Let these expressions serve as a reminder that behind the results presented in this thesis, are real persons dealing with a severe chronic disease.

"*I have received a questionnaire from you and I just want to tell you a bit more about the symptoms in my feet…"*

"During recent time my feet have become numb and I have a burning pain in them. I was not aware that it could be due to my diabetes until I received the questionnaire from you. Is it a rare complication?"

"I take care of my diabetes; I take my diabetes-medication as prescribed, and my blood sugar is normal. Still, I have diabetic polyneuropathy. I think it is unfair and I do not understand why I have a got this complication! Can you tell me why?"

"Thank you for putting focus on diabetic polyneuropathy. I have constant burning pain in my feet and the medication I have got from my doctor does not have any effect."

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List of papers

This PhD dissertation is based on the following four studies, which, in the thesis, will be referred to by their roman numerals.

I. **Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort of newly diagnosed patients with type 2 diabetes: a cohort profile.**
Christensen DH, Nicolaisen SK, Berencsi K, Beck-Nielsen H, Rungby J, Friborg S, Brandslund I, Christiansen JS, Vaag A, Sørensen HT, Nielsen JS, Thomsen RW. *BMJ Open* 2018;8:e017273. doi:10.1136/bmjopen-2017-017273

II. **Diabetic polyneuropathy and prevalence, pain, and patient characteristics: A cross-sectional questionnaire study of 5,514 patients with recently diagnosed type 2 diabetes.**
Gylfadottir SS*, Christensen DH*, Nicolaisen SK*, Andersen H, Callaghan BC, Itani M, Khan KS, Kristensen AG, Nielsen JS, Sindrup SH, Andersen NT, Jensen TS, Thomsen RW**, Finnerup NB**. Resubmitted to *Pain*.
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III. **Metabolic factors, lifestyle habits, and polyneuropathy in early type 2 diabetes: A nationwide study of 5,249 patients in the Danish DD2 cohort.**
Christensen DH, Knudsen ST, Gylfadottir SS, Christensen LB, Nielsen JS, Beck-Nielsen H, Sørensen HT, Andersen H, Callaghan BC, Feldman E, Finnerup NB, Jensen TS, Thomsen RW. *In draft*

IV. **Can diabetic polyneuropathy and foot ulcers in patients with type 2 diabetes be accurately identified based on ICD-10 hospital diagnoses and drug prescriptions?**
Christensen DH, Knudsen ST, Nicolaisen SK, Andersen H, Callaghan BC, Finnerup NB, Jensen TS, Thomsen RW. *Clin Epidemiol*. 2019;11:311-321. doi: 10.2147/CLEP.S197474

Abbreviations

ATC	Anatomical Therapeutic Chemical Classification
BMI	Body Mass Index
CI	Confidence interval
CPR	Central Personal Registration
CPT	Current Procedural Terminology
CRS	Civil Registration System
DDDA	Danish Diabetes Database for Adults
DD2	The Danish Centre for Strategic Research in Type 2 Diabetes
DN4	Douleur Neuropathique en 4 Questions
DNHSP	The Danish National Health Service Prescription Database
DNPR	The Danish National Patient Registry
DPN	Diabetic polyneuropathy
GP	General Practitioner
HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
hsCRP	High-sensitive C-reactive protein
ICD	International Classification of Diseases
IDNC	International Diabetic Neuropathy Consortium
IENFD	Intra epidermal nerve fiber density
LDL	Low-density lipoprotein
MNSI	Michigan neuropathy screening instrument
MNSIq	Michigan neuropathy screening instrument questionnaire
NCS	Nerve conduction studies
NeuPSIG	Neuropathic Special Interest Group
NOMESCO	Nordic Medico-Statistical Committee
NRS	Numeric rating scale
OR	Odds ratio
PPV	Positive predictive value
PR	Prevalence ratio
PROMIS	Patient Reported Outcome Measurement Information System
QoL	Quality of Life
SNRIs	Serotonin–noradrenalin reuptake inhibitors
TCA	Tricyclic antidepressants

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1. Introduction

1.1 Diabetes

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. Diabetes is classified into four overall groups; type 1 diabetes, type 2 diabetes, gestational diabetes, and a group of other types.[1] Of these, type 1 and type 2 diabetes are the main types with type 2 diabetes accounting for around 90% of all diabetes.[2] Type 1 diabetes is pathophysiologically characterized by insulin depletion due to destruction of the insulin-producing pancreatic beta-cells. Type 2 diabetes is characterized by peripheral insulin resistance (liver, muscles and adipose tissue) and varying degrees of beta cell dysfunction leading to relative insulin deficiency.

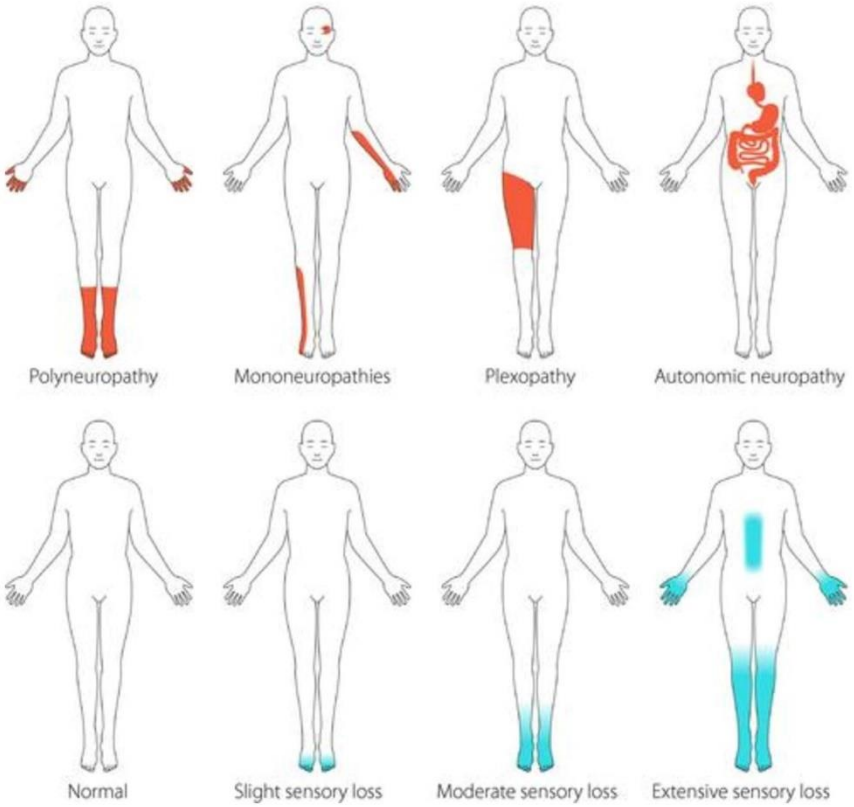
By 2017, an estimated 425 million adult individuals lived with diagnosed diabetes worldwide and the number is expected to reach 629 million by 2045, with the fastest increase expected to occur in developing countries.[2] Factors contributing to the rising diabetes epidemic include massive exposure to diabetes risk factors due to increasing urbanization, adaption to energy-dense diets and sedentary lifestyle, and resulting increased prevalence of obesity. Other important contributing factors are the demographic shift towards an elderly population and an improved survival of diabetes.

1.2 Diabetes complications

One of the main consequences of diabetes is the development of diabetic complications with major impact on morbidity and mortality. An estimated 8.4% of the total population mortality among people aged 20-79 years is attributable to diabetes.[3] The excess mortality in diabetes is related to development of diabetes complications, divided into the classical macrovascular complications like myocardial infarction, stroke, and peripheral arterial disease, the classical microvascular complications i.e. diabetic nephropathy, diabetic retinopathy, and diabetic neuropathy, and a number of "non-classical" complications including e.g. infections and cancer.[4 5] Mortality in diabetes patients has decreased in recent years as a consequence of improved diabetic care.[6 7] However, taking the diabetes epidemic into account there is an urgent need of identifying ways to prevent diabetes complications in order to further improve morbidity and mortality. In this aspect, diabetic neuropathy has been a rather neglected research topic as compared to macrovascular and other microvascular complications.[8]

Diabetic neuropathy is a heterogeneous group of conditions caused by damage to the peripheral nervous system. Diabetic neuropathy can be classified into different subtypes based on the pattern of nerve injury; *the symmetric and diffuse neuropathies* e.g. diabetic polyneuropathy (DPN) and diabetic autonomic neuropathy and *the asymmetric focal/multifocal neuropathies* e.g. mononeuropathy, radiculopathy, and radiculoplexopathy.[9-11] Of these, DPN is by far the most common type[9-11] and also - the focus of this dissertation.

Figure 1. *Diabetic neuropathy. Upper panel shows common types of diabetic neuropathy. Lower panel shows the gradual progression of diabetic polyneuropathy. Reprinted from Gylfadottir et al[8]. with permission under the Creative Commons Attribution License (CC BY), J Diabetes Investig.*



1.3 Diabetic polyneuropathy

DPN is defined as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes".[11] It is a symmetrical peripheral polyneuropathy that demonstrates a "stocking and glove" distribution of nerve affection. DPN develops insidiously starting distally in the toes with a progressive proximal involvement of the feet and legs and eventually the upper extremities (starting in the fingertips), which reflects the dying-back process of damage of the peripheral nerve axons (Figure 1).[8 10 12] Somatic sensory nerves are predominantly and initially affected, but the autonomic and motor functions can be affected too.[10] Symptoms of DPN fall in to a broad spectrum e.g. numbness, tingling, pain, weakness, and unsteadiness.[13] The clinical presentation reflects the type of affected sensory nerves i.e. whether there is only large-fiber dysfunction (large myelinated nerve-fibers), small-fiber dysfunction (small myelinated and unmyelinated nerve fibers), or mixed small-and large fiber dysfunction (most common). Proprioception, and light touch and vibration sensations are mediated by large fibers, whereas sharp pain and temperature sensations are mediated by small fibers. The type of neuronal

dysfunction can be assessed by testing these nerve functions clinically, e.g. by pinprick (sharp pain). In accordance, different quantitative diagnostic tests exist for large- and small fiber function. The gold standard for assessing and quantifying large fiber dysfunction is nerve conduction studies (NCS) measuring nerve conduction velocity and amplitude. Small fiber dysfunction can be assessed and quantified by a skin-biopsy with measurement of the intra-epidermal nerve fiber density (IENFD), but other non-invasive tests exist like quantitative sensory testing (QST) and corneal confocal microscopy. Besides these diagnostic tests, several feasible clinical scoring instruments have been developed and validated for use in the diagnosis of DPN, including the Michigan Neuropathy Screening Instrument.[8 14-19] These different instruments are based on interviews/questionnaires and/or clinical bedside tests.[8 14-18] The support for using these tools in the diagnosis of DPN is evident from the Toronto Diabetic Neuropathy consensus definition of typical DPN that includes a classification with increasing levels of certainty of the diagnosis:[20]

"Possible DPN": presence of either symptom(s) indicative of DPN (predominantly in toes, feet, or legs), sign(s) of distal decreased sensitivity (e.g. light touch, vibration, temperature), or decreased/absent ankle reflexes.

"Probable DPN": presence of at least two of the following; symptom(s) indicative of DPN (predominantly in toes, feet, or legs), sign(s) of distal decreased sensitivity (e.g. light touch, vibration, temperature), or decreased/absent ankle reflexes.

"Confirmed DPN": presence of abnormal NCS/abnormal validated measure of small fiber damage and at least one symptom or sign of DPN.

The importance of being able to identify people with DPN – for research and clinical care purposes – is emphasized by the serious consequences that DPN may exert on the affected patients and on health care costs. DPN may be complicated by falls, fractures, diabetic foot ulcers, amputations, and death.[11] Lifetime risk of developing a foot ulcer may be as high as 25% in diabetic patients.[21] A feared consequence of diabetic foot ulcers is lower extremity amputations. Every 30 second a lower limb/part of a lower limb is amputated somewhere in the world due to diabetes,[22] and estimated one-year mortality after a lower extremity amputation is 48%.[23] Besides the considerable impact DPN may have on the individual patients, DPN also has a substantial impact on health care costs. Total global diabetes cost in 2015 was estimated to be 1.31 trillion US dollars,[24] with foot complications accounting for up to 20%.[25]

1.3.1 Painful diabetic polyneuropathy

Symptoms of DPN vary within a broad spectrum and some patients are even asymptomatic.[11] One of the most critical symptoms is neuropathic pain. The International Association for the Study of Pain (IASP)

Special Interest Group on Neuropathic Pain (NeuPSIG) defines neuropathic pain as "pain caused by a lesion or disease of the somatosensory nervous system".
Neuropathic pain is often described as burning, shooting, aching, squeezing, electric shock, "pins and needles" etc. It can generally be divided into spontaneous (i.e. stimulus-independent) and evoked pain. Examples of the latter include allodynia, in which non-painful stimuli leads to painful sensations, and hyperalgesia characterized by an abnormal increased sensation to a painful stimulus.
Besides neuropathic pain, patients with DPN may experience co-existing non-neuropathic pain caused by e.g. musculoskeletal diseases, peripheral arterial disease etc. which complicates the diagnosis of painful DPN.[8]
A grading system for the diagnosis of neuropathic pain, similar to the Toronto criteria used in the diagnosis of DPN, has been developed by NeuPSIG:[26]

"Possible neuropathic pain": Requires: 1) Presence of pain in combination with pain descriptors suggestive of neurological lesion/disease e.g. burning, hot, electric shocks. 2) A history of a relevant lesion or disease of the somatosensory system (a close temporal relationship between lesion and pain provides strength), 3) A pain distribution anatomically consistent with the location of the lesion/disease.

"Probable neuropathic pain": Possible neuropathic pain + sensory signs in the same neuroanatomical plausible location.

"Definite neuropathic pain": Probable neuropathic pain + an objective diagnostic test confirming the lesion/disease; skin biopsy with IENFD measurement in DPN, computed tomography to confirm the presence of stroke etc.

As for DPN, a number of different screening tools to assess neuropathic pain has been developed[27-33] and validated in general populations or in specific pain conditions. Of these, the Douleur Neuropathique en 4 questions has specifically been validated for use in painful diabetic polyneuropathy.[34 35]
As the pain descriptions indicate (e.g. "electric shock"), painful DPN can be invalidating and pain management is important. Fortunately during recent decades, a huge work has been done to improve the pharmacological treatment of painful DPN.[13 36] Consensus guidelines find the best efficacy of calcium-channel a2δ ligands (gabapentin, pregabalin), serotonin–noradrenalin reuptake inhibitors (SNRIs, e.g. venlafaxine, duloxetine), and tricyclic antidepressants (TCA, e.g. amitriptyline, imipramine) in the treatment of neuropathic pain[37 38] and these drug-classes are recommended as first-line therapy.[36] Still, the major proportion of treated patients do not achieve sufficient pain relief.[13 36] In addition, pharmacological pain treatment comes at the expense of numerous side effects. Therefore, there is a compelling need of improving our understanding why some people with DPN develop neuropathic pain while others do not.

1.3.2 Prevalence

A number of studies have reported estimates of DPN and painful DPN prevalence. Reported estimates vary widely between 2.7%-75.1% for DPN[39-52] and 8%-30% for painful DPN in diabetes populations.[42-45 53-58] This large variation may be partly explained by various ways of assessing DPN and neuropathic pain as described above, leading to a range of diverse definitions used in existing studies. Other possible explanations include differences in;

-*Diabetes duration:* Most studies have investigated patients with longstanding diabetes (8-17 years).[40 41 43 44 46-49 51 55 57] Increasing diabetes duration is a well-known risk factor for DPN and it is increasingly accepted that small-fiber involvement often precedes large-fiber involvement.[59-61] Thus, the timing of DPN assessment according to the course of diabetes disease may have impact also on painful DPN prevalence.

-*Calendar time:* Guidelines for diabetes care has evolved over time emphasizing early pharmacological treatment initiation and a more comprehensive approach, not only targeting hyperglycemia but also diabetes-associated metabolic and lifestyle factors. New glucose-lowering drugs for the treatment of type 2 diabetes have emerged from 2006 onwards and have gained increasing foothold in developed countries,[62] offering more possibilities of reaching normoglycemia. Thus, studies reporting the prevalence of DPN and painful DPN in the 1980s, 1990s and the early 2000s may not reflect the present prevalence of DPN.[41 49-51 56 58]

-*Populations:* A large number of studies have reported the prevalence of DPN and painful DPN in e.g. Asian,[45 47 54] Middle Eastern,[40 63] American,[50 58] and African[52] populations. However, ethnic differences,[63] the higher prevalence of obesity in the US, and non-similar access to health care may lead to a DPN prevalence not comparable to that in e.g. Scandinavian countries. Moreover, some studies have focused solely on populations sampled from outpatient clinics[29 40 41 43 45 46 48 49 52] or from primary care[56 58] and DPN prevalence may vary across these populations *per se* but also over calendar time due to changes in referral practices from primary care.

Of note, not all studies on painful DPN prevalence[53-58] have reported the *simultaneous prevalence of DPN overall or of non-painful DPN.* Therefore, these studies do not allow an evaluation of the distribution of painful and non-painful DPN. Finally, some studies have been of *small size* and/or do not provide confidence intervals for their prevalence estimates.

We aimed to explore the prevalence of DPN and painful DPN in recently diagnosed Danish type 2 diabetes patients.

1.3.3 Risk factors

The common feature of type 1 and type 2 diabetes is hyperglycemia. Large intervention trials have investigated the effect of enhanced glycemic control on the risk and progression of DPN. A meta-analysis[64] of these trials provides firm evidence for an effect on prevention of DPN and on improvement of nerve

function with intensive versus standard therapy in patients with type 1 diabetes. The combined annualized risk difference (RD) of developing clinical neuropathy was -1.84% (95% CI: -2.56; -1.11), N = 1,228 and the combined annual mean difference of peroneal nerve motor conduction velocity was 0.61 m/s (95% CI: 0.51; 0.71), N = 1371. In contrast, in type 2 diabetes, intensive therapy has shown only a modest preventive effect on DPN development with a combined annualized RD for clinical DPN of -0.58% (95% CI: -1.17; 0.01), N = 6,669.[64] These different findings of the impact of hyperglycemia on DPN risk have raised the question whether DPN is the same or two different diseases in type 1 and type 2 diabetes?[65 66] The phenotype of type 1 and type 2 diabetes differs substantially, thus type 1 diabetes is classically found in a lean or normal weight person, whereas the most classic type 2 diabetes patient is characterized by the components of the metabolic syndrome including obesity, hypertension, and dyslipidemia. Accordingly, during recent years, effort has been put into understanding the complex pathogenesis underlying DPN in type 1 and type 2 diabetes. Both different and shared mechanisms have been suggested, as illustrated in Figure 2.

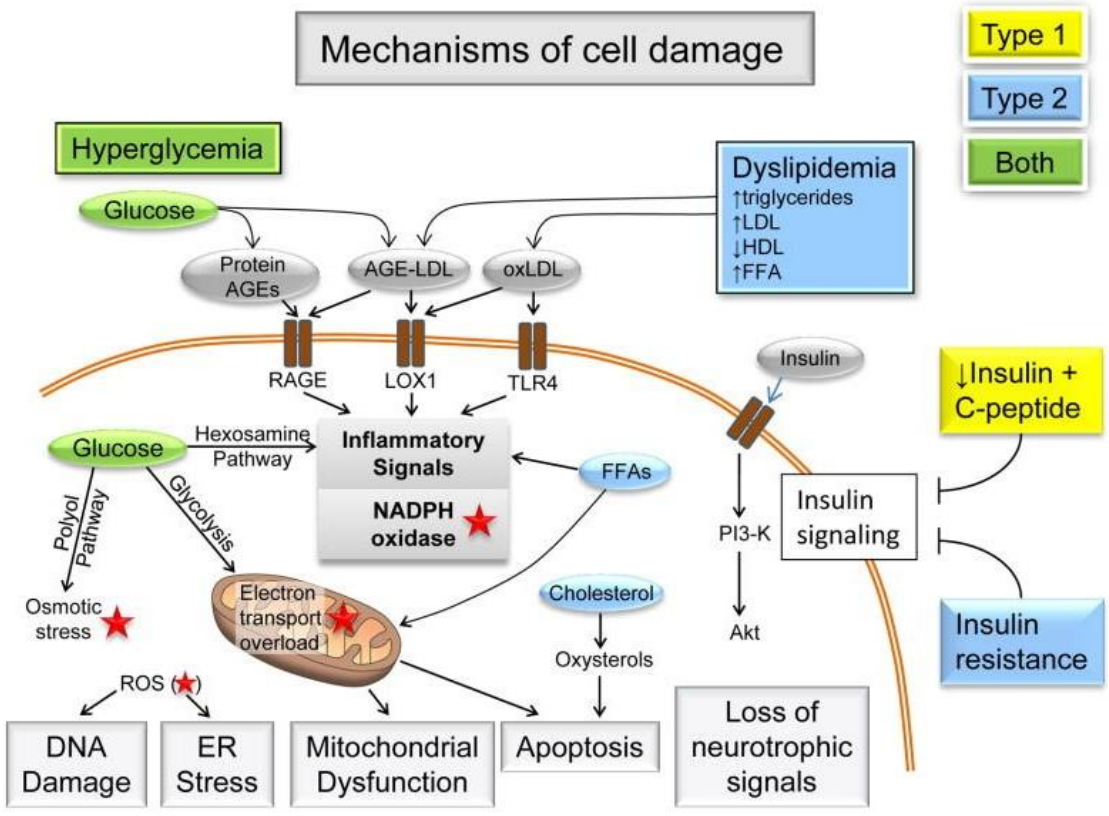
The traditional hypothesis of the pathogenesis underlying DPN includes *prolonged hyperglycemia* causing activation of the polyol pathway and the hexosamine pathway, mitochondrial dysfunction, and generation of advanced glycation end products all together leading to inflammation, oxidative stress, DNA damage and ultimately nerve injury.[13 67] Experimental studies suggest a more *extended metabolic pathogenesis* including also dyslipidemia and impaired insulin signaling (due to insulin resistance in type 2 diabetes) and resulting mitochondrial dysfunction, inflammation and oxidative stress.[13 67] Also, *vascular mechanisms*[68] including *capillary dysfunction*[69] may be part of the pathogenesis through impaired vascular supply of the nerves, disturbed capillary flow patterns, and nerve hypoxia. However, despite the prevailing view of a multifactorial pathogenesis of DPN, a complete understanding of the causes of DPN is lacking.

Metabolic syndrome factors like obesity, hypertension, insulin resistance, and dyslipidaemia as well as related factors like low-grade inflammation have been linked to peripheral polyneuropathy in clinical and epidemiological studies.[43 70-84] Of these, the most convincing evidence exists for obesity.[43 72-76 82 83] Both general obesity (reflected by body mass index [BMI]) and central obesity (reflected by waist circumference, waist-hip-ratio, and waist-to-height ratio) have been linked to DPN in type 2 diabetes,[75 76 82] but the underlying mechanisms remain unclear. Visceral fat accumulation is accompanied by low-grade inflammation, dyslipidemia, and hyperinsulinemia[85] and has been shown to predict other diabetes complications independent of – and better than - BMI in type 2 diabetes and in general populations.[86-88] No study has investigated the association of DPN and central obesity independent of general obesity in type 2 diabetes. The evidence of the relationship between peripheral neuropathy and other metabolic syndrome-related factors like hypertension, dyslipidemia and insulin resistance is less consistent across studies,[66 72-74 79 81 82 84 89] but these factors may also be important risk factors for DPN in type 2 diabetes.[65] Additionally, unhealthy lifestyle e.g. tobacco smoking, alcohol overconsumption (of note, also considered a cause of non-

diabetic peripheral polyneuropathy) and low levels of physical activity may also be potential modifiable factors associated with DPN.[66 72 90]

However, many of the previous studies investigating risk factor and polyneuropathy associations have been based on *exclusively type 1 diabetes populations*[72] *or mixed study populations* e.g. mixed type 1 and type 2 diabetes patients,[43 68 75] or mixed diabetes and non-diabetes populations.[71 73 74 76 84] The latter implies that peripheral polyneuropathy and DPN are investigated as one common disease. In light of the hypothesis of DPN being two different diseases in type 1 and type 2 diabetes, studies of potential risk factor associations in

Figure 2. *Pathogenic mechanisms underlying diabetic polyneuropathy. Factors linked to type 1 diabetes (yellow), type 2 diabetes (blue), and both (green) cause mitochondrial dysfunction, endoplasmatic reticulum stress, DNA damage, and nerve injury. Reprinted and adapted from Callaghan et al*[13]*, with permission from Elsevier.*



ER = endoplasmatic reticulum, AGE = advanced glycation end products, LDL = low-density lipoprotein, HDL, high-density lipoprotein, FFA = free fatty acids, RNS = reactive nitrogen species, ROS = reactive oxygen species, P13-K = phosphatidylinositol-3-kinase,

type 2 diabetes populations are of interest. Also, some studies specifically investigated *the metabolic syndrome or the number of metabolic syndrome factors* but not exactly which of the factors that contributed to the association with DPN.[70 73 78 84]
Moreover, studies have often been based on *long-standing diabetes*[43 70 77] rather than recently diagnosed diabetes patients in which the potential for preventing complications may be largest. Finally, many studies are of *smaller size*.[73 75 79 80]

Only some patients with DPN develop painful DPN and the reason for this is still unknown. Knowledge on the potential differences of patient characteristics associated with painful DPN as compared to non-painful DPN could possibly contribute to gain more insight in the answer of this question. Previous studies point towards some potentially shared characteristics e.g. obesity, but results are inconsistent.[91 92] The most consistent finding is that painful DPN associates with more severe DPN.[91-94] Many existing studies are limited by the use of *non-validated assessment* of neuropathic pain.[55 56 58 91 95 96] Also, many studies of painful DPN suffer from either *uncertainty of the control group or the use of patients without DPN as control group.*[43 56 58 91 96 97] These studies do not allow to disentangle whether an observed risk factor association is related to DPN itself or to neuropathic pain. Finally, given the fact that risk factors for DPN in *type 1 and type 2 diabetes* may differ, it is relevant to distinguish between these patient classes in studies of associations of potential risk factors and painful DPN as well.[60]

In summary, there are gaps in the current knowledge on risk factors associated with DPN and painful DPN in type 2 diabetes. Currently, no disease-modifying treatment for DPN exists,[11] thus, identifying modifiable risk factors for DPN in patients with type 2 diabetes is important in order to prevent DPN development.
We hypothesized, that in recently diagnosed type 2 diabetes patients, central obesity is strongly – and independently of general obesity – associated with DPN. We also hypothesized that other metabolic factors and unhealthy lifestyle is associated with DPN as well, and that distinct metabolic factors may be associated with painful versus non-painful DPN.

1.3.4 Quality of life and mental health

Diabetes is associated with reduced quality of life (QoL)[98] and the prevalence of depression is doubled in patients with type 2 diabetes compared to those without diabetes.[99] Recently, in a study of type 2 diabetes patients in 14 countries, 17% reported moderate/severe levels of depressive symptoms.[100] Neuropathic pain and painful DPN have been linked to reduced QoL, poor sleep, and symptoms of depression and anxiety in diabetes patients in several studies[43 55 57 93 101-104] and likely counts for some of the higher prevalence of these conditions in diabetes patients. Vileikyte *et al.* reported that pain, symptoms of decreased sensitivity in the feet, and unsteadiness each was independently associated with symptoms of depression among diabetes patients with moderate to severe neuropathy, thus linking DPN itself – not only neuropathic pain – to depressive symptoms. Other smaller studies supported that both painful and non-painful DPN are associated

with depression.[105] In contrast, a large study only found an association of reduced QoL with painful DPN, whereas no relation with non-painful DPN was observed,[43] and in general less is known about the impact of DPN itself – independent of neuropathic pain – on QoL, sleep disturbance and symptoms of anxiety. Moreover, existing literature is limited by *small study size*[55 93 103-106] and inclusion of *both type 1 and type 2 diabetes patients*,[43 57 93 101-105] where the underlying prevalence of reduced QoL and of mental health comorbidities may differ due to the substantial differences of these two types of diabetes (i.e. living with a chronic disease since childhood/youth, diabetes duration, prevalence of insulin use and risk of hypoglycemia, comorbidities including obesity). Moreover, *other pain* often coexists with neuropathic pain and may also have an impact on QoL and mental health, however, studies have seldom adjusted for other pain.[43 101-106] We hypothesized, that painful DPN and DPN itself (independently of neuropathic pain) is associated with reduced QoL, sleep disturbances, and symptoms of depression and anxiety.

1.4 Register-based research and diagnostic validity

Denmark has a very long tradition of registering health-related data in numerous medical databases (i.e. administrative, health, and clinical care databases).[107] All these data can be accurately linked due to a unique central personal registration (CPR) number assigned to all Danish residents assigned at birth or upon immigration.[108] These databases may offer a great potential for studying DPN risk and prognosis in a cost-effective manner, however, a premise is that DPN and its complications like diabetic foot ulcers can be validly identified. In the International Classification of Diseases (ICD) system, diabetic neuropathy can be coded using diabetes-specific neuropathy diagnosis codes e.g. G63.2 *diabetic polyneuropathy*, G59.0 *diabetic mononeuropathy* or it can be coded using neuropathy-specific diagnosis codes in diabetes patients like G62.0 *polyneuropathy unspecified* or G56.9 *mononeuropathy of upper limb*. The validity of diagnosis-, procedure-, and drug prescription coding for DPN and diabetic foot ulcers have been evaluated in a few existing studies. An ICD-9 based algorithm for painful diabetic *peripheral* neuropathy, i.e. including non-polyneuropathy conditions like diabetic mononeuropathy, revealed a positive predictive value (PPV) of 79%.[109] A second study reported a PPV of 91.4% of the diabetes-specific *poly*neuropathy code.[110]
One study validated 5 different methods to identify diabetic foot ulcers based on ICD-9 diagnosis codes alone or in combination with Current Procedural Terminology, edition 4 (CPT-4) procedure codes. The methods varied in complexity ranging from requiring only one diagnosis code (707.1x, ulcer of lower limb) to the most complex method requiring a procedure code in addition to a diagnosis code (for most diagnosis codes).[111] PPVs ranged from 62-82%.[111] In a study of patients with multiple diabetes complications a PPV of 88.5% for the ICD-9 diagnosis code for ulcer of the lower limb (707.1x) was reported.[112]
Thus, existing evidence is somewhat encouraging with regard to the use of medical databases in DPN research, although one of the studies did not report data on diabetic *poly*neuropathy[109] and only reported on *painful* peripheral neuropathy.[109] Nevertheless, the coding practices used in a tax-supported uniform Danish

healthcare system, may not necessarily be similar to those applied in the ICD-9-based[109-112] and CPT-4-based[111] systems as well as in populations selected on private insurance plans[109] or professions[111]. A large number of validation studies on various diseases have been performed on the Danish National Patient Registry,[113 114] and in general the validity is high, but the validity of DPN and diabetic foot ulcer coding has not been studied.

We hypothesized, that the positive predictive values of non-painful and painful DPN as well as diabetic foot ulcers identified by the use of diagnosis codes, surgery codes, and drug prescription codes in Danish registries are high.

2. Aims

The specific aims for the four studies included in this dissertation were:

Study I To provide a detailed description of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort including biobank data and linked register data.

Study II To examine the prevalence of DPN and painful DPN in recently diagnosed type 2 diabetes patients, to examine patient characteristics associated with DPN and painful DPN, and to investigate the impact of DPN and painful DPN on mental health in recently diagnosed type 2 diabetes.

Study III To clarify the association of obesity, other metabolic risk factors, and lifestyle factors assessed at type 2 diabetes diagnosis with prevalence of DPN at a median of 2.8 years later, and to identify factors associated with painful DPN.

Study IV To investigate the positive predictive values of hospital-diagnosed DPN - both non-painful and painful – and diabetic foot ulcers using diagnosis codes, surgery codes, and drug prescription codes in Danish registries.

Study I will be an integrated part of the Methods section since it describes the cohort on which study II and III were based.

3. Methods

Table 1 provides an overview of the studies.

3.1 Setting

Denmark has a tax-financed health care system ensuring free access to health care for all residents including partial reimbursement for most prescription drug costs.[108] The general practitioners (GPs) are the fundament of the primary health care sector and are responsible for nearly all referrals to the secondary health care sector.[107] Health and social services are comprehensively documented at an individual level in various registers. As already mentioned, data can be unambiguously linked using the unique and permanent CPR-number.[108] In Denmark, an estimated 80% of type 2 diabetes patients are treated at the GPs' office, while the remainder receive diabetes care at the hospital outpatient clinics.[115]

3.2 The International Diabetic Neuropathy Consortium

The International Diabetic Neuropathy Consortium (IDNC) was established in May 2015 owing to a six-year Challenge Grant from the Novo Nordisk Foundation.[116] The IDNC is an international interdisciplinary consortium consisting of basic, clinical and epidemiological researchers from Aarhus and Odense, DK, Michigan, US, and Oxford, UK. Overall, the IDNC aims to perform a detailed investigation of DPN including the pathophysiology, epidemiology and risk factors, and the clinical profile taking advantage of existing (e.g. DD2) data and new collected data (e.g. the IDNC/DD2 questionnaire data).[116]

3.3 Data sources

3.3.1 Administrative and health care databases

The Civil Registration System (CRS)[108] was established in 1968 and is responsible for assigning the CPR-number. The CRS is updated on a daily basis and holds dates of birth, death, immigration, and emigration as well as civil status, place of residence, and family relationship (children, partner, and parents).

The Danish National Patient Registry (DNPR)[113] has recorded data on all non-psychiatric hospital admissions since 1977, on non-psychiatric outpatient specialist clinics and emergency room visits since 1995, and on all psychiatric hospital contacts from 1995 onwards (inpatient, outpatient, emergency room). Data collected include e.g. admission/discharge dates, type and date of surgery (according to the Nordic Medico-Statistical Committee [NOMESCO] classification of surgical procedures since 1996), info on major treatments and procedures (since 1999) and one primary discharge diagnosis (the primary reason for contact) and, if relevant, a number of secondary diagnoses. All diagnoses were coded according to the ICD, 8th revision until the end of 1993 and the ICD-10 thereafter.

Table 1: *Summary of materials and methods.*

	Study I	Study II	Study III	Study IV
Objectives	To provide a detailed description of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort including biobank data and linked register data.	To examine the prevalence of DPN and painful DPN in recently diagnosed type 2 diabetes, to examine patient characteristics associated with DPN and painful DPN, and to investigate the impact of DPN and painful DPN on mental health in recently diagnosed type 2 diabetes.	To clarify the association of central and general obesity and a range of metabolic and lifestyle factors with DPN and with neuropathic pain occurrence in early type 2 diabetes.	To investigate whether hospital-diagnosed DPN, both non-painful and painful DPN – and diabetic foot ulcers can be accurately identified using diagnosis codes, surgery codes, and drug prescription codes in Danish registries.
Setting	Denmark, 2010-2016.	Denmark, 2016.	Denmark, 2010-2016.	Central Denmark Region, 2009-2016.
Design	Cross-sectional.	Cross-sectional.	Cross-sectional.	Cross-sectional validation study.
Data sources	DD2 core data, DD2 biobank data, and linked Danish health register data (CRS, DNPR, DNHSP, DDDA)	Neuropathy questionnaire data	Neuropathy questionnaire data, DD2 core data, DD2 biobank data, and linked Danish health register data (CRS, DNPR, DNHSP, DDDA)	Danish health register data (CRS, DNPR, DNHSP) and medical record data.
Study population	All DD2 patients enrolled by February 2016, N = 7,011.	All DD2 patients enrolled by February 2016 returning a filled out questionnaire, N = 5,514 (prevalence part), and all patients with valid data on DPN/painful DPN (patient characteristics/mental health part), N = 5,249.	All DD2 patients enrolled by February 2016 with valid data on DPN and neuropathic pain, N = 5,249. Subpopulation linkable to the DDDA, N = 3,623.	Randomly selected validation cohorts for diagnosis/prescription-based definitions of non-painful DPN (N = 60), painful DPN (N = 60), and diabetic foot ulcer (N = 60).
Exposures	-	-Risk factor part: age, gender, diabetes duration, alcohol consumption, smoking, BMI, height.* -Mental health part: DPN, painful DPN.	-General and central obesity measures. -Low-grade inflammation, c-peptide (proxy for insulin resistance), HbA1c, blood pressure, lipid levels, medication, macro-microvascular complications, albumin/creatinine ratio. -Physical activity level (baseline + change), alcohol (baseline), smoking (baseline + change).	The diagnosis/prescription-based definitions of non-painful DPN, painful DPN, and diabetic foot ulcer.
Outcomes	Description of patient characteristics registered in the DD2.	-Prevalence of DPN/painful DPN. -Risk factor part: DPN, painful DPN.* -Mental health: QoL, depression, anxiety, sleep disturbance.	DPN, neuropathic pain occurrence in those with DPN = painful DPN.	Positive predictive value of diagnosis and prescription codes.
Statistical analysis	Descriptive data.	Descriptive data. Calculation of prevalence. Logistic and linear regression.	Descriptive data. Log-binomial and Poisson regression.	Calculation of PPVs.

**As described in section 4.8 and further discussed in section 5.2.5, we included DPN and painful DPN as the independent variables in the regression models used to examine the association of patients characteristics with DPN and painful DPN. However, DPN and painful DPN was interpreted as the outcomes*.

The Danish National Health Service Prescription Database (DNHSP)[117] has collected individual-level data on filled prescriptions of reimbursable drugs since 2004. Data includes CPR-number, prescriber-related information, and dispensing details (e.g. date of dispensing, type of drug according to the Anatomical Therapeutic Chemical [ATC] classification system, and strength and amount of drug).

The Danish Diabetes Database for Adults (DDDA)[118] is a national clinical quality database established in 2005 with the purpose of monitoring the quality of care given to diabetes patients. Data on a number of specified variables (e.g. anthropometric measures, blood pressure, glycemic control, lipid levels, smoking habits) are provided from GP offices and hospital outpatient clinics annually or biennially. Reporting from the GP offices became mandatory in 2013, but discontinued in 2014 due to legal issues concerning automated data transmission.

3.3.2 The Danish Centre for Strategic Research in Type 2 Diabetes Cohort (Study I)[119]

The DD2 cohort was established in 2009, the first patient was enrolled in November 2010 and enrolment is still ongoing today. The overall goal of the DD2 project is to provide a large and data-rich cohort of newly diagnosed type 2 diabetes patients that can serve as a resource for extensive type 2 diabetes research within many fields - including studies of type 2 diabetes complications. The DD2 patients are patients diagnosed with type 2 diabetes in the routine clinical practice relying on the WHO criteria for diabetes. Enrolment into the DD2 cohort can take place from either the GPs or the hospital specialist outpatient clinics (by 2016, 53% and 47%, respectively) throughout Denmark. At time of enrolment, the physician/nurse completes a registration form including interview items e.g. weight at 20 years of age, family history of diabetes, info on selected lifestyle factors as well as a few items requiring a physical examination e.g. measure of waist- and hip circumference (DD2 core data). Finally, urine- and fasting blood samples are collected and stored in a corresponding biobank and have currently been examined for a number of variables including c-peptide and high-sensitivity C-reactive protein (hsCRP). The DD2 aimed at developing a flexible, simple and fast enrolment procedure that could be implemented as part of the everyday clinical practice in order to enroll as many newly diagnosed type 2 diabetes patients as possible. Thus, the DD2 core data collected at baseline was kept to a minimum and substantial additional baseline and follow-up data has been achieved through linkage with the Danish health registers including DDDA (Appendix I; overview of data collection available in figure 2). Moreover, a number of projects within the DD2 cohort has been initiated and adds additional data. Such a project is the IDNC/DD2 neuropathy questionnaire survey in 2016.

3.3.3 The IDNC/DD2 neuropathy questionnaire survey

In 2016, as part of this thesis work, we conducted a questionnaire survey among all alive DD2 patients with valid addresses (N = 6,267). The main purpose was to gain knowledge on DPN, including presence of neuropathic pain and mental health to be used in study II and study III (Appendix II; full questionnaire

available in Supplementary Table 1). Besides forming the basis for my own and other epidemiological studies, the IDNC/DD2 neuropathy questionnaire data also served as material for recruiting patients for a number of clinical studies on DPN performed by the IDNC-researchers. Two reminders were sent and the total response rate was 86% (N = 5,755). Of these, 5,514 (82%) returned one partly or fully filled questionnaire. The IDNC/DD2 questionnaire included questions on weight, height, lifestyle i.e. tobacco smoking, alcohol, physical activity, neuropathy, QoL and other mental health measures, and a number of questions related to pain, in particularly pain in the feet.

3.3.3.1 Neuropathy and pain - scales included in the IDNC/DD2 questionnaire

The Michigan Neuropathy Screening Instrument (MNSI) is a screening tool developed to identify DPN.[18] The MNSI consists of two parts; a self-administered questionnaire assessing subjectively defined DPN and a minor clinical examination assessing objectively defined DPN. These two parts can be used in conjunction, but has also been validated for use individually. A score of ≥4 on the Michigan Neuropathy Screening Instrument questionnaire (MNSIq) has a specificity of 92% and a sensitivity of 40% in detecting clinically confirmed DPN[19] and was included in the IDNC/DD2 neuropathy questionnaire after double translation into Danish.

The Douleur Neuropathique en 4 Questions (DN4) is a screening tool used to identify neuropathic pain.[33] It includes a minor clinical examination part and a questionnaire part. The 7-item DN4 questionnaire has been validated independently of the clinical examination part and a cut-off of a score ≥3 has a high diagnostic accuracy for identifying neuropathic pain in diabetic polyneuropathy with sensitivity and specificity of 84%.[34] We included the DN4 questionnaire in the IDNC/DD2 neuropathy questionnaire.

3.3.3.2 Mental health – scales included in the IDNC/DD2 questionnaire

We included the Patient-Reported Outcomes Measurement Information System (PROMIS®) 4-item short forms for anxiety, depression, and sleep disturbances after validated translation into Danish.[120] These instruments grade symptoms experienced during the previous seven days using a 5-category response scale e.g. from "bad" to "very god" or from "never" to "always". The resulting scores are converted into PROMIS T-scores that are standardized relative to an American general population with a mean T-score of 50.[121 122] Moreover, the T-scores enable a categorization of the level of symptoms/impairment.[121 122] To assess QoL within the previous seven days, we included an 11-item numeric rating scale (NRS) ranging from 0 (worst QoL possible) to 10 (best QoL possible).

3.3.4 Medical records

For study IV, a manual audit of the medical record from the discharging department was performed for each of the persons included in the validation cohorts.

3.4 Definitions of diabetic polyneuropathy

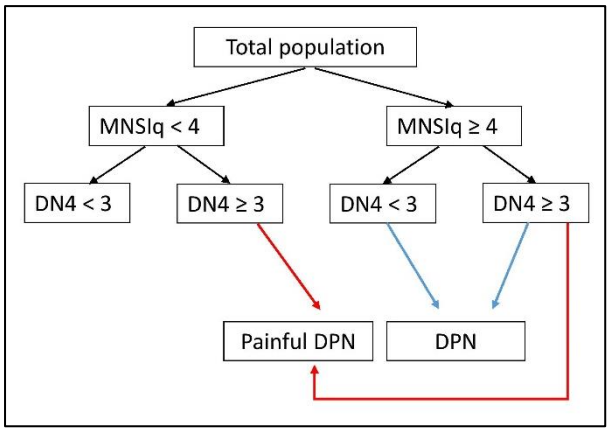
3.4.1 Study II

We used the validated cut-off of a MNSIq-score ≥4 abnormal responses to define DPN.[19]

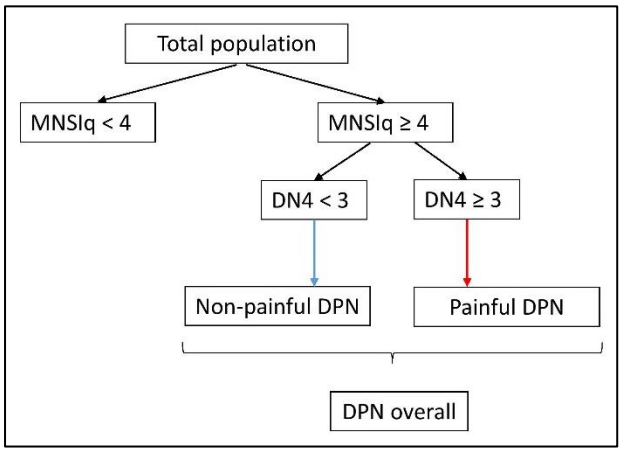
We applied the criteria for possible neuropathic pain defined by the international consensus statement (NeuroPPIC) for genetic studies and the grading system by NeuPSIG:[26 123] 1) pain with neuropathic description (in our study: DN4 ≥3), 2) a history of a relevant disease or somatosensory lesion (in our study: diabetes), and 3) a neuroanatomical plausible distribution of neuropathic pain (in our study: both feet). In line with this definition, in study II, we defined painful DPN as existence of pain in both feet together with a score ≥3 on the DN4 questionnaire, regardless of MNSIq score. As a consequence of this definition, some patients in study II fulfilled the criteria for painful DPN, but not the criteria for DPN (Figure 3, panel a).

Figure 3. *Schematic overview of DPN, non-painful, and painful DPN definitions in study II and study III. DN4 <3 means either no pain in feet or pain in combination with DN4 <3. DN4 ≥3 means pain in feet in combination with DN4 score ≥3.*

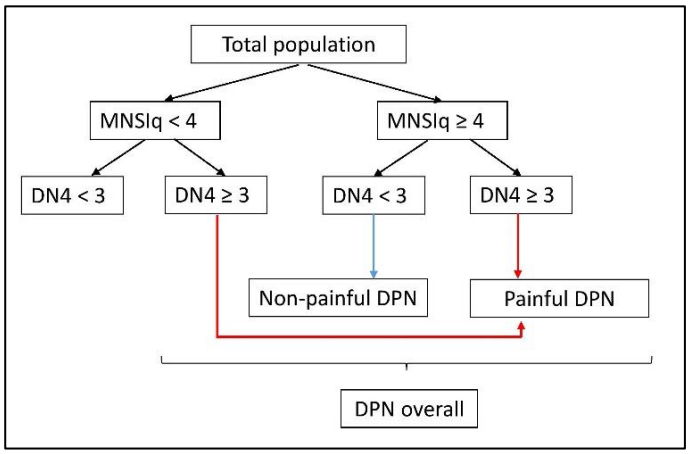
a) Study II



b) Study III, main analyses



c) Study III, sensitivity analyses



3.4.2 Study III

In study III, main analyses, we used a more intuitive definition. Thus, DPN was still defined as a MNSIq-score ≥4. Non-painful and painful DPN was distinguished based on presence or absence of neuropathic pain (DN4 ≥3) in both feet (Figure 3, panel b). In sensitivity analyses, we allocated the small group of patients with MNSIq <4 and neuropathic pain (DN4 ≥3) in both feet to the painful DPN group (Figure 3, panel c). The definitions used in study II and study III enable a diagnosis of DPN and painful DPN at the level of "possible" according to the current grading systems (see section 1.3).[26 93 123]

3.4.3 Study IV

We defined potential DPN as an in- or outpatient hospital diagnosis indicative of DPN among patients with type 2 diabetes (see Table 2 for further details, including diagnosis codes). Both primary (i.e. the primary reason for the hospital contact) and secondary diagnoses were included. DPN-patients who had redeemed at least one prescription for an anti-epileptic medication or a SNRI/TCA[11] used to treat neuropathic pain within the preceding year, and up to half a year after, the DPN diagnosis, were considered to have potential painful DPN, except if they had a diagnosis recorded in the DNPR that was considered an exclusion criteria. Such exclusion diagnoses were epilepsy and depression/anxiety for those with prescription redemption for anti-epilepsy medicine and SNRI/TCA, respectively. Opioid prescriptions were not used to identify DPN patients with neuropathic pain since 1) opioids are not recommended as first/second-line therapies for painful DPN due to safety concerns and high risk of addiction,[10 36] and 2) opioids are used for many other pain-conditions that cannot be validly identified and excluded. Since some DPN patients may develop neuropathic pain over time, a patient could be included in the potential non-painful DPN population and at a later and distinct point in time in the potential painful DPN population.

3.5 Definition of diabetic foot ulcer

We defined potential diabetic foot ulcer as at least one in- or outpatient hospital discharge diagnosis code indicative of foot ulcer or at least one surgery code suggesting a surgical procedure relevant for treating diabetic foot ulcer among patients with type 2 diabetes (see Table 2 for further details). Both primary and secondary discharge diagnosis codes were used.

Table 2. *In- and outpatient discharge codes and prescription codes used to identify patients with painful and non-painful DPN and diabetic foot ulcer.*[124]

Type 2 diabetes[a]
≥ 1 diabetes discharge code (E10-E14, H36.6, O24 [except O24.4], G63.2)
OR
≥ 1 prescription of a glucose-lowering drug (ATC: A10)[b]
Potential DPN:
E-chapter codes
Type 2 diabetes *plus* ≥ 1 discharge code for "diabetes with neurological complication" (E10.4, E11.4, E12.4, E13.4, E14.4)[c]
OR
G-chapter codes
Type 2 diabetes *plus* ≥ 1 discharge code for "diabetic polyneuropathy" (G63.2)
OR
Type 2 diabetes *plus* ≥ 1 discharge code for "polyneuropathy, unspecified" (G62.9)
Potential painful DPN algorithm:
DPN *plus* ≥ 1 prescription code for antiepileptic drugs *minus* an epilepsy discharge code (G40+G41)
OR
DPN *plus* ≥ 1 prescription code for antidepressants (SNRI/TCA) *minus* a depression/anxiety discharge code (F30-F34, F40-42, F48.8 + F48.9)
Potential non-painful DPN algorithm:
DPN patients that do not fulfil the criteria for painful DPN
Potential diabetic foot ulcer:
Type 2 diabetes *plus* ≥ 1 discharge code for "diabetes with peripheral vascular complication" (E10.5, E11.5, E12.5, E13.5, E14.5)
OR
Type 2 diabetes *plus* ≥ 1 discharge code for "ulcer" (L97, L98.4, R02)
OR
Type 2 diabetes *plus* ≥ 1 discharge code for "osteomyelitis" (M86)
OR
Type 2 diabetes *plus* ≥ 1 surgery code for surgery of lower extremity (KQDA, KQDB, KQDG)

[a]All patients younger than 30 years at diagnosis treated with insulin monotherapy were excluded in order to minimize misclassification of type 1 diabetes patients. [b]Except females aged 20-39 prescribed metformin exclusively in order to minimize misclassification of patients with polycystic ovarian syndrome. [c]Excluding patients with ICD-10 codes for G73.0 amyothrophy, G99.0 autonomic neuropathy, G59.0 diabetic mononeuropathy. Abbreviations: DPN; Diabetic polyneuropathy, ICD-10; International classification of diseases, version 10, SNRI; Serotonin-noradrenalin reuptake inhibitors, TCA; Tricyclic antidepressants

3.6 Study designs and study populations

All studies included in this dissertation are cross-sectional studies. The study population in study I was all DD2 patient enrolled by February 2016 (for overview, see flowchart in the result section of this thesis). Study II was based solely on the IDNC/DD2 neuropathy questionnaire data and the study population consisted of all patients, who returned a filled out questionnaire (prevalence part) and all patients with valid

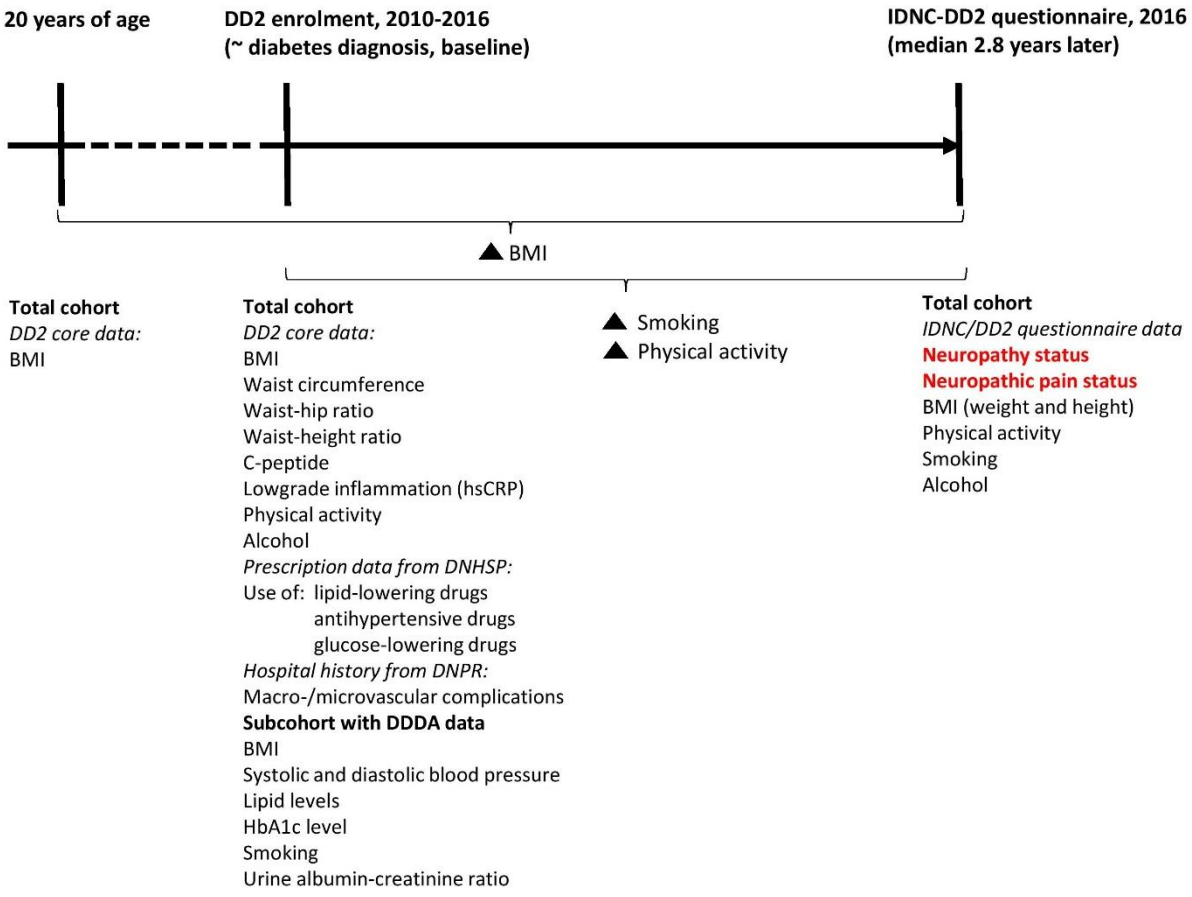
data on criteria for both DPN and neuropathic pain (patient characteristics/mental health part) as described above. Study III relied on the IDNC/DD2 questionnaire data together with additional DD2 core data and linked register data. The study population was similar to the study population for the patient characteristics/mental health part of study II, and consisted of all patients with valid data on both DPN and neuropathic pain. Analyses based on DDDA variables were restricted to a sub cohort (69%) linkable to the DDDA. Finally, study IV (validation study) was based on health register data and medical record data. The study population consisted of three randomly selected validation cohorts (N = 60 in each cohort) of type 2 diabetes patients that had been discharged from one university hospital or four regional hospitals in the Central Denmark region, 2009-2016, with a diagnosis of either non-painful DPN, painful DPN, or diabetic foot ulcer according to our definitions.

3.7 Potential risk factors

For study II, information on patient characteristics stemmed exclusively from the IDNC/DD2 neuropathy questionnaire. Data was collected simultaneous with the information on DPN and neuropathic pain at a median of 2.8 years after the DD2 enrolment date. Variables included height, BMI calculated from self-reported information on height and weight ([weight in kg]/[height in meters x height in meters]), alcohol overconsumption (> 7/14 units per week [female/male]), and smoking status (ever [current + former] vs. never) as well as biological sex, age, and diabetes duration achieved from the DD2 data.

In study III, we used additional DD2 data and the linked register data to study risk factor-DPN associations more comprehensively. Our main focus was the obesity- and metabolic profile as well as lifestyle profile at time of DD2 enrolment as a proxy for time at type 2 diabetes diagnosis (from now on referred to as *baseline*). For a few variables, we also used the data from the neuropathy questionnaire in order to perform analyses of the change of a risk factor between baseline and the IDNC/DD2 neuropathy questionnaire. Thus, obesity measures included BMI (measure of general obesity)[125] at three different time points; 1) recalled BMI at 20 years of age, 2) BMI at baseline, and 3) self-reported BMI at time of the IDNC/DD2 neuropathy questionnaire in 2016. We used waist circumference, waist-hip ratio, and weight-height ratio as measures of central obesity.[125] Metabolic risk factors of interest available for the total cohort and for the DDDA subcohort as well as the corresponding data sources are shown in Figure 4. For hsCRP we excluded values ≥10 mg/L since they may represent ongoing infection;[126 127] physical activity was measured as day per week with more than 30 minutes of physical activity (official recommendation from the Danish Health Authority); we did not investigate changes in alcohol consumption from baseline to IDNC/DD2 questionnaire due to the use of different cut-offs at the two assessments (≥21/14 units vs. ≥14/7 units [male/female]); and we included information on antihypertensive- and lipid-lowering treatment since some patients may have normal blood pressure and lipid levels due to relevant pharmacological treatment.

Figure 4. *Schematic overview and timeline of assessment of obesity measure, other metabolic and lifestyle factors, and DPN-status.*



3.8 Validation – reference standard

We used medical record data as the reference standard in the validation study.

3.8.1 Diabetic polyneuropathy

Prior to the medical record review, we defined a checklist of symptoms, signs, and diagnostic test results that were used to confirm the diagnosis (Appendix IV; checklist available in table 2). Patients from the potential non-painful and painful DPN validation cohorts were categorized as having DPN if they fulfilled at least one of the following criteria: 1) at least one symptom of DPN (including neuropathic pain) in both feet, 2) at least one sign of DPN in both feet, 3) positive nerve conduction test supporting DPN, or 4) physician notes documenting presence of DPN. Moreover, it was noted if neuropathic pain was described in the medical record. Patients with a more likely cause of polyneuropathy than diabetes were not classified as having DPN.

Exceptions included alcohol overuse and vitamin B12 deficiency[128] unless it was explicitly stated that polyneuropathy was caused by these conditions.

3.8.2 Diabetic foot ulcer

An explicit notification of "diabetic foot ulcer" or at least one ulcer on the toes/feet in the absence of another more likely pathogenesis of foot ulcer than diabetes were used as criteria for confirming a diagnosis of diabetic foot ulcer.

3.9 Statistical analysis

In all four studies, we provided descriptive data of the total cohorts and in study II-IV also according to neuropathy groups.

In study II, we calculated the prevalence of DPN and painful DPN and corresponding 95% confidence intervals (CI) using the exact method for binomial distributions. For the risk factor part of study II, we used multivariable logistic (categorical patient characteristics) and linear (continuous patients characteristics) regressions. The cross-sectional study design precludes investigation of temporal relationships and the associations between patient characteristics and DPN were investigated while including DPN and painful DPN as independent variables in the models. This approach allowed us to include the two DPN-variables simultaneous in the same model, thus enabling investigation of the association of patient characteristics with painful DPN (neuropathic pain) independent of DPN and to examine whether interaction between the two methods of identifying DPN and painful DPN was present. Thus, each patient characteristic was modeled as a function of DPN, painful DPN, and an interaction term between DPN and painful DPN and if no statistically significant interaction was observed, the regressions were rerun without the interaction term. All analyses were adjusted for biological sex, age, and diabetes duration. For the mental health part in study II, we used a similar approach. Thus, we modelled QoL and T-scores for depression, anxiety, and sleep disturbance as functions of DPN, painful DPN, and an interaction term between DPN and painful DPN in linear regressions, while controlling for biological sex, age, diabetes duration, and BMI (model 1). Since pain other than neuropathic pain in the feet may possibly confound the associations, we reran all regressions including a variable for pain in other bodily locations (model 2). If no statistically significant interaction between DPN and painful DPN were observed, all models were repeated without the interaction term. We performed a sensitivity analysis in which we excluded BMI from the models, because the relationship between BMI and mental health outcomes may be bidirectional.

In study III, we calculated the prevalence proportion of DPN, non-painful DPN, and painful DPN. We used log-binomial and Poisson regressions (with robust error variance)[129 130] and calculated prevalence ratios (PRs) of DPN associated with each of the obesity measures and metabolic and lifestyle factors under study. We examined the continuous risk factors as both categorical and continuous variables using clinical relevant

cut-points/units. Moreover, for obesity measures we also used a unit of 1 SD for the continuous analyses in order to compare the magnitude of the association across central and general obesity. All PRs were adjusted for biological sex, age, and diabetes duration. The models for waist circumference, waist-hip ratio, and waist-height ratio were also adjusted for BMI in order to elaborate further on the association of central obesity and DPN.[125] The analyses of change of physical activity level (from baseline to DPN assessment at a median of 2.8 years later) were additionally stratified according to the baseline activity level. After restricting the cohort to those with DPN, we calculated the PRs of painful DPN associated with each of the risk factors under study in order to examine factors associated with neuropathic pain presence in DPN. In study III, we performed three sensitivity analyses. First, we repeated the analyses, while restricting the population to those with a registered diabetes duration <½ year and <1 year at baseline. The purposes of these analyses were to focus on newly diagnosed diabetes exclusively and to increase the proportion of patients with likely incident DPN at the subsequent DPN assessment. Second, we extended the DPN/painful DPN definition and included those with neuropathic pain (DN4 ≥3 and pain in both feet), but MNSIq score <4 (Figure 3, panel c). Third, we repeated the analyses after exclusion of patients with alcohol overconsumption, since peripheral neuropathy in these patients may be either DPN, alcoholic polyneuropathy or a mixture.

In study IV, we computed PPVs as an indirect measure of the specificity of the codes included in the algorithms. The PPVs were calculated as the proportion of the coded patients, who were classified as having the corresponding diseases according to the medical records. For the painful and the non-painful DPN algorithms, we estimated the PPVs for having DPN (either painful or non-painful). Additionally, we estimated the PPV for having painful DPN for the painful DPN algorithm and for having non-painful DPN with the non-painful algorithm. We stratified the analyses by administrative covariates (e.g. type of hospital, department, diagnosis codes), and we investigated other combinations of diagnosis codes.

In study II-IV, we calculated 95% confidence intervals to quantify precision of the estimates.

3.10 Ethical considerations

All studies were approved by the Danish Data Protection Agency. The DD2 project is approved by the Danish Scientific Ethical Committee. All DD2 patients volunteered to participate in the DD2 project and gave their written informed consent. For the validation study, we obtained permission from the Danish Health and Medicine Authorities and from the head of all participating departments to access medical records without individually informed patient consent.

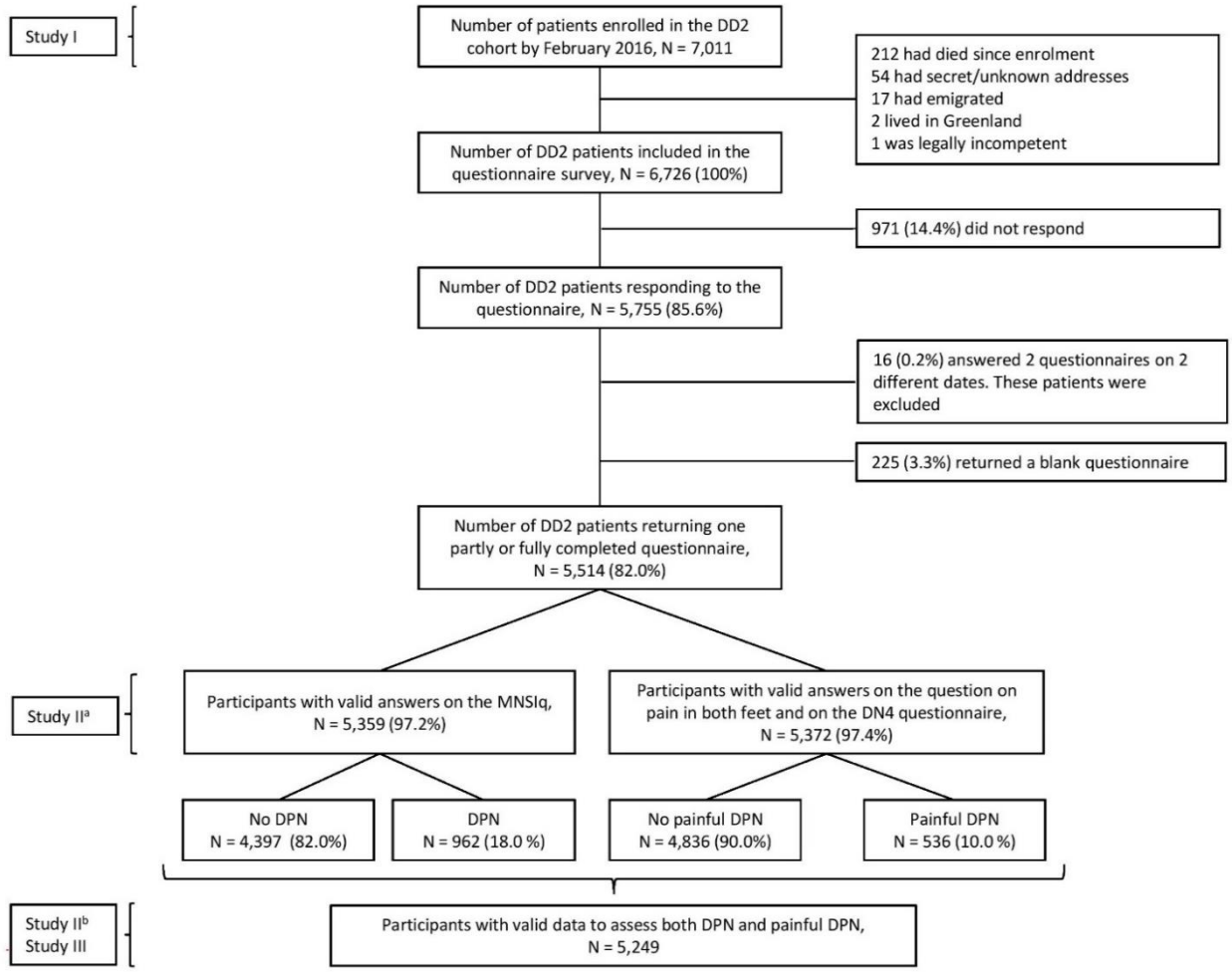
4. Summary of results

Main findings are summarized below. Results are reported in details in appendices I-IV.

4.1 DD2 cohort – patient characteristics

In study I-III, the total DD2 cohort and relevant subsets of the DD2 cohort are described at different time points (at DD2 enrolment and at time of the IDNC/DD2 neuropathy questionnaire in 2016). These data are available in the corresponding appendices I-III.

Figure 5. *Flowchart of study populations (study I-III). Study II[a] refers to the prevalence part of study II, while study II[b] refers to the risk factor associations part. "Valid answers" means that enough items had been answered to unambiguously allocate a given patient as having a MNSIq score ≥ or <4, example: a patient with ≥4 positive responses was allocated as having MNSIq score ≥4 even in case of missing answers to 1 or more of the remaining items encompassed by the MNSIq. Likewise for DN4.*

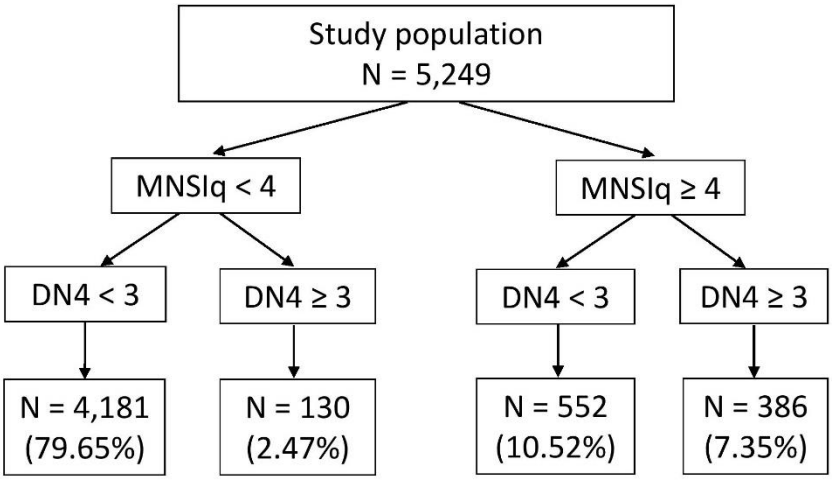


4.2 Prevalence (study II and II)

Of the 6,726 DD2 patients who were sent an IDNC/DD2 neuropathy questionnaire, 5,514 returned a partly or fully completed questionnaire (Figure 5). Among the 5,359 patients with data to assess DPN, 962 had a score ≥4, corresponding to a DPN prevalence of 18.0% (95% CI: 16.9; 19.0). The prevalence of painful DPN among the 5,372 with data to assess painful DPN was 10.0% (95% CI: 9.2; 10.8), corresponding to 536 patients. Of those with painful DPN, 130 patients had a MNSIq-score <4.

5,249 patients had valid data on both MNSIq, DN4, and pain location in both feet. Using the more intuitive definition of painful DPN in study III (Figure 3, panel b) resulted in a slightly lower prevalence of painful DPN compared to study II, i.e. 7.4% (Figure 6). In sensitivity analyses, the 130 patients with MNSIq <4 were added to the painful DPN group in accordance with the NeuPSIG definition (Figure 3, panel c), resulting in a prevalence of DPN (painful and non-painful) of 20.3% and of painful DPN of 9.8%.

Figure 6. *Distribution of the 5,249 patients included in the study population in study II (risk factor and mental health associations) and study III according to MNSIq and DN4 status.*



DN4 <3 means either no pain in feet or pain in combination with DN4 <3. DN4 ≥3 means pain in feet in combination with DN4 score ≥3.

4.3 Potential risk factors

4.3.1 IDNC/DD2 questionnaire (study II)

We did not observe any interaction between DPN defined by MNSIq and painful DPN defined by DN4 and pain location.

Table 3 shows the adjusted estimates. DPN was associated with female sex, younger age, longer diabetes duration, ever tobacco smoking, and higher BMI, whereas painful DPN only showed a clear association with ever tobacco smoking.

Table 3. *Associations between DPN and painful DPN and patient characteristics at questionnaire time, study II*

	Female	Ever smoking	Alcohol over-consumption	Age, year	BMI, kg/m²	Diabetes duration, year	Height, cm
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
DPN	1.24 (1.05; 1.46)	1.36 (1.14; 1.63)	0.94 (0.74; 1.18)	-1.90 (-2.78; -1.02)	1.67 (1.19; 2.14)	0.25 (0.06; 0.44)	0.43 (-0.11; 0.96)
Painful DPN	1.11 (0.90; 1.37)	1.52 (1.20; 1.92)	1.09 (0.81; 1.46)	0.45 (-0.68; 1.57)	0.35 (-0.26; 0.95)	0.06 (-0.18; 0.31)	0.21 (-0.47; 0.90)

The estimates are adjusted for age, sex, diabetes duration, and DPN or painful DPN.

4.3.2 DD2 and linked register data (study III)

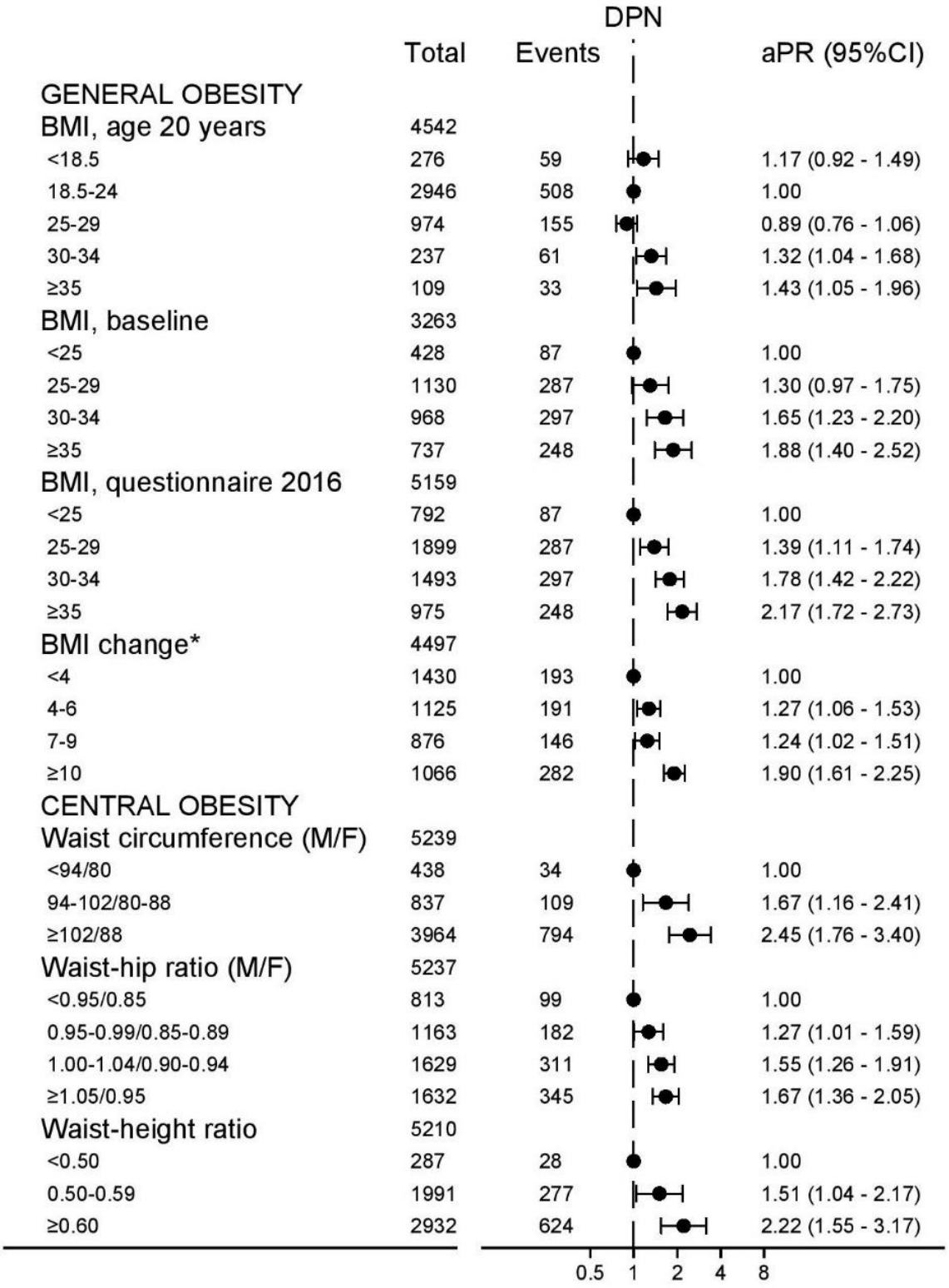
Adjusted PRs for DPN associated with the different obesity measures and other metabolic/lifestyle factors of interest are shown in Figure 7 and Figure 8, respectively, as well as in Appendix III, Supplementary Table 3-5.

4.3.2.1 Obesity

Higher BMI and increasing waist circumference, waist-hip ratio, and waist-height ratio were associated with increased prevalence of DPN in categorical and continuous analyses. The magnitude of the DPN associations did not differ for general and central obesity measures in analyses using 1 SD as unit (Appendix III; Analyses of continuous measures available in Supplementary Table 3).

All central obesity measures remained positively associated with DPN after further adjustment for BMI, thus for a given BMI the prevalence of DPN was higher with increasing central obesity, e.g. aPR was 1.85 (95% CI: 1.32; 2.60) for a waist circumference of ≥102/88 cm (male/female) vs. <94/80 cm (Appendix III; Data available in Supplementary Figure 3).

Figure 7. *Prevalence ratios of DPN associated with general and central obesity measures, study III*



All estimates are adjusted for age, sex, and diabetes duration.

4.3.2.2 Other metabolic and lifestyle factors

As shown in Figure 8, our results suggest that DPN is associated with a worse metabolic profile including lower high-density lipoprotein (HDL) cholesterol levels, higher triglycerides levels, low-grade inflammation, higher c-peptide levels (proxy for insulin resistance), higher hemoglobin A1c (HbA1c) levels, and use of antihypertensive drugs, though not high systolic and diastolic blood pressure. Moreover, sedentary lifestyle and smoking at baseline as well as continued smoking from baseline until DPN assessment were associated with higher DPN prevalence. Finally, presence of other diabetic complications (including micro-and macroalbuminuria), and use of insulin vs. other antidiabetic drugs were also associated with DPN (Appendix III, Supplementary Table 3-5)

4.3.2.3 Pain occurrence

The statistical precision in the internal analyses of neuropathic pain associations among DPN patients was limited. However, several metabolic factors seemed to associate with neuropathic pain presence in DPN including dyslipidemia (total cholesterol, low-density lipoprotein [LDL] cholesterol, and triglyceride), central obesity (waist circumference and waist-hip ratio), and high systolic blood pressure. Moreover, being a smoker, high alcohol intake, and decreasing activity level from baseline to DPN assessment were associated with neuropathic pain presence (risk estimates are available in appendix III, Table 1 and Supplementary Table 6-8).

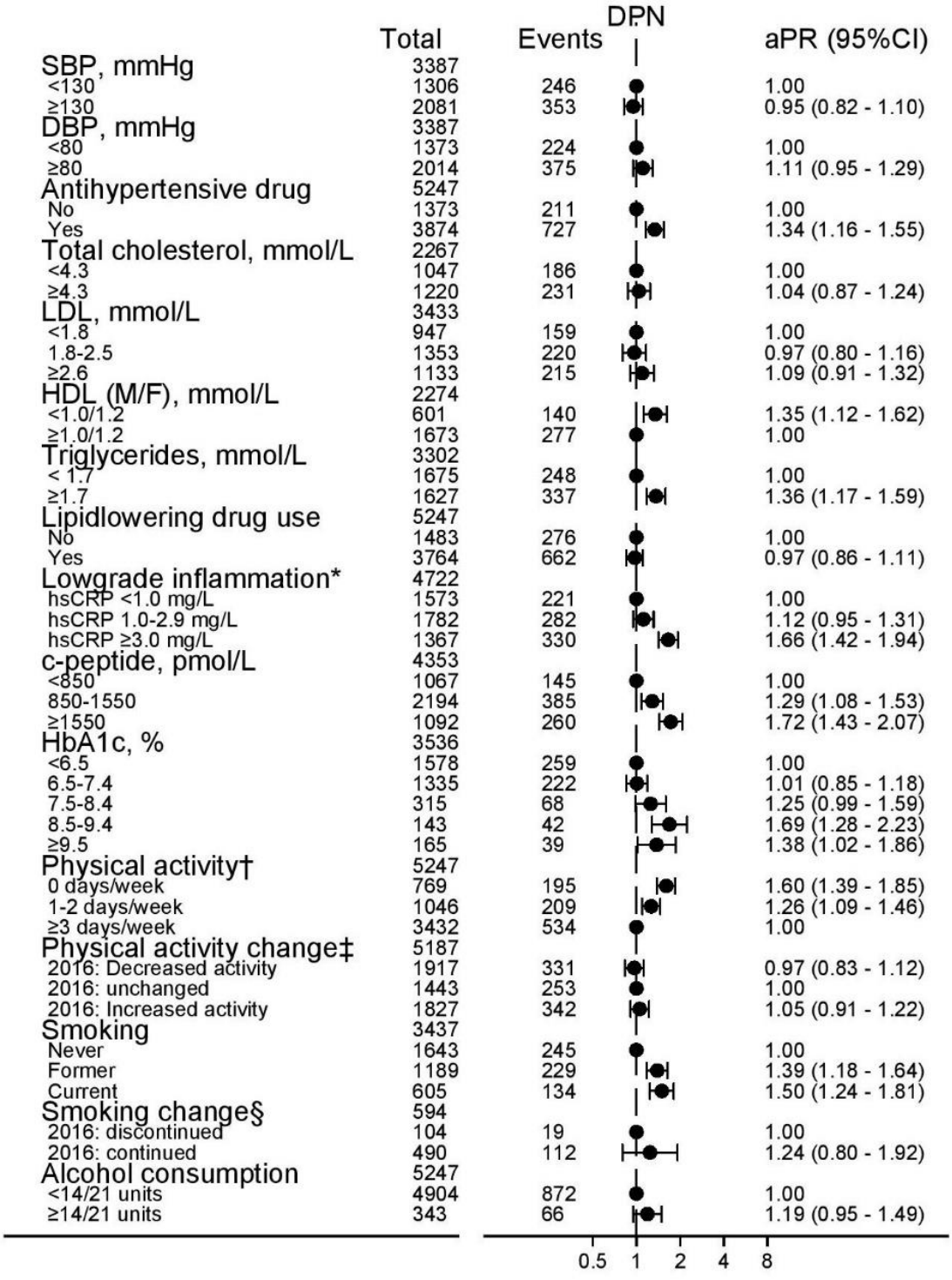
4.3.2.4 Sensitivity analyses

Restricting the cohort to those with <½ year and <1 year of diabetes duration at baseline in general supported the findings from the main analyses, but with reduced statistical precision (data not shown). High systolic blood pressure was an exception and associated with low DPN prevalence; <½ year diabetes duration (aPR 0.59 [0.43; 0.81]) and <1 year diabetes duration (aPR 0.79 [0.61; 1.02]).

Using the extended painful DPN definition (Figure 3 panel c), led to inclusion of an additional 130 (2.5%) patients with painful DPN. These patients were more similar to those without any DPN with regard to risk factor profile and therefore most relative estimates were slightly reduced in these sensitivity analyses (data not shown), however, did not lead to changes in our conclusions.

Excluding DPN-patients with alcohol overconsumption did not change the estimates, thus not leading to any changed conclusions (data not shown).

Figure 8. *Prevalence ratios of DPN associated with metabolic and lifestyle factors at baseline (time of DD2 enrolment ~ type 2 diabetes diagnosis), study III.*



**hsCRP values above 10 mg/L were excluded in order to exclude values reflecting ongoing infections †Days per week with minimum 30 minutes of physical activity. ‡Change from baseline to IDNC/DD2 questionnaire. §Among those who were current users at baseline. All estimates are adjusted for age, sex, and diabetes duration.*

4.4 Mental health (study II)

We did not observe interaction between DPN and painful DPN.

DPN and painful DPN were independently and additively associated with lower QoL and higher T-scores of depression, sleep disturbances, and anxiety (Table 4). The associations were stronger for DPN than painful DPN, e.g. for depression, DPN: β: 4.20 (95% CI: 3.54; 4.86) and painful DPN: β: 3.35 (2.51; 4.18). Additional adjustment for pain in other bodily localizations attenuated all associations, however, did not change any conclusions, e.g. for depression, DPN: 2.96 (95% CI: 2.32; 3.61), painful DPN: 2.12 (95% CI: 1.30; 2.93). The estimates increased slightly when BMI was left out of the models (Appendix II: Data available in Supplementary Table 6). Pain intensity was negatively correlated to reported QoL (spearmans rho -0.24, p<0.001) and positively correlated to reported symptoms of poor sleep, depression, and anxiety within the prior 7 days (spearmans rho 0.26, 0.23, and 0.25, respectively, p<0.001), but the correlations were weak (all below 0.3).

Table 4. *Associations between DPN and painful DPN and mental health.*

	Quality of Life (NRS 0-10)		**Depression, T-scores**		**Sleep disturbance, T-scores**		**Anxiety, T-scores**	
	Model 1	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
DPN	-1.16	-0.85	4.20	2.95	4.65	3.46	3.97	2.82
	(-1.31 ; -1.01)	(-1.00; -0.71)	(3.53; 4.84)	(2.30; 3.59)	(4.04 ; 5.27)	(2.86; 4.06)	(3.31 ; 4.64)	(2.17; 3.48)
Painful	-0.85	-0.57	3.35	2.12	2.22	1.05	2.73	1.61
DPN	(-1.04; -0.67)	(-0.76; -0.39)	(2.51; 4.18)	(1.30; 2.93)	(1.44 ; 3.00)	(0.30; 1.81)	(1.89; 3.58)	(0.78; 2.44)
Number of other pain locations								
1	-	-0.60	-	1.30	-	1.95	-	1.28
		(-0.73; -0.46)		(0.71; 1.89)		(1.40; 2.50)		(0.69; 1.88)
2	-	-0.97	-	3.47	-	3.95	-	3.37
		(-1.11; -0.83)		(2.86; 4.09)		(3.37; 4.52)		(2.74; 3.99)
3	-	-1.29	-	5.57	-	5.26	-	5.20
		(-1.46; -1.13)		(4.83; 6.31)		(4.57; 5.95)		(4.45; 5.96)
4	-	-1.82	-	7.67	-	6.45	-	6.86
		(-2.05; -1.58)		(6.62; 8.72)		(5.49; 7.41)		(5.80; 7.93)
5	-	-1.58	-	8.22	-	7.04	-	7.42
		(-2.13; -1.02)		(5.81; 10.62)		(4.78; 9.30)		(4.89; 9.94)

Model 1: Adjustment for age, sex, diabetes duration, BMI, and DPN or painful DPN, respectively. Model 2: Adjustment for age, sex, diabetes duration, BMI, number of pain locations other than extremities (head/face, shoulders, stomach, lower or upper back, or "other location" [category capturing locations not listed here]), and DPN or painful DPN, respectively.

4.5 Validation of diabetic polyneuropathy and diabetic foot ulcer (study IV)

The corresponding medical records were identified for 53 of the 60 (88%) randomly selected patients in the painful DPN validation cohort, for 54 of the 60 (90%) patients in the non-painful DPN validation cohort, and 53 of the 60 patients in the diabetic foot ulcer validation cohort.

After medical record review, 38 of the 53 patients with potential painful DPN were classified as having DPN; PPV 72% (95% CI: 58; 83). Of these, 19 had neuropathic pain, thus the PPV for painful DPN was 36% (95% CI: 58; 83). Of the 54 patients with potential non-painful DPN, 30 were classified as having DPN; PPV 56% (95% CI: 41; 69). Of these, 27 had non-painful DPN corresponding to a PPV of 50% (95% CI: 36; 64) for non-painful DPN. Diagnosis codes from the E-chapter of the ICD-10 were often used for other neurological conditions than DPN (e.g. stroke and mononeuropathies), especially when listed as secondary diagnoses. If the algorithms were restricted to primary and secondary G-chapter codes and only primary E-chapter codes, the PPVs for DPN increased to 78% (95% CI: 63; 89) and 74% (95% CI: 56; 87) for the painful and non-painful DPN algorithms, respectively. Restricting to only G-chapter codes increased the PPV for DPN further for the painful DPN algorithm, but not the non-painful algorithm (table 5).

Only 18 out of the 53 patients with potential diabetic foot ulcer, had the diagnosis confirmed; PPV 34% (95% CI: 22; 48).

Table 5. *Numbers and positive predictive values of DPN and diabetic foot ulcers algorithms.*[124]

	Potential painful DPN, N = 53				
	Medical record review, conclusion			PPV (95% CI)	
	Non-painful DPN	Painful DPN	Not DPN	DPN (+/- pain)	Painful DPN
Total	19	19	15	72 (58; 83)	36 (23; 50)
All G-codes + primary E-codes	17	18	10	78 (63; 89)	40 (24; 54)
All G-codes	15	15	5	86 (70-95)	43 (26-61)
	Potential non-painful DPN, N = 54				
	Medical record review, conclusion			PPV (95% CI)	
	Non-painful DPN	Painful DPN	Not DPN	DPN (+/- pain)	Non-painful DPN
Total	27	3	24	56 (41; 69)	50 (36; 64)
All G-codes + primary E-codes	22	3	9	74 (56; 87)	65 (47; 80)
All G-codes	16	1	7	71 (49; 87)	67 (45; 84)
	Diabetic foot ulcer, N = 53				
	Medical record review, conclusion			PPV (95% CI)	
	Diabetic foot ulcer	Not diabetic foot ulcer			
Total	18	35		34 (22; 48)	

5. Discussion

Among recently diagnosed type 2 diabetes patients in the DD2 cohort, we found that approximately 1 in 5 had DPN and 1 in 10 had painful DPN. We showed that DPN associates with modifiable metabolic and lifestyle factors including general and central obesity, hyperglycemia, dyslipidemia, low-grade inflammation, high c-peptide level, tobacco smoking, and low levels of physical activity. Neuropathic pain presence in DPN was associated with unhealthy lifestyle including tobacco smoking, high alcohol intake, and decreasing physical activity level after diabetes diagnosis. Also, we observed an association with neuropathic pain and metabolic syndrome factors (central obesity, dyslipidemia, and high systolic blood pressure), yet statistical precision in the pain analyses was limited.

Moreover, we reported that not only painful DPN, but also DPN itself - independent of neuropathic pain - was associated with lower QoL, poorer sleep, and more symptoms of depression and anxiety, and showed stronger association with mental health than neuropathic pain.

Finally, we found a reasonable PPV of diagnosis codes for DPN in the DNPR suggesting a potential for future register-based research on DPN, although use of additional prescription data did not allow for a valid separation into non-painful and painful DPN. Our algorithm for diabetic foot ulcers was not valid.

5.1 Comparison with existing literature

In the following paragraphs, our results will be compared to previous literature and possible explanations of our findings will briefly be touched.

5.1.1 Prevalence

Prior to a discussion of our results against the existing literature, a word on the slightly varying DPN and painful DPN definitions in study II and III is required. The prevalence of DPN and painful DPN depend on the tools and criteria used to define these conditions. Different tools perform in different ways and may potentially identify different features of DPN and painful DPN. We used the MNSIq and the DN4 (in combination with anatomical pain location), which allows for a DPN/painful DPN diagnosis at the level of "possible" (see section 1.3), thus our prevalence estimates reflect the prevalence of possible DPN and possible painful DPN. The sensitivity of a MNSIq score ≥4 is 40%, when validated against a diagnosis of confirmed DPN in patients with longstanding type 1 diabetes in the Epidemiology of Diabetes Interventions and Complications (EDIC) study.[19] The MNSIq likely underestimates the prevalence of DPN in type 2 diabetes patients also. Thus, the 130 patients who fulfilled the criteria for painful DPN in study II, but had a MNSIq <4, could possibly be a consequence of the low sensitivity of the MNSIq. On the other hand, the largest relative difference of positive responses (i.e. assigning a point) to MNSIq was observed for question 6 (allodynia) and question 7 (temperature sensation) comparing those with painful DPN and MNSIq ≥4 to those with painful DPN and MNSIq <4 in study II. Thus, those with painful DPN and MNSIq <4

specifically reported symptoms related to pain or small fiber dysfunction to a lesser extent than those with painful DPN and MNSIq ≥4, thus complicating the correct grouping of these 130 patients. Nevertheless, whether these patients were allocated to the painful DPN group or not, only changed the DPN prevalence slightly in this large cohort (DPN ~ 18-20%, painful DPN ~ 7-10%).

A Danish study[82] using the MNSIq and a cutoff similar to ours (≥4) reported a DPN prevalence of 13.1% at screen-detected diabetes diagnosis in type 2 diabetes patients, whereas a Nigerian[52] and a French[42] study using a MNSIq score ≥7, found a prevalence of DPN of 6.9% and 11%, respectively, in mixed type 1 and type 2 diabetes populations with median diabetes duration of 5 and 15 years. Our prevalence of 18% corroborates these findings taking into account the different cut-offs and diabetes durations. Recent European studies have reported varying prevalence of DPN in type 2 diabetes. A Swedish population-based study[131] found a DPN prevalence of 23% (median diabetes duration 7.0 years) based on presence of symptoms and signs, and an Irish study[132] reported a prevalence of 14 % (diabetes duration unknown) using one question about symptoms (tingling pain or lack of feeling in feet) to define DPN. A higher DPN prevalence was reported by both a Belgian,[43] an Austrian study,[49] and an Italian study[46] (50.8%, 37.5%, and 30.6%, respectively), possibly explained by the study populations being based on outpatient clinics only, the longer diabetes duration, and the DPN definitions, which was based on clinical examinations in these studies. However, the Italian study[46] also reported a DPN prevalence of only 3.7% if based solely on a MNSIq score ≥7, which is considerably lower than our finding. This study excluded all patients with a previous amputation which is one of the questions encompassed by the MNSIq (positive in 3,5% of those with DPN in our study). This may – together with the higher cut-off – explain some of the observed difference. A German/UK study[39] on general practice data reported a DPN prevalence of 2.4% (UK) and 5.7% in patients with diabetes duration <1 year. The DPN diagnosis was based on the diagnosis code E11.4 or the original diagnosis text of the treating physician, thus the lower prevalence may be a consequence of low sensitivity of the diagnostic coding and/or it may be a marker of inadequate focus on DPN in primary care (i.e. DPN is not diagnosed).[133]

A large UK study[58] found a prevalence of painful DPN of 21.5% in type 2 diabetes patients (median diabetes duration 4 years), i.e. twice as high as in our study. The diagnosis of painful DPN included a clinical examination which may explain part of the difference. Moreover, the diagnosis of pain was based on the Neuropathy Symptom Score (NSS) which also includes non-pain related symptoms like fatigue, cramping, and numbness, thus possibly overestimating the prevalence of *painful* DPN. Likewise, other European studies[29 43 55 56] have reported higher prevalence of painful DPN in type 2 diabetes populations than us, which may likely be explained by the use of different diagnostic criteria as well as longer diabetes durations in those studies. For instance, in a Canadian study,[53] the prevalence of painful DPN increased successively from 10% in those with diabetes duration <5 years, to 26% in those with diabetes duration 5-9 years, and 64% in those with ≥ 10 years.

5.1.2 Risk factors

To the best of our knowledge, our studies of associations between potential risk factors and DPN/painful DPN are the largest questionnaire studies in recently diagnosed type 2 diabetes patients using validated screening tools to identify DPN and neuropathic pain.

5.1.2.1 Obesity, low-grade inflammation, and hyperinsulinemia

Our results corroborate previous findings showing that increasing degree of obesity is associated with DPN in type 2 diabetes.[77 79 81-83] In study III, we extended previous research on obesity and DPN; waist circumference and BMI are correlated and when evaluated individually each reflect both general and central obesity, however, we showed that central fat distribution is associated with DPN independently of general obesity. In support, we observed an association of higher DPN prevalence with increasing levels of c-peptide (as a proxy for hyperinsulinemia) and triglyceride as well as increasing low-grade inflammation, which may act as mediators on the pathway from central obesity to DPN.[76 85] Neuropathic pain occurrence in DPN, on the other hand, was not associated with general obesity, c-peptide, and low-grade inflammation, which is in contrast with some previous studies,[43 71 96 97] but support findings for BMI from other studies.[44 93 94] One contributing mechanism for the divergence may be the choice of reference group in some previous studies.[43 91 96] If the reference group include or solely consist of patients without DPN, an association between a given risk factor and painful DPN may be driven by the association of that risk factor with DPN – and not necessarily with neuropathic pain. *If* we had analyzed our data in that way, painful DPN would have shown to be strongly associated with both general and central obesity, c-peptide and low-grade inflammation as well as a number of other factors investigated in study III (Appendix V). However, these associations would have been partly or fully driven by the associations with DPN, as revealed by our results from the internal pain analyses among patients who all had DPN. Three recent cross-sectional studies performing a detailed neurological investigation and using a diagnosis of confirmed DPN and confirmed neuropathic pain compared painful DPN to non-painful DPN. They did not find an association between BMI and neuropathic pain in DPN.[44 93 94] Of note, these studies included fewer DPN patients (N = 191-293), included both type 1 and type 2 diabetes patients, and only performed univariate analyses of pain associations.

5.1.2.2 Hyperglycemia

Despite that meta-analyses have concluded that enhanced glycemic control confers a less preventive effect for DPN development in type 2 diabetes than in type 1 diabetes,[64 134] hyperglycemia is still a contributing risk factor for DPN development in type 2 diabetes.[77 79 83] In accordance, higher HbA1c levels were associated with higher DPN prevalence in our study, however, not with neuropathic pain presence in DPN. The latter contrast recent findings from a study of confirmed painful DPN.[93]

5.1.2.3 Dyslipidemia

The higher DPN prevalence with lower HDL cholesterol level in our study corroborates findings from the longitudinal ADDITION study of screen-detected type 2 diabetes patients[82] as well as studies of type 2 diabetes[79 135] and mixed diabetes patients with longstanding diabetes.[43 97] Likewise, the link between hypertriglyceridemia and DPN is in accordance with previous results of longstanding diabetes.[43 79 135 136] In contrast, existing literature on LDL cholesterol in type 2 diabetes is more uncertain. Experimental studies suggest that glycated and oxidized LDL may play a role in DPN pathogenesis.[13] Yet, the ADDITION study found that high baseline LDL cholesterol[82] and a steeper increase over 10 years[83] predicted a lower risk of DPN. The authors pondered whether their findings could be explained by statin treatment, since statins may exert a preventive effect on DPN risk,[137-139] though evidence for this association is still controversial.[140 141] Another type 2 diabetes study reported more peripheral nerve injury with low LDL cholesterol levels,[142] and suggested that insufficient cholesterol supply may impair peripheral nerve regeneration. Neither LDL cholesterol nor lipid-lowering drug use was associated with DPN in our study. Dyslipidemia was not associated with neuropathic pain in univariate internal analyses among DPN-patients in a type 2 study by Spallone *et al*,[97] or in two multivariable studies of mixed diabetes and non-diabetes patients.[96 143] In contrast, a multivariable analysis by Van Acker *et al*.[43] showed an association between painful DPN and hypertriglyceridemia and high total cholesterol; yet, uncertainty about the reference group complicates interpretation of the association with neuropathic pain. Also, one of the three recent studies examining confirmed painful DPN held data on dyslipidemia. In that study, the proportion of patients with any dyslipidemia gradually increased over the DPN groups: without pain (52.7%), with mild pain (53.8%), with moderate/severe pain (61.3%), particularly driven by hypertriglyceridemia.[94] In our study, total cholesterol, LDL, and triglyceride seemed associated with higher occurrence of neuropathic pain in the internal analyses among patients with DPN, however, power was limited.

5.1.2.4 Hypertension

The role of hypertension in DPN is uncertain. Just as for hyperglycemia there appears to be a difference in the role of hypertension on DPN risk between type 1 and type 2 diabetes,[66 89 144] although no meta-analysis has been performed. Thus, high blood pressure have been associated with DPN in type 1 diabetes studies, whereas no association has been found in most type 2 diabetes studies.[66 72 79 82 83 89 144] Likewise, in our main analyses, we did not find an association of blood pressure with DPN whereas antihypertensive drug use was associated with higher DPN prevalence. In our cohort, 74% were treated with antihypertensive drugs and the blood pressures were well-controlled and showed little variation, which may have hampered the identification of an association. However, further adjustment for or stratification by antihypertensive drug use did not reveal an association between blood pressure values and DPN. Restricting the cohort to individuals with the shortest diabetes duration at DD2 enrolment (i.e. increasing the likelihood of incident

DPN) resulted in lower DPN risk with higher systolic blood pressure, which persisted after adjusting and stratifying for antihypertensive drug treatment. The reason for this unexpected observation is unknown.

5.1.2.5 Unhealthy lifestyle

Smoking was strongly associated with DPN and with painful DPN in our studies. A recent meta-analysis of 10 prospective and 28 cross-sectional studies on type 1 and type 2 diabetes patients concluded that smoking may be associated with DPN, but evidence was graded as low-strength. Of note, several included studies allocated former smokers or smokers with fever pack-years to the non-smoking reference group. If a detrimental effect of smoking is irreversible, this may dilute an association. This possibility is supported by the fact that higher risk estimates were reported in studies comparing ever smokers (former and current) with never smokers. Both DPN and neuropathic pain occurrence in DPN seemed to be higher in those who continued versus discontinued smoking in our study. This may reflect 1) a stronger association with higher cumulative exposure (time and dose), 2) a reversible effect of smoking on nerve damage, or 3) reverse causation, i.e. smoking as self-medication in patients with DPN and painful DPN. However, the association between former smoking and DPN argues against reverse causation as the only explanation for the association between smoking and DPN. Also, it argues against a reversible harmful effect of smoking on nerve damage.

Previous literature on alcohol and DPN associations has been conflicting.[66 89] The paradoxical findings of no association between alcohol overconsumption and neuropathic pain occurrence in DPN in study II and a positive association in study III can be ascribed the higher cut-off point for alcohol overconsumption used at DD2 enrolment and may indicate a dose-response relationship. It is difficult to distinguish between alcohol as a potential risk factor of DPN and neuropathic pain or as a causal factor of alcoholic polyneuropathy in diabetes patients. We did not have information on nutritional deficiencies that could contribute to a more in-depth understanding. Moreover, reverse causation (i.e. alcohol as self-medication for pain) cannot be ruled out as a consequence of the cross-sectional study design. Of note, the potential misclassification of alcoholic polyneuropathy as DPN did not explain other observed risk factor-DPN associations as evident from the sensitivity analysis restricted to those without alcohol overconsumption.

Encouraging data supports physical exercise as a therapy option to prevent and treat DPN.[145-147] In line with these findings, we observed an association of higher DPN prevalence with sedentary lifestyle as well as a lower prevalence of neuropathic pain among DPN patients who increased their activity level between the two assessments of physical activity. Conversely, increased physical activity level from baseline to DPN assessment did not affect DPN prevalence in our study. But if we stratified by physical activity level at baseline, we observed a lower DPN prevalence in those who had low baseline physical activity level, but had increased their activity level at the time of questionnaire. Equally, in those who had high baseline activity level, but decreased their activity level over time, a higher DPN prevalence was observed. The cross-sectional nature of our study prevents us from drawing firm conclusion on whether these observations

support physical exercise as DPN prevention or whether they result from reverse causation, i.e. symptoms of DPN leading to less activity.

5.1.2.6 Sex

We observed a higher prevalence of DPN among females, which contrasts some previous findings.[43 44 71 105] We speculate, whether the symptom-based definition of DPN may explain this observation. Females may be more susceptible to report symptoms than males, supported by results from other studies using a similar MNSIq-based definition of DPN.[42 83] On the other hand, we did not find an association with female sex and painful DPN, arguing against reporting bias as the sole explanation.

5.1.2.7 Age and diabetes duration

Diabetes duration is a well-established risk factor for DPN,[89 144] probably reflecting a longer cumulative exposure to risk factors. In accordance, we observed an association between longer diabetes duration and DPN after adjustment for age, however, not with painful DPN. Older age is also a well-recognized risk factor for DPN.[89 144] Surprisingly, we observed an association of younger age with DPN. An explanation may be that the DD2 enrolls recently diagnosed type 2 diabetes patients and young onset type 2 diabetes is an indicator of a worse diabetes phenotype and risk factor profile.[148] Also, non-responders were slightly younger at questionnaire time. It cannot be ruled out that the observed association with younger age may be partly explained by a responder bias if non-responders were both young and had a lower DPN prevalence.

5.1.3 Mental health

The associations between painful DPN and lower QoL, poor sleep and symptoms of anxiety and depression in our study are in line with results from other studies on type 1 diabetes,[106] type 2 diabetes[55] and mixed populations.[93 101 103-105] Likewise, the observed association between DPN itself and depressive symptoms independent of neuropathic pain is in accordance with other studies,[57 101 102 105 106] whereas the observation that DPN itself is associated with lower QoL contrasts results from two large studies.[43 57] These studies did not find an association of non-painful DPN[43] or of MNSI-score (questionnaire or examination) independently of pain[57] with mental or physical QoL assessed using the Medical Outcomes Study 12-item Short-Form Health survey (SF-12). We used a rather crude measure for QoL (NRS 0-11), which may possibly explain this divergence. However, falls, foot ulcers (which may be painless in patients with DPN) and lower extremity amputations could be intermediates on a pathway from DPN to reduced QoL. Also, depressive symptoms and QoL are interrelated,[149] thus, we do not find an association of DPN itself with QoL implausible. We found an association of DPN – independently of pain – with sleep disturbance contrasting results by Bouhassira *et al.*[57] but corroborating results from studies linking sleep apnea to DPN,[150-152] possible via sleep apnea-induced hypoxemia and resulting oxidative stress,[150] i.e. reverse causation. Painful

DPN may be linked to sleep disturbance via the same mechanism,[152] but the association may be bidirectional with nocturnal exacerbations of pain leading to poor sleep.[103]

The observed associations of DPN and painful DPN with mental health measures were independent and additive, i.e. those who fulfilled both the DPN and the painful DPN criteria had lower QoL and more symptoms of depression, anxiety and sleep disturbance, which is consistent with findings from an Italian study on depressive symptoms.[105]

Apart from neuropathic pain, DPN patients often have co-existing pain in other bodily locations,[93 153] which was also observed in our study. Most studies investigating painful DPN and mental health outcomes did not adjust for other pain, thus previous observed associations could theoretically be confounded by other pain. In our study, pain in other bodily locations was a strong confounder and considerably diminished the estimates, though, did not eliminate the associations, thus supporting previous literature.

All patients with DPN/painful DPN reported pain in other locations more often than those without DPN and we cannot exclude that positive answers to the MNSIq and the DN4 represent other causes than DPN.

5.1.4 Diagnostic validity

To our knowledge, only a single validation study has examined *diabetic poly*neuropathy coding, reporting a PPV of 91% for DPN for the ICD-9 code 357.2 (polyneuropathy in diabetes) which corroborates our findings for the ICD-10 code 63.2 (diabetic polyneuropathy). However, DPN identification based solely on this code would lower completeness. Including additional diagnosis codes in a DPN-algorithm revealed acceptable PPVs in our study, if secondary diagnosis codes of the less specific "diabetes with neurological complication" was left out. Hartsfield et al.[109] validated an algorithm for painful *diabetic peripheral* neuropathy including also mononeuropathies, autonomic peripheral neuropathy etc. Consequently their PPV of 79% cannot be compared directly to our results. Despite different underlying mechanisms, they also found that data on pain treatment was not useful for identifying patients with painful neuropathy.[109] Since neuropathic pain may not necessarily be recorded in the medical records if present, the low PPV for painful DPN in our study may be an underestimate. In contrast, data on treated depression/anxiety diagnosed by the GPs was not available, thus, our exclusion of therapies prescribed for other reasons than neuropathic pain may have been insufficient, resulting in a true low PPV for painful DPN. However, the gabapentinoids, which are primarily prescribed for neuropathic pain or hospital specialist diagnosed epilepsy (which was excluded) was prescribed to half of the patients with verified DPN and missing pain description in the painful DPN validation cohort, thus suggesting that missing descriptions of true neuropathic pain had led to a false low PPV for painful DPN. Sensitivity, specificity, and negative predictive values of coding for *diabetic poly*neuropathy was neither evaluated in previous studies nor in our study. Applying the G-chapter and primary E-chapter ICD-10 diagnosis codes on the 5,249 DD2 patients included in study III, showed that only 3.8% had hospital-diagnosed DPN prior to the IDNC/DD2 questionnaire response, indicating a low

sensitivity of the ICD-10 codes. This makes the codes unsuitable for use in studies of prevalence, incidence, and absolute risk of DPN, whereas the moderately high PPVs indicate a potential for use in studies of relative risk and when identifying DPN-cohorts for studies of prognosis.[154]

Our PPV of 34% for diabetic foot ulcer is much lower than the PPVs of 61%-82% reported in previous studies.[111 112] This difference is likely explained partly by our inclusion of the frequent ICD-10 codes E10.5-E14.5 "diabetes with peripheral vascular complication" which was not part of the previously validated algorithms.[111 112] These codes were included because peripheral vascular disease also contributes to the pathogenesis of diabetic foot ulcers, however, they turned out to mainly represent hospital contacts for e.g. peripheral vascular bypass/intermittent claudication and more unspecific conditions (e.g. callosities, clavus) thus diminishing the PPV. The surgery code KQDB "treatment of ulcer of lower limb" was likewise frequent in the diabetic foot ulcer validation cohort. No further subclassification of this code in regards to the exact anatomical location at the lower limb exists, and the code frequently represented ulcers above malleoli.

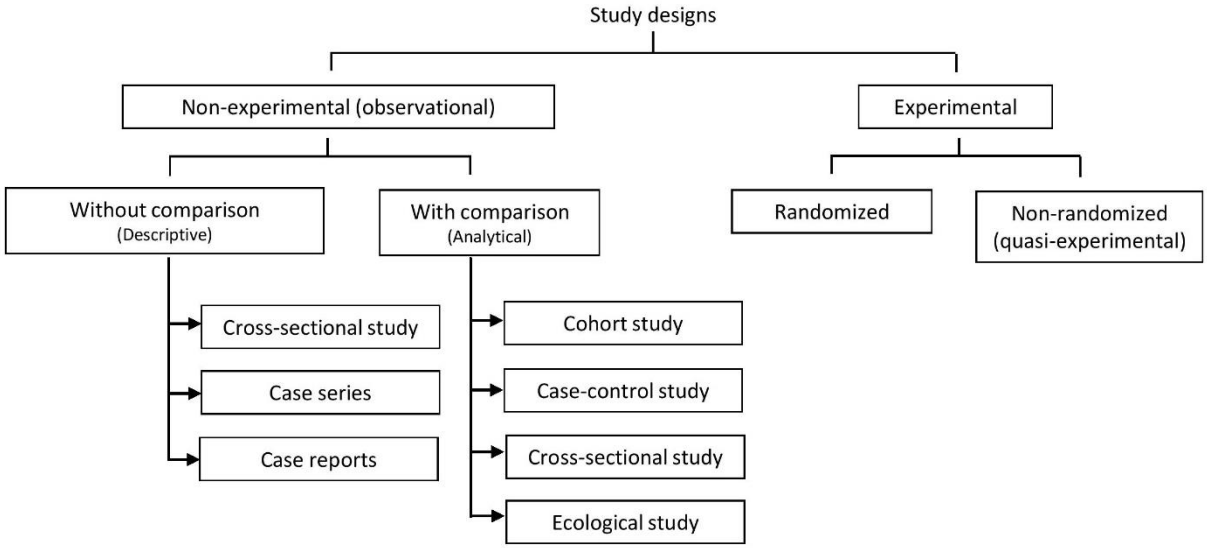
The ICD-9 diagnosis code for "ulcer of lower limbs, except decubitus" 707.1x has shown PPVs of 82%-92%.[111 112] Despite the low PPV of our diabetic foot ulcer algorithm, Danish registers may still hold a potential for studying diabetic foot ulcers based on the corresponding ICD-10 code L97 (our PPV: 75%, n = 4), however, prior validation in a larger study is required.

5.2 Methodological considerations

The purpose of epidemiologic studies is to achieve valid and precise estimates of disease occurrence or of the effect of an exposure on an outcome,[155] the latter with the optimal goal of estimating causal inference. However, accuracy of the estimates may be threatened by random error (affects precision) and systematic error (affects validity) of which information bias, selection bias, and confounding are the main sources of the latter.[155] Traditionally, analytical observational study designs (Figure 9) have been ranked according to their potential impact, ranking cohort and case-control studies above the cross-sectional study again ranked above the ecological study due to potential limitations inherent to the study design. For the cross-sectional study design such limitations include e.g. difficulties in determining the time order of events such as whether exposure is leading to outcome (universal premise for causal association) or vice versa and whether a factor is a potential confounder or an intermediate factor, thus potentially compromising internal validity. Study II was a traditional cross-sectional study ascertaining exposure and outcome status simultaneous, whereas in study III outcome status was assessed subsequent to exposure status assessment. Still, a classical longitudinal study could not be conducted since it was not possible to determine who were DPN-free at exposure assessment. However, in sensitivity analyses, we restricted the cohort to patients newly diagnosed with diabetes. We thereby increased the likelihood that the DPN events were incident and thus tried to mimic a cohort study design, though, still keeping in mind that diabetic complications may be present already at time of type 2 diabetes diagnosis.[156]

Below, a critical appraisal of potential limitations of the internal validity of our descriptive measures and estimates of associations follows.

Figure 9. *Study designs.*



5.2.1 Random error (chance)

We used the width of the confidence intervals to quantify precision in our studies, which together with the strength of the estimate enables an evaluation of the inference.[157] Current epidemiological practice argues against the use of *P*-values and the exact location of CI boundaries in the evaluation of inference, because such evaluation is based on statistical – and not necessarily clinical – significance.[157-159]

The rather large number of patients and outcomes in study II and III ensured an overall acceptable precision, however, despite these studies being some of the largest to examine DPN associations in type 2 diabetes, the precision in subgroup (e.g. neuropathic pain) and sensitivity analyses was compromised and these findings must be interpreted with caution.

Gaining access to the medical record data for the validation study required a number of time-consuming steps; 1) achieving permission from relevant authorities including Danish Health and Medicine Authorities (total waiting time for the latter was ~10 months), 2) applying for and receiving data from the CRS, DNPR, and DNHSP, 3) gaining permission from the heads of all participating departments, 4) instruction of the health person allocated to identify the electronic medical records at each department, and 5) waiting for achieving the medical record data. Consequently, our validation sample sizes were relatively small resulting from a compromise between practical feasibly and precision.

5.2.2 Selection bias

Selection bias is a systematic error that arises if the exposure-outcome association differs between those participating in a study and the target population (those theoretically eligible for the study), including those who do not participate.[155]

One source of selection bias is self-selection, referring to a bias that arise if participation in a study associates with the outcome,[155] which may cause bias to the prevalence estimates. Self-selection bias may have been introduced at the enrolment step into the DD2 cohort and again at the participation step into the IDNC/DD2 neuropathy questionnaire, if patients with DPN were more (or less) likely to participate. Regarding the first step, the overall aim of the DD2 project is not specifically to investigate DPN, thus the patients were not aware of the studies encompassed in this thesis at the DD2 enrolment. Still, patients that experience complications may be more willing to participate than those without which would lead to an overestimation of the true DPN prevalence. In contrast, patients that are more health-cautious may also be more likely to participate. Comparing the prevalence of other microvascular complications in the DD2 cohort with the prevalence reported in other cohorts might give a hint of whether self-selection bias into the DD2 cohort is an issue, since microvascular complications often co-exist.[160] However, different definitions, e.g. clinical examination/laboratory measurements[161 162] vs. diagnosis codes (and the actual codes and lookback periods used)[163 164] and calendar years (earlier detection of diabetes and improvements in clinical management in recent years)[165] make such comparisons very difficult. However, taking these difficulties into account, a cautious guess is that the prevalence of microvascular complications other than DPN in our cohort represents that of other type 2 diabetes cohorts. Regarding self-selection into the IDNC/DD2 questionnaire, we reassuringly observed similar estimates of DPN prevalence across questionnaire intervals (original contact - 1st reminder – 2nd reminder) and achieved a high response rate. Still, it cannot be ruled out that the non-responding DD2 patients may have another prevalence of DPN.

Although enrolment in the DD2 cohort in the first years (2010-2012) primarily took place from outpatient hospital clinics (thereby including patients with potentially more advanced diabetes),[119] baseline characteristics of the DD2 cohort reassuringly seem to be representative of other newly diagnosed type 2 diabetes cohorts.[163 164] Likewise, baseline data of the study population in study III are in general similar to non-responders (Appendix VI), except for the non-responders being slightly younger at questionnaire time as discussed in section 5.1.2.7. Principally, this reduces the likelihood of Berksonian bias, that is if both exposure and outcome determines participation into a study leading to bias of the relative estimates.[155] Nonetheless, only two-thirds of the patients in study III were linkable to the DDDA. Within the DDDA subcohort, some variables had missing data, particularly HDL and total cholesterol. Within the total cohort, some variables (besides the DDDA variables) were also affected by missingness, mainly c-peptide. For other variables we had nearly complete data. Missing data can be considered a selection problem and can be handled in different ways.[166-168] We have analyzed our data using complete case analysis, whereas another

approach could have been to perform multiple imputation, for example.[167 168] The use of complete case analysis reduces precision and may potentially lead to bias. In our study, the 69% of patients linkable to the DDDA were similar to those not linkable for many variables for example female gender (42.3% vs. 42.1%) and waist circumference (median 106 cm (97; 116) vs. 106 cm (97; 116)), but differed on some variables, e.g. presence of macrovascular complications (23.9% vs. 21.9%). Within the DDDA subcohort, levels of other lipids that were less affected by missingness, i.e. triglyceride and LDL cholesterol, were comparable among those with and without HDL cholesterol measurements. Nevertheless, our results for the variables affected by missing data should be interpreted with caution. Finally, besides the self-selected response to the IDNC/DD2 questionnaire, another potential source of selection bias relates to the proportion of patients who died (or emigrated/had secret addresses) in the time-window from DD2 enrolment to the IDNC/DD2 questionnaire and thus were not eligible for participation. Fortunately, this proportion was small (4%).
In study IV, selection bias may potentially have been introduced at two levels. First, the study was restricted to the Central Denmark region. However, since the Danish health care system is homogeneous with regards to structure (e.g. demographic and socioeconomic composition) and practice,[169] this choice seemed reasonable and was favorable in terms of feasibility. Second, medical record data could not be identified for 10-12% of the validation cohorts. However, there was no systematic pattern of the missing records regarding type of hospital, department, diagnosis etc. and we do not expect that coding validity differed for these hospital contacts. If we assume that patients with missing medical record data did *not* have DPN, a worst case scenario analysis results in a PPV for DPN of 69% (95% CI: 54; 81) for the potential painful DPN validation cohort based on G-codes and primary E-codes. Opposite, a best case scenario analysis yields a PPV of 80% (95% CI: 67-90%).

5.2.3 Information bias and problems

Information bias can arise if exposure or outcome data are determined erroneously.[155]
Exposure data in our studies of associations was either clinically measured (e.g. waist circumference), self-reported (e.g. lifestyle), or extracted from routine registers (e.g. drug use), whereas outcome data was either self-reported (DPN, pain) or extracted from medical records (e.g. symptoms and signs of DPN).
Estimates based on self-reported data may be prone to recall bias if patients with symptoms of DPN/pain report or recall e.g. anthropometric and lifestyle data differently from those without and vice versa if patients with e.g. depressive symptoms are more likely to report symptoms of DPN. We cannot rule out that such recall bias may have affected our results. However, knowledge of our study hypotheses cannot have led to systematic erroneous reporting for most reported exposure variables in study III, since this information was reported prior to collection of DPN/pain data. In addition, some patients likely developed DPN and neuropathic pain after DD2 enrolment, reducing the risk of recall bias. Moreover, self-reported

anthropometric data have been reported to be reasonable accurate for use in large epidemiological studies[170] - relevant for the self-reported BMI measures at age 20 years and at INDC/DD2 questionnaire time.
Also, some of the questions included in the MNSIq are less susceptible to subjective judgement for example "Has your doctor ever told you that you have diabetic neuropathy?", "Have you ever had an open sore on your foot?", "Is the skin on your feet so dry it cracks open?" and are likely less prone to be erroneously reported as compared to e.g. pain questions included in the DN4. Thus, if the observed associations with mental disorders were solely explained by recall biases, we would not expect that DPN itself was stronger associated with symptoms of mental health disorders than neuropathic pain as observed. Finally, since the MNSIq and the DN4 questionnaires were filled out without knowledge about the hypotheses and the metabolic and lifestyle factors to be studied, we find no reason why DPN and painful DPN status would be systematically erroneous reported among exposure groups. As previously written, the sensitivity of the MNSIq is rather low in type 1 diabetes[19] and likely also in type 2 diabetes patients. This results in misclassification of some true DPN patients as DPN-free causing an underestimation of DPN prevalence. However, for studies of relative risk, a high specificity is more important.[154 155] We therefore expect that bias of the prevalence ratios (as a measure of the risk ratios) due to low sensitivity will be minor.
Another source of information problems related to our work is the medical records used as reference standard in the validation study. Incomplete information may have led to an underestimation of the true PPVs of DPN as described section 6.4.1. On the other hand, in order to account for this potential information problem, we applied less stringent criteria to verify polyneuropathy than those outlined by the Toronto Consensus Panel on DPN20 171 holding a risk of overestimating the PPVs.

5.2.4 Confounding

In simple terms, confounding is a confusion of effects meaning that the observed association between exposure and outcome is distorted because the actual exposure effect is mixed with an effect of an extraneous factor. To act as a confounder, a factor must be 1) imbalanced across exposure categories, 2) an independent cause or a proxy of the cause of the outcome, and 3) it cannot be an intermediate factor on the causal pathway from exposure to outcome.[155] However, confounders could not be so unambiguously defined in our studies, because the pathophysiological pathways between obesity, other metabolic factors under study and lifestyle habits are still not fully understood. Many of the examined factors may be intermediates/clusters in the same causal pathways.
Confounding can be addresses in the design phase e.g. by restriction and matching and in the analysis phase by e.g. stratification, standardization, and adjustment.[155] In study II and study III, we handled potential confounding by adjustment. Including all possible covariates in one multivariable model without considering whether the confounder criteria were fulfilled, would have been the choice if we aimed for a prediction study, not caring about etiology. However, even though we are fully aware that causal inference cannot be

drawn from cross-sectional studies (which are not ideal for prediction studies either), we wear "etiological glasses" while conducting our risk factor association studies. For instance, obesity leads to low-grade inflammation[172] which again may be a risk factor for DPN.[80 173] Thus, adjustment for low-grade inflammation would had led to a lower obesity risk estimate reflecting only the direct association and eventually other indirect associations between obesity and DPN, but not the full association of obesity with DPN. One could argue that the association between hsCRP and DPN, should have been adjusted for obesity then, however, the pathways become more complex when physical activity, smoking, lipid levels etc. are added. Moreover, the link between low-grade inflammation and obesity may be bidirectional.[174] As a consequence of the complex and unclear interrelationship between the variables under study, we only adjusted for a restricted number of confounders, similar to other studies.[82 156]

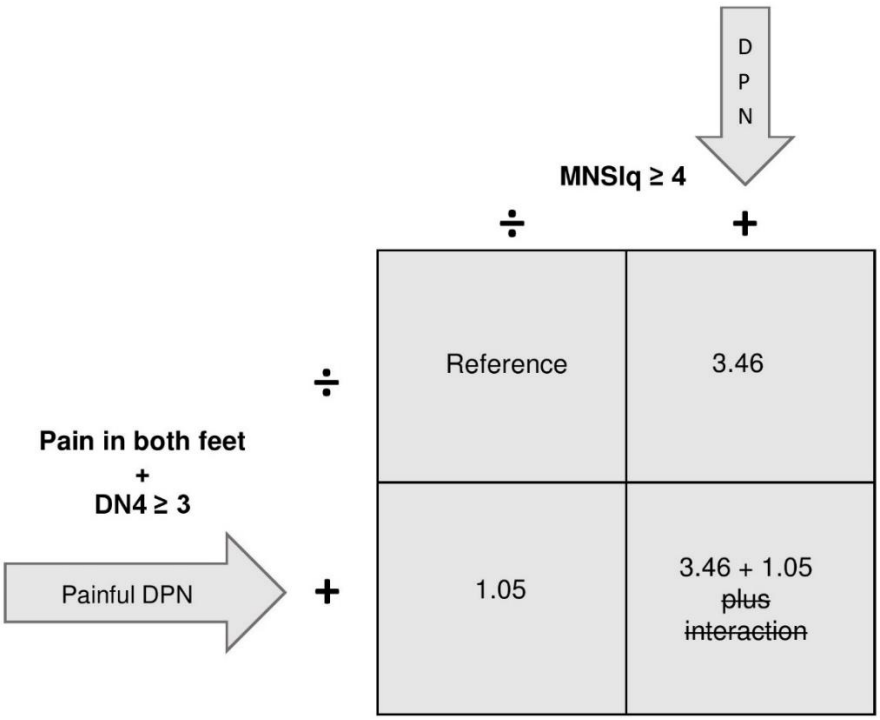
5.2.5 Statistical considerations

A few extra words on some of our statistical analyses are required.

First, the diagnostic tools and definitions of DPN and painful DPN used in study II, resulted in only partly overlapping DPN groups, i.e. one could have painful DPN without fulfilling the criteria for DPN (Figure 3, panel a and Figure 10). Consequently, we included DPN and painful DPN simultaneous as independent variables in the regression models and we examined whether DPN *defined by MNSIq* and painful DPN *defined by DN4 and pain in feet* was independent of each other without statistical interaction. Since interaction was not observed in the regressions for patient characteristics/mental health, we could assume that the association of DPN (i.e. defined by MNSIq) with a given dependent variable was independent of the association of painful DPN (i.e. defined by DN4 and pain in both feet) with the same dependent variable. Thus, the size of the total association for a patient fulfilling both the criteria for DPN and for painful DPN was the sum of each independent estimate in the linear regressions (or the product of the estimates for DPN and painful DPN in the logistic regressions), as illustrated in Figure 10.

Second, in study III, we analyzed the continuous metabolic factors as both categorical and continuous variables (using clinical relevant units and, for obesity measures, also restricted cubic splines regressions. The latter are not presented in the "Methods" and "Summary of results" sections in thesis, but are available in Appendix III). Categorization of continuous measures adhere to some disadvantages including loss of power and precision. Moreover, the information gained from the data is simplified, e.g. the use of categories principally rely on an unrealistic assumption of homogeneity of the risk within categories.[175] Still, we choose to present our data in both ways, since many of the included risk factors have well-defined clinical categories and cut points for treatment goals used in the clinical setting, e.g. BMI, blood pressure, and lipid levels.[176-178] However, the results from the categorical and continuous analyses complements each other and show similar results.

Figure 10. *Example for sleep disturbance.*



Someone fulfilling both the DPN and the painful DPN criteria had a 3.46 + 1.05 point higher T-score of sleep disturbance than one not fulfilling any of the two criteria. Of note, the associations for DPN and painful DPN from the logistic regressions e.g. for smoking are multiplicative.

6. Main conclusions and perspectives

In this thesis, we have shown that DPN is a prevalent condition in early type diabetes and around half of DPN patients have neuropathic pain. We have shown that both DPN and painful DPN was associated with lower QoL, with poorer sleep, and with symptoms of depression and anxiety and that DPN itself was even stronger associated with self-reported mental health comorbidities than neuropathic pain, showing the seriousness of this frequent diabetes complication. We have conducted one of the largest studies (number of participants and number of different risk factors) of potential risk factors associated with DPN in early type 2 diabetes and found that unhealthy lifestyle habits and modifiable metabolic risk factors were associated with DPN. This supports an encouraging potential for other targets than hyperglycemia in the prevention of DPN in type 2 diabetes. Neuropathic pain in DPN may be associated with some of the risk factors, but the statistical power of the neuropathic pain analyses was limited even in our large study. Finally, we have provided an algorithm to identify hospital-diagnosed DPN based on diagnosis codes. This may be a useful tool for future register-based research on DPN relative risk and prognosis, however, not for studies of DPN prevalence, incidence and absolute risk.

Taking into account the above discussion about methodological considerations and potential threats to internal validity, in particularly the cross-sectional study design, our association-studies may be considered as *hypothesis-screening* studies, i.e. studies in which a given hypothesis is tested, but further confirmation of the results in studies of better internal validity is needed.[179] As suggested by acknowledged epidemiologists,[179] we avoid the term *hypothesis-generating* studies, since our studies were conducted based on pre-existing hypotheses. In order to enhance the knowledge on effective prevention of DPN and targets for disease-modifying treatments, future studies should be longitudinal and focus on a single/few risk factors with the potential of in-depth confounder control.

Moreover, the effect of e.g. reducing excess body weight and hyperglycemia, of smoking cessation, and of physical training programmes on reducing risk of DPN should be explored. Randomized controlled trial design is one opportunity for such studies, but register-based studies also offer a potential for example for studies based on codes for bariatric surgery[180] and with exact BMI-measures.[181] Recently, the Danish Health and Medicine Authorities allowed the use of national laboratory data for research purposes which renders nationwide longitudinal studies of the effect of biomarker changes possible, and provides possibilities for extensive confounder adjustment in future studies. Also, while writing this thesis, we have achieved an agreement on getting data from a new Danish database holding detailed clinical data from all Danish podiatrists. These data will allow for a more detailed validation of the diagnosis codes of DPN and foot ulcers and for more well-powered studies of risk factors for painful DPN. Finally, other hypotheses have gained interest during the course of our work, including whether some of the newer glucose-lowering drugs are effective in the prevention of DPN in type 2 diabetes. For instance, the incretin-based therapies, that is the glucagon-like peptide 1 receptor agonists (GLP-1RA) and the dipeptidylpeptidase 4 inhibitors may be

drug-classes of special interest due to potential neuroprotective effects[182 183] and beneficial effects both on glycemic control, weight-reduction (GLP-1RA), and inflammation.[184-187]

7. Summary

Diabetic polyneuropathy (DPN) is a devastating diabetes complication. Besides sensory discomfort, DPN may be followed by invalidating neuropathic pain and is associated with substantial morbidity including diabetic foot ulcerations and lower extremity amputations. Understanding of risk factors for DPN in type 2 diabetes and knowledge on why only some patients develop pain is limited. Thus, current prevention of DPN restricted to glycemic control, which unfortunately has limited effect in type 2 diabetes. In this thesis, we aimed to study the occurrence of DPN and painful DPN and its association with mental health and with lifestyle and metabolic risk factors in patients with early type 2 diabetes. Finally, we wanted to examine the potential for using diagnosis and prescription codes to study non-painful and painful DPN as well as diabetic foot ulcers in future epidemiological studies.

In **study I**, we provided a detailed description of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) Cohort, which forms the basis for study II and study III. The enrolment process, the DD2 data collection and data sources linked to the DD2, the baseline characteristics of the enrolled patients, as well as strength and limitations of the cohort were discussed.

In 2016, we conducted a large questionnaire survey in the DD2 cohort (N = 6,726) including validated questionnaire tools on neuropathy, pain, and mental health outcomes as well as a few items on current anthropometric measures and lifestyle. We received a remarkable total response rate of 86% (78% with valid data on both DPN and neuropathic pain).

In **study II**, based on the questionnaire data, we reported a prevalence of DPN and painful DPN of 18% and 10%, respectively, among patients with early type 2 diabetes (median diabetes duration 4.6 years). Moreover, we showed that female sex, younger age, longer diabetes duration, higher BMI, and smoking were associated with DPN, whereas only smoking was clearly related to painful DPN. Finally, we reported that DPN and painful DPN both was independently associated with lower quality of life, sleep disturbances, and symptoms of depression and anxiety. DPN itself was stronger associated with mental health comorbidities than neuropathic pain.

In **study III**, we extended our investigation of the association of DPN and lifestyle and metabolic factors further and included detailed data from additional data sources on the metabolic and lifestyle profile at time of type 2 diabetes diagnosis. We showed that DPN is related to modifiable risk factors including central and general obesity, low grade inflammation, glycemic control, c-peptide levels (insulin resistance), hypertriglyceridemia and low levels of HDL cholesterol, antihypertensive drug use, and unhealthy lifestyle (tobacco smoking and low physical activity level). Pain occurrence in DPN may share some of these risk factors, e.g. smoking. However, precision was limited for this part of the study and further research on risk factor and pain associations is needed.

Finally, in **study IV**, we found a positive predictive value of 74-78% for hospital-diagnosed DPN, thus suggesting a potential for future register-based research on DPN risk and prognosis. Non-painful and painful

DPN could not be accurately distinguished by adding analgesic prescription redemption to our algorithms. Finally, our algorithm for diabetic foot ulcers did not perform well with a positive predictive value of 34% (95% CI: 22-48).

8. Dansk resumé (Danish summary)

Diabetisk nervebetændelse i form af diabetisk polyneuropati (DPN) er en alvorlig diabetisk komplikation. Udover ubehagelige føleforstyrrelser kan DPN være forbundet med svære og invaliderende nervesmerter samt med betydende sygelighed inklusiv forekomst af diabetiske fodsår og amputationer. Vores viden om hvilke risikofaktorer, der er forbundet med DPN samt vores viden om, hvorfor det kun er nogle patienter, der udvikler smertefuld DPN, er begrænset. Af denne grund er den nuværende forebyggende indsats mod DPN rettet mod opnåelse af normalt blodsukkerniveau. Desværre har studier vist, at blodsukkerkontrol kun har en begrænset reducerende effekt på DPN-risiko blandt patienter med type 2 diabetes.

Formålet med denne afhandling var at undersøge forekomsten af DPN og smertefuld DPN samt at undersøge hvordan DPN og smertefuld DPN er relateret til mental sundhed og til både livsstilsfaktorer og metaboliske risikofaktorer blandt patienter med tidlig (relativ nydiagnosticeret) type 2 diabetes. Sluttelig ønskede vi at undersøge, om det er muligt at identificere patienter med smertefuld og ikke-smertefuld DPN samt patienter med diabetiske fodsår ved brug af registerdata (diagnosekoder og receptindløsning) med henblik på fremtidige register-baserede studier.

I **studie I** gav vi en detaljeret beskrivelse af Dansk Center for Strategisk Forskning i Type 2 Diabetes (DD2) kohorten, som danner baggrund for studie II og studie III i denne afhandling. Vi beskrev, hvordan patient- og dataindsamling til DD2 foregår, vi beskrev patientkarakteristika på rekrutteringstidspunktet for de patienter, der indgik i DD2 kohorten per 2016, samt DD2 kohortens styrker og begrænsninger.

I 2016 udførte vi en stor spørgeskemaundersøgelse blandt alle DD2 patienter (N = 6.726). Spørgeskemaet indeholdt en række validerede spørgeskemaskemainstrumenter indenfor DPN, smerte, mental sundhed samt enkelte spørgsmål om antropometriske mål og livsstil. Vi opnåede en høj total besvarelsesprocent på 86% (78% med data på DPN og nervesmerte).

I **studie II** som var baseret alene på data fra spørgeskemaundersøgelsen, fandt vi en DPN-forekomst på 18% og en smertefuld DPN-forekomst på 10% blandt patienter med tidlig type 2 diabetes (median diabetes varighed 4,6 år). DPN var relateret til kvindeligt køn, yngre alder, længere diabetes varighed, højere BMI og tobaksrygning. Smertefuld DPN var kun tydeligt relateret til tobaksrygning. Endelig rapporterede vi i studie II, at både DPN og smertefuld DPN var forbundet med dårligere livskvalitet, søvnforstyrrelse samt angst- og depressive symptomer. DPN i sig selv – uafhængig af smerteforekomst - var stærkere associeret til mental sundhed end nervesmerter.

I **studie III**, udvidede vi vores undersøgelse af DPN og relationen til livsstilfaktorer og metaboliske risikofaktorer. Vi anvendte supplerende datakilder indeholdende data omkring livsstil og metaboliske faktorer omkring tidspunktet for type 2 diabetesdiagnosen. Vi fandt, at DPN er relateret til både generel overvægt og mavefedme, inflammation, højere c-peptid niveauer (insulin-resistens), højere blodsukkerniveauer, højere triglycerid- og lavere HDL kolesterol niveauer, brug af blodtrykssænkende medicin og usund livsstil inklusiv tobaksrygning og lavt fysisk aktivitetsniveau. Forekomst af nervesmerter

var relateret til nogle af disse risikofaktorer fx rygning, men ikke alle. Generelt var præcisionen lav for den del af studiet, der omhandlede neuropatisk smerte og yderligere forskning på dette område er nødvendigt. Sluttelig, **i studie IV**, fandt vi en positiv prædiktiv værdi for hospitalsdiagnosticeret DPN på 74-78% og dermed et muligt potentiale for fremtidig register-baseret forskning indenfor DPN risiko og prognose. Data på receptindløsning af smertestillende medicin kunne derimod ikke bidrage til at adskille patienter med ikke-smertefuld og smertefuld DPN. Og endelig kunne vores algoritme for diabetisk fodsår ikke anvendes til at identificere de rette patienter, positiv prædiktiv værdi 34% (95% CI: 22-48).

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10. Appendices

Appendix I

Appendix II

Appendix III

Appendix IV

Appendix V

Appendix VI

Appendix I

BMJ Open

Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort of newly diagnosed patients with type 2 diabetes: a cohort profile

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ABSTRACT

Purpose The aim of this article is to provide a detailed description of the ongoing nationwide Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort and biobank. The DD2 cohort continuously enrols newly diagnosed patients with type 2 diabetes (T2D) throughout Denmark. The overall goal of the DD2 project is to establish a large and data-rich T2D cohort that can serve as a platform for exhaustive T2D research including (1) improved genotypic and phenotypic characterisation of T2D, (2) intervention studies of more individualised T2D treatment, (3) pharmacoepidemiological studies and (4) long-term follow-up studies on predictors of T2D complications and prognosis.

Participants Between 2010 and 2016, 7011 individuals with T2D have been enrolled and assessed at baseline. Information collected include interview data (eg, body weight at age 20 years, physical activity and alcohol consumption), clinical examination data (eg, hip–waist ratio and resting heart rate) and biological samples (whole blood, DNA, plasma and urine) stored at −80°C and currently analysed for a range of biomarkers and genotypes.

Findings to date Registry linkage has provided extensive supplemental continuous data on glycosylated haemoglobin A, lipids, albuminuria, blood pressure, smoking habits, body mass index, primary care contacts, hospital diagnoses and procedures, medication use, cancer and mortality. Cross-sectional associations between biomarkers, family history, anthropometric and lifestyle measures and presence of complications at baseline have been reported.

Future plans During 2016, a detailed follow-up questionnaire has been answered by 85% of initial participants, providing follow-up information on baseline variables and on presence of diabetic neuropathy. The DD2 cohort has now been followed for a total of 18 862 person-years, and nested intervention trials and follow-up studies are ongoing. In the future, the cohort will serve as a strong national and international resource for recruiting patients to nested case studies, clinical trials, postmarketing surveillance, large-scale genome studies and follow-up studies of T2D complications.

Strengths and limitations of this study

- The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort is an ongoing Danish cohort and biobank of newly diagnosed patients with type 2 diabetes (T2D) continuously enrolled on a nationwide level since 2010.
- By 2016, 7011 patients have been recruited from general practitioners (53%) and hospital specialist outpatient clinics (47%).
- The cohort includes baseline interview data, physical examination data, biological samples (DNA, blood and urine) as well as baseline and follow-up data on glycosylated haemoglobin A, lipids, albuminuria, blood pressure, smoking habits, body mass index, primary care contacts, hospital diagnoses and procedures, medication use, incident cancers, mortality and causes of death.
- The cohort may represent patients with more advanced T2D than average, and 85% already had initiated glucose-lowering treatment at enrolment that hampers studies of drug-naive patients with T2D.
- In the future, the DD2 cohort will serve as a strong national and international resource for recruiting patients to nested clinical trials, large-scale genome studies and long-term follow-up studies of T2D complications through linkage with the large network of existing population-based registries in Denmark.

INTRODUCTION

Type 2 diabetes (T2D) is associated with a wide range of devastating complications, classically including cardiovascular disease, end-stage renal disease, blindness, diabetic foot ulcers and amputations. T2D also increases risk of infections, some cancers and neuropsychiatric disease.[1] These complications inflict a burden on the healthcare system as well as on the individual patients with diabetes. Since it is hard to prevent T2D, there is a compelling

need for cost-effective research to target and improve diabetes treatment in order to prevent complications. Fortunately, over the last decades, the number of available glucose-lowering treatment options has increased,[2] facilitating individually tailored treatment. In this context determining how genotype and phenotype may influence the optimal choice of treatment is essential.[3]

Denmark has a tax-supported healthcare system that includes free access to care at general practitioners (GPs) and hospitals as well as partial reimbursement for most prescribed drugs, including diabetes drugs.[4] GPs—the gatekeepers of the Danish National Health Service—are responsible for nearly all referrals to hospitals and specialists. Patient contacts with health and social services are extensively documented at the individual level in national databases using the unique civil personal registration number (CPR number) that has been assigned since 1968 to all Danish residents at birth or on immigration.[4]

In this setting, the nationwide Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project was established in 2010. The Project's overall goal is to provide an international resource for T2D research. It encompasses establishment of the nationwide and data-rich DD2 cohort and a linked biobank of DNA, blood and urine. The study rationale has been to provide a platform for improved genotypic and clinical characterisation of T2D, for developing more individualised treatment and for testing new antidiabetic drugs in nested intervention studies. Moreover, to establish a unique T2D patient cohort by linkage with the large network of existing population-based registries in Denmark, through which patients can be followed longitudinally for a variety of clinical and socioeconomic outcomes for decades, allowing improved understanding of the predictors of long-term diabetes prognosis.

The aim of this cohort profile paper is to provide a description of the DD2 cohort as an international T2D research resource, including an overview of the collected data and a description of the baseline characteristics.

COHORT DESCRIPTION

Study participants and recruitment

Since 1 January 2009, all patients with newly or recently diagnosed T2D in Denmark have been eligible to participate in the DD2 cohort. The first patient was enrolled in November 2010, and the cohort now consists of 7011 patients. The DD2 project is ongoing, with continuous enrolment.

The process of enrolling in the DD2 cohort has been described in detail elsewhere[5]: (1) clinical providers (usually either the patient's GP or a hospital physician/nurse) identify patients newly diagnosed with T2D in routine clinical practice. (2) Those patients are informed by the clinical provider about the existence of the DD2 project. (3) Patients interested in participating receive detailed oral and written information and are asked to sign a written informed consent document for enrolment. (4) Patient clinical information is then collected: GPs or hospital physicians/nurses complete an online questionnaire, including items requiring a physical examination. (5) Urine and fasting blood samples are collected for storage in a biobank.[6]

Approximately 80% of patients with T2D in Denmark receive care at GPs' offices and the remainder at hospital specialist outpatient clinics. Enrolment into the DD2 cohort takes place in both settings (figure 1). From 2010 to 2012, most patients were enrolled at hospital outpatient clinics rather than at GP offices (1559 vs 739 patients). From 2013 on, the number of patients recruited by GPs increased rapidly,[7] reaching 3688 in February 2016. As of that month, the corresponding number of patients recruited by outpatient specialist clinics was 3323 (table 1).

During the entire DD2 enrolment period, all diagnosing of T2D in routine clinical practice has been made according to WHO criteria, before 2012 primarily based on OGTT and after 2012 primarily based on glycosylated haemoglobin A (HbA1c) >48 mmol/mol (6.5%). No further diagnostic criteria for enrolment have been applied in the DD2 project. The DD2 project explicitly aims to comprehensively study T2D as diagnosed in everyday clinical care, as one of the project aims is to document pitfalls in initial T2D diagnosing, including investigation of subtypes and subphenotypes in the cohort, occurrence of secondary diabetes, autoimmune diabetes and so on.

While the DD2 cohort from the beginning aimed to focus on newly diagnosed T2D patients, in clinical practice, the referral to DD2 may not happen at first diabetes notice when other clinical activity may be more pertinent. Individuals who have had prevalent T2D for some time after 2009 are also accepted for participation. While we do not have complete data on exact date of diabetes diagnosis for all individuals, average time from first recorded glucose-lowering drug initiation to enrolment date in the DD2 cohort is 1–1.5 years.

The exact proportion of all patients with incident T2D in Denmark that is enrolled into the DD2 cohort is unknown. With an average enrolment in the order of 1000–1200 DD2 patients per year, the project enrols an estimated 5% of newly diagnosed patients with T2D nationwide.

The number of enrolled patients and recruitment sites vary across the five Danish healthcare regions. Currently, the largest proportion (35%) of the cohort patients has been recruited from the region of Southern Denmark, which comprises 21% of Denmark's population, followed by the Central Denmark Region (with 24% of cohort patients) and the Capital Region (with 19% of cohort patients), comprising 22% and 31% of Denmark's population, respectively.

Loss of patients from the DD2 cohort can occur due to emigration and death. These events are identified by linkage to the Danish Civil Registration System (CRS), which has recorded vital status for the entire Danish population since 1968, with daily electronic updates.[4] Enrolled

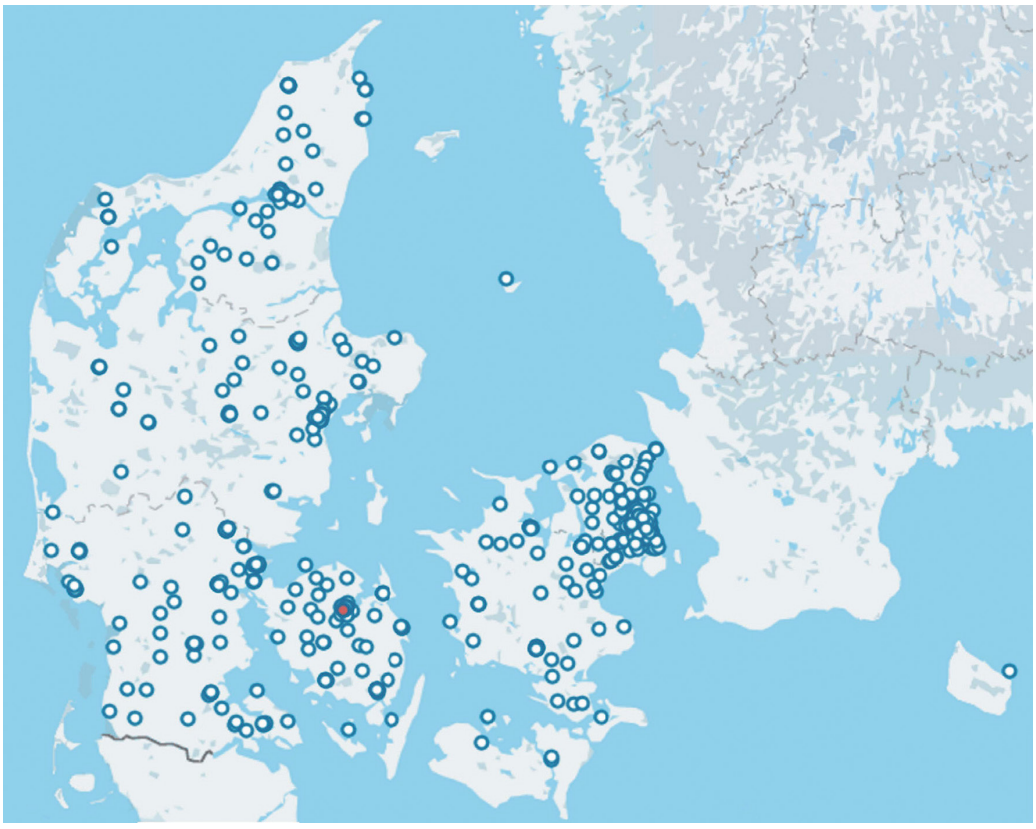

Figure 1 Enrolment sites throughout Denmark. Every circle represents an enrolment site, either at a general practitioner's office or at an outpatient clinic. The administrative headquarter of the DD2 is in Odense (red dot). DD2, Danish Centre for Strategic Research in Type 2 Diabetes.

patients have the right to withdraw from the DD2 cohort, but only four individuals have done so thus far.

Data collection

Baseline data recorded in the DD2 database include each patient's CPR number, date of enrolment and DD2 variables collected directly at the enrolment visit (interview data, clinical examination data and biobank data). Furthermore, linkage with a wide range of Danish health and administrative registries, including the Danish Diabetes Database for Adults (DDDA), one of several Danish nationwide public clinical quality improvement registries,[7] ensures important additional baseline data (figure 2).

The cohort is followed prospectively through linkage with the DDDA and other Danish administrative and health registries. Moreover, directly collected data are used to follow DD2 patients for outcomes not routinely available in medical registries as described below.

Directly collected DD2 baseline variables

These include waist–hip ratio, recalled body weight at age 20 years, maximum lifetime body weight, alcohol consumption, family history of diabetes, resting heart

Table 1 Geographical distribution of DD2 participants by recruitment setting and year of enrolment

	Hospital outpatient clinics, N								General practitioners' offices, N						
Region	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**Total**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**Total**
Capital	0	23	246	197	199	85	5	755	7	144	259	134	39	5	588
Central Denmark	18	211	356	299	222	195	7	1308	0	38	192	84	72	5	391
North Denmark	0	2	52	16	73	19	5	167	0	31	276	161	30	0	498
Zealand	0	0	0	6	20	4	0	30	3	1	432	239	71	11	757
Southern Denmark	0	334	303	168	160	93	5	1063	184	324	237	147	471	50	1413
Unspecified	–	–	–	–	–	–	–	–	–	2	29	7	1	2	41
Total Denmark	18	570	957	686	674	396	22	**3323**	194	540	1425	772	684	73	**3688**

DD2, Danish Centre for Strategic Research in Type 2 Diabetes.

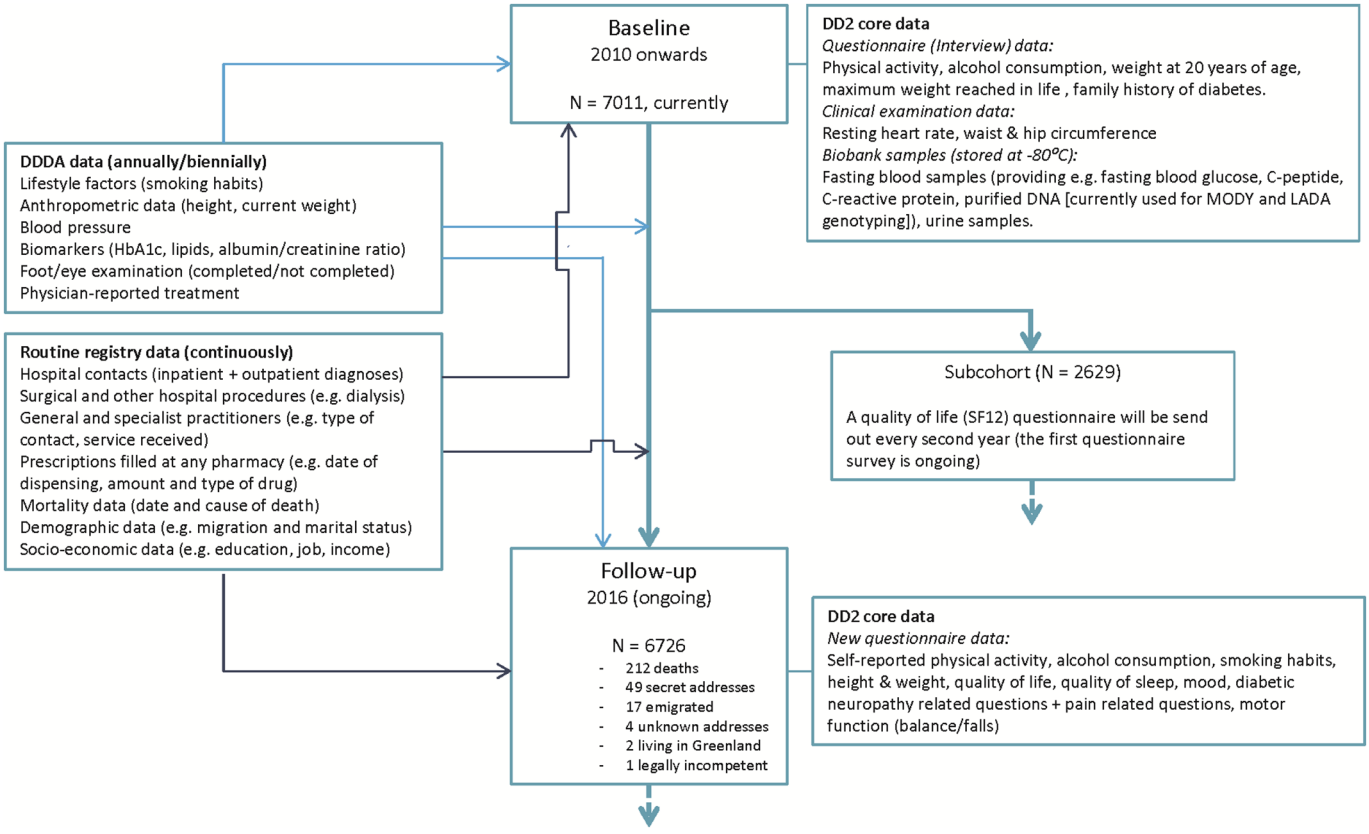

Figure 2 Flow chart of data collection in the DD2 cohort study. DD2, Danish Centre for Strategic Research in Type 2 Diabetes; DDDA, Danish Diabetes Database for Adults; HbA1c, glycosylated haemoglobin A; SF-12, 12-Item Short Form Health Survey.

rate, physical activity level and (added in 2015) self-reported date of first T2D diagnosis. The self-reported physical activity level is currently being validated by placing accelerometers (AX3, Axivity, Newcastle, UK) directly on the skin of the thigh and back of persons in a subcohort of DD2 participants (n=1000), and all physical activity is recorded over a 10-day period. Moreover, a smartphone application (www.interwalk.dk) holding an interval-based training program has been developed. DD2 participants are encouraged to download and use this application, which guides and monitors their physical exercise. The logged exercise data are then transferred to the DD2 cohort and allow future investigations of for example, the impact of exercise on risk of T2D complications. Urine and blood samples (whole blood, serum, plasma and purified DNA) are stored in a biobank[6] and can be used for myriad purposes. To date, plasma samples have been analysed for a number of baseline variables, including fasting blood glucose, C-peptide (used in homeostatic model assessment of insulin resistance), glutamic acid decarboxylase antibodies, alanine-aminotransferases (ALAT), amylase and C reactive protein (CRP). Several currently initiated and planned studies will provide a larger range of analysed biobank variables. For instance, DNA has been purified from 6000 patients and 'maturity-onset diabetes of the young' (MODY) genes are now being sequenced in order to identify individuals carrying likely pathogenic variants. As well, studies of the prevalence of 'latent autoimmune diabetes in adults' in cohort members and the impact of the inflammatory system on diabetic complications have been initiated and will lead to analyses of additional biomarkers, including mannan-binding lectin.

Variables provided by the DDDA

The DDDA, established in 2005, provides a key source of supplemental individual-level data.[7] Quality of care data (variables outlined below) are submitted from GP offices and outpatient clinics to the DDDA annually or biennially. The DDDA thus can provide baseline and follow-up data for members of the DD2 cohort including data on the T2D diagnosis date first recorded in the healthcare system, tobacco smoking, completion of examinations for diabetic foot and eye disease, body mass index (BMI), physician-reported antidiabetic treatment, antihypertensive treatment, hypolipidaemic treatment and routine laboratory measurements such as HbA1c, plasma lipids and albuminuria.

Administrative and health registries

Migration and civil status can be ascertained from the CRS[4] (figure 3). The Danish National Patient Registry (DNPR)[8] maintains complete data on all hospital admissions since 1977 and on all hospital outpatient clinic and emergency room contacts since 1995, including dates of admission and discharge, visit dates, surgical procedures

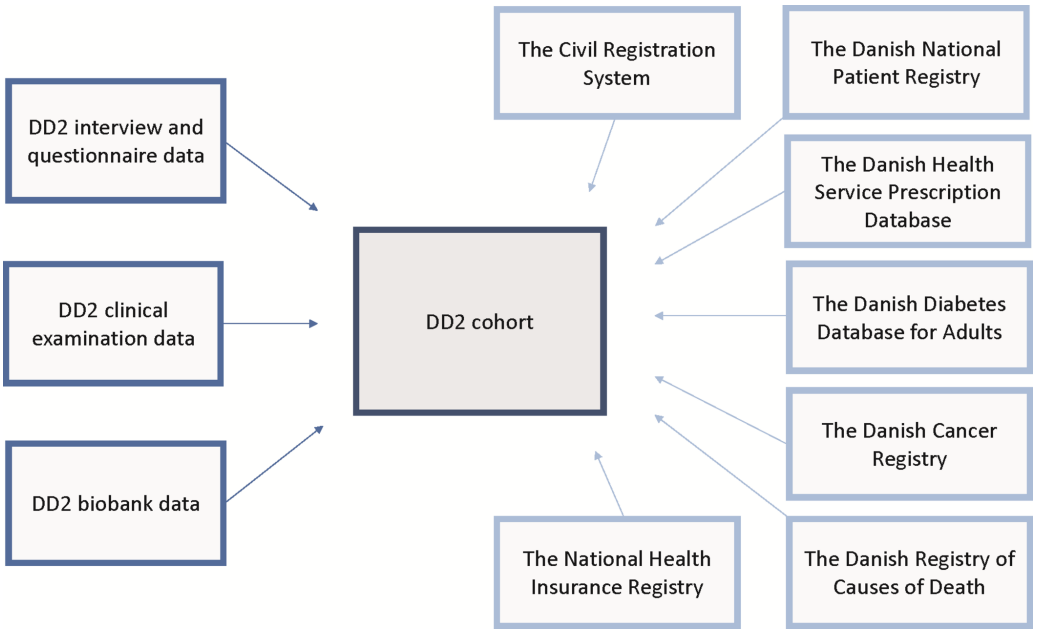

Figure 3 Schematic overview of individual-level data linkage in the DD2 cohort using the civil registration number as personal identification. DD2, Danish Centre for Strategic Research in Type 2 Diabetes.

performed and primary and secondary discharge diagnoses coded according to the *International Classification of Diseases*, 8th Revision until the end of 1993 and 10th Revision thereafter. Linkage to the DNPR makes it possible to obtain individual-level information at baseline and during follow-up on diseases and treatments relevant to diabetes, such as cardiovascular events, microvascular complications and dialysis. On the basis of diagnoses in the DNPR, the baseline comorbidity burden of each DD2 participant is calculated using Charlson Comorbidity Index (CCI) scores[9] (low: 0 points, medium: 1–2 points, high ≥3 points; diabetes is excluded as it constitutes the index disease). Linkage to the Danish National Health Service Prescription Database (DNHSP)[10] provides individual-level information on reimbursable drugs dispensed at all pharmacies in Denmark allowing to track treatment history of each DD2 participant. Pharmacy data include the date of dispensing, as well as amount and type of drug prescribed according to the Anatomical Therapeutic Chemical classification system. The Danish Cancer Registry (DCR)[11] has recorded data on cancer diagnoses in Denmark since 1943 and is a valuable resource for studying cancer in patients with T2D. Linkage to the Danish Register of Causes of Death (DRCD)[12] allows evaluation of cause-specific mortality, while linkage to the Statistics Denmark database provides socioeconomic data, for example, on education, job level and income. The National Health Insurance Service Registry contains information on all services provided by GPs and specialists in Denmark since 1990, including physiotherapists, dentists and chiropractors. Data on services include the number of contacts, health service provider and type of service (eg, consultation, blood glucose measurement or vaccination).[13]

Questionnaire data 2016

By June 2016, all enrolled DD2 patients were sent questionnaires to gather follow-up data (described below) as well as data on the prevalence of diabetic neuropathy. Neuropathy-related questions included, among others, the Michigan Neuropathy Screening Instrument questionnaire, the Douleur Neuropathique 4 questionnaire and other pain-related questions. A random sample of patients reporting symptoms of painful and non-painful neuropathy as well as a random sample of control patients without symptoms will be invited for an in-depth neuropathy examination during 2016–2018, which will allow comprehensive investigation of this late diabetic complication. The questionnaires also maintained follow-up questions regarding the lifestyle factors included in the baseline study interview, including alcohol use, smoking habits and physical activity (figure 2). Preliminary data from this questionnaire survey have revealed a remarkable response rate of 85%.

Also, quality of life 12-Item Short Form Health Survey (SF-12) questionnaires have recently been emailed to all DD2 cohort members with a known email address (n=2629). As of August 2016, 1002 patients (38% of those with email addresses) had responded to this questionnaire. Follow-up SF-12 questionnaires will be emailed automatically every second year.

Baseline characteristics of study participants

An overview of the interview and clinical examination data collected from the 7011 patients enrolled in the DD2 between November 2010 and February 2016 is provided in table 2.

As of February 2016, the DD2 cohort consisted of 4065 (58%) men, the median age was 61 years (IQR:

Table 2 Characteristics of patients with newly diagnosed type 2 diabetes enrolled in the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project during November 2010–February 2016

Variables	Patients with type 2 diabetes
Persons enrolled in DD2 by February, 2016, n (%)	7011 (100)
Enrolled from general practitioners' offices, n (%)	3688 (53)
Enrolled from hospital clinics, n (%)	3323 (47)
Median age (IQR), years	61 (52–68)
Minimum–maximum age, years	17–95
Gender, n (%)	
Male	4065 (58)
Female	2946 (42)
Resting heart rate, median (IQR)	70 (63–79)
Waist circumference, median (IQR), cm	106 (97–116)
Waist–hip ratio in men, median (IQR)	1.02 (0.97–1.06)
Waist–hip ratio in women, median (IQR)	0.92 (0.87–0.97)
Median weight at 20 years of age (IQR), kg	70 (60–80)
Weight unknown at age 20, n (%)	929 (13)
Median maximum lifetime weight (IQR), kg	100 (86–115)
Maximum lifetime weight unknown, n (%)	75 (1)
Alcohol use, n (%)	
Maximum 14/21 drinks/week (women/men)	6539 (93)
More than 14/21 drinks/week (women/men)	472 (7)
Days per week with 30+ min of physical activity (days)	
0	1161 (17)
1	488 (7)
2	891 (13)
3	954 (14)
4	622 (9)
5	681 (10)
6	317 (5)
7	1897 (27)
Regular sports activities, n (%)	
Yes	2748 (39)
No	4262 (61)
Level of physical activity during the past year, n (%)	
Hard physical training and competitive sports several times a week	55 (1)
Leisure sports, heavy garden work or similar activity at least 4 hours per week	1370 (20)
Walking, cycling or other light exercise at least 4 hours per week	4298 (61)
Reading, television watching or other sedentary activity	1288 (18)
Family history of diabetes, n (%)	
Yes	3717 (53)
No	2696 (39)
Don't know	598 (9)
Median fasting blood glucose (IQR), mmol/L*	7.1 (6.4–8.3)
Median C-peptide†‡ (IQR), pmol/L	1161 (865–1570)
Glutamic acid decarboxylase antibodies§	
≤30 kU/L, N (%)	5655 (97)
>30 kU/L, N (%)	161 (3)
Median alanine-aminotransferases¶ (IQR), U/L	27 (20–38)
Median pancreatic amylase¶ (IQR), U/L	23 (16–30)
Median C reactive protein¶ (IQR), mg/L	2.1 (1.0–4.8)

*Currently analysed for the first consecutive 5363 DD2 patients.
†Reference range: 400–1600 pmol/L.
‡Currently analysed for the first consecutive 5800 DD2 patients.
§Currently analysed for the first consecutive 5816 DD2 patients.
¶Currently analysed for the first consecutive 1018 DD2 patients.

52–68 years) and 3717 (53%) patients had a known family history of diabetes. In regard to exercise, 1161 patients (17%) reported less than 30 min and 1897 patients (27%) reported at least 30 min of physical activity 7 days per week. Overall, 2748 patients (39%) reported regular sports activities. At baseline, 7% (n=472) consumed more alcohol per week than the recommended maximum safe amount in Denmark (maximum of 14/21 drinks per week for women/men). Weight at 20 years of age was available for 6082 study participants, with a median value of 70 kg (IQR: 60–80 kg). The median value of maximum lifetime weight was 100 kg (IQR: 86–115 kg) in the cohort. Median waist–hip ratios were 1.02 (IQR: 0.97–1.06) in men and 0.92 (IQR: 0.87–0.97) in women. To date, biological samples have been obtained from more than 93% of cohort members, with a median fasting blood glucose measurement of 7.1 mmol/L (minimum–maximum: 2.5–29 mmol/L) and a median C-peptide measurement of 1161 pmol/L (IQR: 865–1570 pmol/L).[14]

By September 2015, 5115 patients (73%) had been linked to the DDDA quality-of-care database (table 3).

Of the 3835 patients with a known BMI value, 37% of men were overweight at DD2 enrolment and 52% were obese, while 29% of women were overweight and 55% were obese. Seventeen per cent (n=886) were

Table 3 Characteristics of 5115 patients enrolled in the nationwide Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project, which currently can be linked to the Danish Diabetes Database for Adults

Variables	Patients with type 2 diabetes
N (%) of DD2 participants who could be linked to the Danish Diabetes Database for Adults, as of September 2015.	5115 (73)
Tobacco smoking, n (%)	
Never smoker	2254 (44)
Former smoker	1649 (32)
Current smoker, daily	886 (17)
Current smoker, occasionally	55 (1)
Smoking status listed as unknown	271 (5)
Men (n=2984)	
Median height (IQR), cm	178 (174–183)
Height missing, n (%)	709 (24)
Current median weight (IQR), kg	96 (85–110)
Weight missing, n (%)	229 (8)
Current BMI, n (% of those with known BMI)	
<18.5	2 (0)
18.5–24.9	250 (11)
25–29.9	833 (37)
30+	1183 (52)
BMI missing	716
Women (n=2131)	
Median height (IQR), cm	164 (160–169)
Height missing, n (%)	561 (26)
Current median weight (IQR), kg	84 (71–96)
Weight missing, n (%)	188 (4)
Current BMI, n (% of those with known BMI)	
<18.5	8 (1)
18.5–24.9	240 (15)
25–29.9	458 (29)
30+	861 (55)
BMI missing	564
HbA1c value	
<7%	3592 (70)
7.0%–8.0%	824 (16)
8.0%–9.0%	303 (6)
≥9.0%	313 (6)
HbA1c missing	83 (2)
Albuminuria, N (%)	
Albumin–creatinine ratio <30 mg/g	3565 (70)
Albumin–creatinine ratio 30–300 mg/g	830 (16)
Albumin–creatinine ratio ≥300 mg/g	110 (2)
Albuminuria–creatinine ratio missing	610 (12)
Blood pressure, median (IQR), mm Hg	
Systolic	130 (124–140)
Diastolic	80 (74–85)
Lipids, median (IQR), mmol/L	
Total cholesterol	4.4 (3.7–5.1)
HDL cholesterol	1.2 (1.0–1.5)
LDL cholesterol	2.2 (1.7–2.8)
Triglycerides	1.6 (1.2–2.4)
Physician-reported antidiabetic treatment, n (%)*	
Insulin only	59 (1)
Insulin and oral antidiabetic treatment	306 (6)
Oral antidiabetic treatment	3993 (78)
None	757 (15)
Physician-reported antihypertensive treatment, n (%)	3523 (69)
Physician-reported ACE inhibitor or ATII-antagonist treatment, n (%)	2786 (54)
Physician-reported hypolipidaemic treatment	3373 (66)
Eye screening completed	2762 (54)
Foot examination completed	4362 (85)

*Within the year prior to enrolment in the DD2 cohort.
ATII-antagonist, angiotensin 2 antagonist; BMI, body mass index; HbA1c, glycosylated haemoglobin A; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

current daily smokers, and 32% (n=1649) were former smokers. Most had an HbA1c value below 7.0% (70%) and 4358 (85%) had received antidiabetic treatment within the year prior to enrolment; 59 patients (1%) were taking only insulin and 3993 (78%) received only oral antidiabetic treatment, as confirmed by routine prescription data from DNHSP. A diabetic eye examination had been performed on 2762 (54%) cohort members and a foot examination on 4362 (85%). At DD2 enrolment, microalbuminuria was present in 16% of patients, and macroalbuminuria was present in 2%. Median systolic/diastolic blood pressure was 130/80, and 3523 patients (69%) were being treated for hypertension. Median total cholesterol was 4.4 (IQR: 3.7–5.1) mmol/L; 3373 patients (66%) were receiving hypolipidaemic treatment. Linkage of the DD2 cohort to the DNPR and DNHSP revealed that approximately one-third of patients had hospital-diagnosed comorbidity (CCI score ≥1 point) prior to enrolment. Twenty-two per cent had hospital-diagnosed macrovascular disease, and 15% had microvascular complications prior to enrolment (table 4).

Table 4 Comorbidities (determined through linkage with the DNPR) and use of medications (determined through linkage with the DNHSP) at enrolment among the first 7011* patients with newly diagnosed type 2 diabetes enrolled in the DD2 project

Variables	Patients with type 2 diabetes
Persons enrolled in DD2 by February 2016, n (%)	7011 (100)
Charlson Comorbidity Index score†	
0	4807 (69)
1–2	1820 (26)
3+	384 (5)
Charlson Comorbidity Index conditions at baseline, n (%)	
Myocardial infarction	345 (5)
Congestive heart failure	283 (4)
Peripheral vascular disease	309 (4)
Cerebrovascular disease	465 (7)
Dementia	13 (0.2)
Chronic pulmonary disease	510 (7)
Connective tissue disease	183 (3)
Ulcer disease	126 (2)
Mild liver disease	111 (2)
Hemiplegia	19 (0.3)
Moderate to severe renal disease	117 (2)
Any tumour	508 (7)
Leukaemia	15 (0.2)
Lymphoma	26 (0.4)
Moderate to severe liver disease	16 (0.2)
Metastatic solid tumour	43 (0.6)
HIV/AIDS	9 (0.1)
Any macrovascular complications	1511 (22)
Any microvascular complications	1035 (15)
Diabetic neuropathy	230 (3)
Diabetic retinopathy	682 (10)
Diabetic nephropathy	209 (3)
Mental disorder (based on discharge and treatment codes for mental illness)*	1456 (21)
Antidiabetic treatment, n (%)*‡	
Insulin only	80 (1)
Insulin and oral antidiabetic treatment	396 (6)
Oral antidiabetic treatment	5387 (78)
None	1053 (15)
Glucose-lowering drugs, n (%)*‡	
Biguanides (metformin)	5631 (81)
Insulin	476 (7)
Sulfonylureas	463 (7)
DPP-4 inhibitors	653 (9)
GLP-1 analogues	378 (5)
SGLT-2 inhibitors	57 (0.8)
Meglitinides	7 (0.1)
Thiazolidinediones (glitazones)	1 (0.01)
Alpha-glucosidase inhibitors	4 (0.06)
Antihypertensive treatment excluding loopdiuretics, n (%)*‡	4991 (72)
ACE inhibitors or ATII-antagonist treatment, n (%)*‡	4157 (60)
Hypolipidaemic treatment, n (%)*‡	4886 (71)
Oral steroids, n (%)*‡	430 (6)

*At the time of analysis, full prescription data were available only until 31 December 2015. Therefore, variables based on data from the DNHSP are for a subcohort numbering 6916 patients.
†Diabetes mellitus not included in the score as it constitutes the index disease. Comorbidity categories include DNPR derived data on cancer; more detailed cancer data are available from the Danish Cancer Registry.
‡Within the last year prior to DD2 enrolment.
ATII-antagonist, angiotensin 2 antagonist; DD2, Danish Centre for Strategic Research in Type 2 Diabetes; DNPR, Danish National Patient Registry; DNHSP, Danish National Health Service Prescription; DPP-4-inhibitors, dipeptidylpeptidase 4 inhibitors; GLP-1 analogues, glucagon-like peptide 1 analogues; SGLT-2 inhibitors, sodium glucose cotransporter 2 inhibitors.

FINDINGS TO DATE

Mortality ratios

The DD2 cohort has been followed for a total of 18 862 person-years (PY) and a mean of 2.7 years. A total of 212 patients have died, yielding an all-cause mortality rate (MR) of 1.12 per 100 PY (95% CI 0.97 to 1.29), with differences by gender (MR for women: 0.81 (95% CI 0.62 to 1.03); MR for men: 1.36 (95% CI 1.15 to 1.60)). As expected, all-cause MR increased with age (table 5).

Table 5 Number of deaths and overall, age-specific and gender-specific mortality rates (per 100 person-years) for the baseline cohort of 7011 DD2 patients

Variables	Number of deaths	MR (95% CI)
Total DD2 cohort	212	1.12 (0.97 to 1.29)
Gender		
Women	65	0.81 (0.62 to 1.03)
Men	147	1.36 (1.15 to 1.60)
Age (years)		
<40	2	0.23 (0.03 to 0.83)
40–59	37	0.50 (0.35 to 0.69)
60–79	149	1.47 (1.25 to 1.73)
≥80	24	4.97 (3.19 to 7.40)

Median follow-up time from date of enrolment to death or to date of latest data extraction from the Civil Registration System was 2.68 years (IQR: 1.67–3.63 years).
DD2, Danish Centre for Strategic Research in Type 2 Diabetes; MR, mortality rate.

Key findings

Several baseline cross-sectional studies based on preliminary findings for parts of the current DD2 cohort were published in 2014–2016.[15–20] Parental history of T2D was associated with younger age at diagnosis (adjusted prevalence ratio (aPR) for age <40 years: 1.66 (95% CI 1.19 to 2.31); aPR for age 40–60 years: 1.36 (95% CI 1.24 to 1.48)), as well as poorer glucose control at diagnosis (aPR for fasting blood glucose <6.5 mmol/L: 0.80 (95% CI 0.62 to 1.02) and aPR for fasting blood glucose ≥7.5 mmol/L: 1.47 (95% CI 1.20 to 1.80)). No associations were observed for anthropometric and lifestyle factors, while parental history tended to be negatively associated with the high beta cell function phenotype[18] (aPR 0.79 (95% CI 0.61 to 1.01)). This suggests that patients with T2D may not inherit diabetes primarily as a result of family-learned lifestyle habits (physical inactivity and overeating), but rather as a result of a constitutional impairment of insulin action/secretion.[18] In studies of DD2 biobank data, a 16% prevalence of elevated ALAT (>38 IU/L for women and >50 IU/L for men)[15] and a 40% prevalence of elevated CRP (>3.0 mg/L)[16] have been found among newly diagnosed patients with T2D. As well, potentially modifiable predictors of elevated ALAT/CRP have been identified, including low physical activity (ALAT: relative risk (RR) 1.4, 95% CI 1.04 to 1.93; CRP: RR 1.46, 95% CI 1.11 to 1.91).[15 16] Such biomarkers may have an impact on the clinical outcome of diabetes, which future prospective studies using DD2 data will be able to examine.

In another study, patients with incident T2D were followed during their first year postdiagnosis. It was found that 74% were receiving glucose-lowering therapy, with 88% receiving monotherapy, mainly metformin. Factors associated with receiving any glucose-lowering therapy included young age (RR 1.29, 95% CI 1.16 to 1.44), central obesity (RR=1.23, 95% CI 1.04 to 1.44), large weight gain since youth (RR=1.10, 95% CI 1.03 to 1.18), lack of regular physical activity (RR=1.07, 95% CI 1.01 to 1.15), high baseline fasting blood glucose (RR=1.25, 95% CI 1.10 to 1.42) and high comorbidity burden (RR=1.20, 95% CI 1.05 to 1.38).[17]

A publication list is provided at www.dd2.nu. The results of many ongoing long-term prospective studies will be reported in years to come including genotype studies investigating the association between genotypes and pharmacological treatment strategies on relevant endpoints like glycaemic control and albuminuria.

STRENGTHS AND LIMITATIONS

The DD2 project has established a large population-based cohort of newly diagnosed patients with T2D, with demographic, anthropometric, lifestyle and clinical data. Furthermore, the project maintains a valuable biobank. Use of the unique individual-level CPR identifier makes it possible to avoid multiple registrations and to take advantage of existing data in Danish health and administrative registries in a cost-effective way. These registries also provide a source of long-term follow-up data. Since much of the data are recorded by health personnel during diagnosis and treatment, and not for research purposes, investigator bias is reduced. In addition, the validity of Danish registry data is high. As an example, reporting of cancer to the DCR is mandatory, and completeness is secured by cross-checking with other registries including DRCD and the Danish Pathology Registry.[11 21] In the DNPR, the positive predictive value of, for example, hospital discharge diagnoses included in the CCI is 94%–100%.[22]

A few limitations of the DD2 cohort must be noted. First, while DD2 and DDDA data are nearly complete for demographic variables and for many clinical variables such as smoking, physical activity, hip–waist ratio and resting heart rate, the proportion of missing data is large for some variables. For example, in the DDDA, BMI is missing for 26% of women. However, waist–hip ratio is available for 100% of the DD2 cohort and constitutes a better predictor of cardiovascular complications than BMI.[23] Second, linkage to the DDDA currently is only possible for 73% of DD2 participants, partly explained by the data delay associated with the DDDA's role as a clinical quality improvement registry. Its mandate is to report on quality of care based on data collected during the previous year (ie, patients need to have prevalent T2D for 1–2 years before they are eligible for quality-of-care assessment). In contrast, the DD2 project focuses on incident T2D and is tasked with collecting data at time of diagnosis.[7] Moreover, while reporting to the DDDA by GPs became mandatory in 2013, all GP reporting discontinued in September 2014 due to conflicting interpretation of legal issues concerning automated data transmission. Third, while it was planned for the DD2 cohort to recruit newly diagnosed T2D patients, approximately 85% already have initiated glucose-lowering treatment at enrolment. This hampers analyses of biomarkers among treatment-naive patients with T2D. However, the exact start date of glucose-lowering therapy can be ascertained from prescription registries. Fourth, since predominantly patients from outpatient specialist clinics were enrolled in early study years, that is, during 2010–2012, the cohort initially may have contained newly diagnosed patients with T2D with more advanced disease than average. However, the baseline data presented in this paper are similar to baseline data in a recent registry-based cohort study investigating patients with T2D from the Northern Region of Denmark at initiation of their first glucose-lowering therapy,[24] thus reassuring that exposures of interest are represented for Danish patients with T2D in earlier phases of the disease. Finally, a large proportion of patients in the DD2 cohort were enrolled during the last few years, which limits current opportunities for conducting long-term follow-up studies.

In the future, the DD2 cohort will serve as a strong national and international resource for recruiting patients to nested case studies and clinical trials, postmarketing surveillance, large-scale genome studies, intervention

studies, for example, in patients with rare diabetic subtypes, and follow-up studies of diabetes complications.

COLLABORATION

More information about the DD2 cohort can be found at the DD2 website www.dd2.nu. The DD2 project has a Steering Group with members from GP practices/hospital research units in all regions of Denmark. The Steering Group strongly encourages national and international collaboration. Interested researchers can contact Director Dr Henning Beck-Nielsen at henning.beck-nielsen@syd.dk.

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Contributors HB-N, HTS, JSC, JR, AV, SF, IB, JSN and RWT designed the DD2 cohort and raised the funding. IB is in charge of storage and analyses of biological samples. SKN and KB performed all the statistical analyses. DHC and RWT wrote the first draft of this report. All authors critically revised the manuscript and approved the final version.

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Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Ethics approval The DD2 Project was approved by the Regional Committee on Health Research Ethics for Southern Denmark (Record number S-20100082) and the Danish Data Protection Agency (2008-58-0035).

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Appendix II

Title page

PAIN

Diabetic polyneuropathy and pain, prevalence, and patient characteristics: A cross-sectional questionnaire study of 5,514 patients with recently diagnosed type 2 diabetes.

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Abstract

Most studies of diabetic polyneuropathy (DPN) and painful DPN are conducted in persons with longstanding diabetes. This cross-sectional study aimed to estimate the prevalence of DPN and painful DPN, important risk factors, and the association with mental health in recently diagnosed type 2 diabetes. A total of 5,514 (82%) patients (median diabetes duration 4.6 years) enrolled in the Danish Centre for Strategic Research in Type 2 Diabetes cohort responded to a detailed questionnaire on neuropathy and pain. A score ≥ 4 on the MNSI questionnaire determined possible DPN whereas pain presence in both feet together with a score ≥ 3 on the DN4 questionnaire determined possible painful DPN. The prevalence of possible DPN and possible painful DPN was 18% and 10%, respectively. Female sex, age, diabetes duration, BMI, and smoking were associated with possible DPN, whereas only smoking showed a clear association with possible painful DPN (OR 1.52 [95% CI: 1.20; 1.93]). Possible DPN and painful DPN were independently and additively associated with lower quality-of-life, poorer sleep, and symptoms of depression and anxiety. Possible DPN itself had greater impact on mental health than neuropathic pain. This large study emphasizes the importance of careful screening for DPN and pain early in the course of type 2 diabetes.

Introduction

Diabetic polyneuropathy (DPN) is a serious diabetes complication. Previous studies have reported a wide range of prevalence from 26% to 50% for DPN,[1; 25; 37; 38; 44] and between 8-30% for painful DPN.[1; 3; 7; 12; 13; 37; 43] This variation may be explained by different assessment methods and definitions of DPN, and differentially selected study populations.[28; 31; 34; 40; 42] Most studies have examined patients with long duration of diabetes i.e. 8-17 years,[3; 7; 13; 30; 31; 35; 37; 38; 44] whereas little is known about the prevalence of DPN and painful DPN in recently diagnosed diabetes.

Accumulating evidence suggest that not only hyperglycemia, but also factors like increasing diabetes duration, type 2 versus type 1 diabetes, obesity, smoking, and female sex,[2; 3; 7; 11; 13; 23; 30; 31; 35; 37; 38; 44] may be linked to DPN and painful DPN, which particularly may be true in type 2 diabetes. However, existing studies are either old[44], based on mixed population (e.g. non-diabetes, type 1 diabetes, and type 2 diabetes,[3; 7; 12; 13; 38; 44]) include patients with longstanding diabetes,[3; 7; 13; 30; 35; 37; 38; 44] are of smaller size,[3; 7; 12; 30; 35] or only investigate painful DPN.[1; 3; 12] Less evidence on factors associated with DPN and painful DPN in recently diagnosed type 2 diabetes patients is available from large-scale studies.

In diabetes patients, chronic neuropathic pain has been related to decreased quality of life (QoL), poor sleep, and symptoms of anxiety and depression.[4; 7; 11; 13; 18; 19; 21; 35; 38; 41] In contrast, the impact of DPN itself - regardless of pain - on quality of life and mental health comorbidities is uncertain in type 2 diabetes. A study suggested that having DPN without painful symptoms had no effect on mental

health-related measures,[38] whereas other studies found depression to be more common both among diabetes patients with painless and painful DPN.[4; 11]

To fill these knowledge gaps, we conducted a questionnaire survey on neuropathy and pain in the large Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort which enrolls patients with recently diagnosed type 2 diabetes throughout Denmark. The aims of this paper are 1) to explore the prevalence of possible DPN and painful DPN in recently diagnosed type 2 diabetes patients, 2) to investigate patient characteristics and lifestyle factors associated with possible DPN and painful DPN, and 3) to examine the impact of possible DPN and painful DPN on mental health in recently diagnosed type 2 diabetes.

Methods

Setting and patients

This study is based on the 7,011 type 2 diabetes patients consecutively enrolled in the DD2 cohort by February 2016. Detailed information on the logistics and characteristics of this cohort have previously been reported.[10; 26] In brief, the DD2 cohort began enrolment in November 2010 and the project is ongoing. Enrolment of newly or recently diagnosed (median diabetes duration at time of enrolment 1.3 year, IQR 0.3-2.9 years) type 2 diabetes patients takes place at the general practitioner's office and outpatient hospital clinics (Departments of Endocrinology) in Denmark. During the DD2 enrolment period all patients have been diagnosed with diabetes according to the WHO criteria.[10]

Questionnaire

By June 7, 2016, a detailed questionnaire consisting of 41 questions was sent out to all patients alive and living in Denmark with a known address enrolled into DD2 (N=6,726) (Figure 1). A complete version of the questionnaire is available in the supplementary digital content (supplementary Table 1). In September 2016 and again in October 2016 a reminder was sent to those who had not provided a response. All patients were sent a paper version and a link to an electronic version allowing them to answer in their preferred way. All patients were asked to return a blank questionnaire including a note of the reason if they did not want to participate in the questionnaire survey. A subsample of the cohort was invited for a detailed clinical examination, these results will be presented in a separate publication.

Patient characteristics

Patient demographics included in the questionnaire were age, sex, height, and weight. Lifestyle factors included smoking habits, alcohol consumption (> 7/14 units of alcohol [women/men], which is the maximum safe amount recommended by the Danish Health Authority), and questions on physical activity level.

DPN

There is no gold standard for identifying polyneuropathy for epidemiological research purposes, but the Michigan Neuropathy Screening Instrument questionnaire part (MNSIq[15]) is a commonly used symptom based screening tool for identifying DPN.[2; 8; 43] ***We used the MNSIq and the validated cutoff of ≥ 4/13 abnormal responses to define possible DPN [15; 33].*** This cutoff had a sensitivity of 40%

and a specificity of 92% for detecting confirmed clinical neuropathy in a selected group of patients with longstanding type 1 diabetes.[24]

Questions on gait instability and falls, as well as frequency and severity of falls, were also included in the questionnaire.

Painful DPN and other pain

The questionnaire contained questions on general pain (any constant or recurrent pain and location of pain) and pain in both feet. Patients reporting pain in both feet were given more detailed questions about the pain. They filled out the 7-item Douleur Neuropathique en 4 Questions (DN4) which is a screening tool for neuropathic pain and with a high performance in DPN.[32; 35] The DN4 questionnaire comprises 7 “yes” or “no” items related to pain quality; 4 sensory descriptors (tingling, pins and needles, numbness, itching) and 3 pain descriptors (burning, painful cold and electric shock sensation). Only patients with pain in the feet completed the DN4 and it was specified that it concerned characteristics of the pain in their feet (Supplementary table 1). A DN4 score of ≥ 3/7 has a sensitivity and specificity of 84% for identifying clinically confirmed painful DPN.[32] ***Patients with pain in both feet and a DN4-score ≥ 3 were considered to have possible painful DPN [6; 17; 32; 35] regardless of MNSIq-score*** (Figure 2). Our neuropathic pain definition was in accordance with the consensus statement (NeuroPPIC) from the Neuropathic Special Interest Group (NeuPSIG) of the International Association for the Study of Pain (IASP) for the basic “entry level” to identify possible neuropathic pain in questionnaire studies.[39] We included additional questions on pain quality, use of pain medications, and pain duration and pain intensity within the previous 24

hours and 7 days at the time of evaluation were recorded. For the latter we used a numeric rating scale (NRS) ranging from 0-10, with 0 denoting no pain and 10 the worst possible pain. We used the Patient Reported Outcome Measurement Information System (PROMIS®) short form v1.0 – Pain Interference 4a to assess pain interference with daily activities, household, and social activities within the previous 7 days.[22]

Mental health

The patients rated their QoL in the previous 7 days using a NRS ranging from 0 to 10, with 10 being the best QoL possible and 0 the worst.[20] Sleep disturbance and symptoms of depression and anxiety were assessed using the PROMIS Short Forms 4a. The instruments grade symptoms experienced during the previous 7 days with a frequency or severity grading of symptoms from “never” to “always” or from “bad” to “very good” with five options. The scores are converted into PROMIS T-scores, which are standardized relative to an American/US reference population and are used to categorize the level of impairment/symptoms (normal, mild, moderate, severe).[22; 29]

Ethical considerations

All DD2 patients volunteered to participate in the DD2 study and gave written informed consent. The Danish National Committee on Health Research Ethics (record number S-20100082) has approved the DD2 project. The Danish Data Protection Agency (record number 2008-58-0035) has approved the DD2 project and the study is registered at Aarhus University internal notification no. 62908-250.

Statistical analyses

Means (SD) were used to describe normally distributed data, and medians (IQR) to describe non-normally distributed variables. Information on *both* DPN (defined based on MNSIq) *and* painful DPN (defined based on DN4 and pain location in both feet) status was available for a subpopulation of 5,249 patients. Combination of MNSIq-defined possible DPN and DN4-defined possible painful DPN status yielded four distinct groups (Figure 2, Table 2) for which descriptive data were provided. Finally, descriptive data on age, sex, and diabetes duration were provided for responders and non-responders.

We calculated the prevalence of DPN and painful DPN with 95% confidence intervals (CI) using the exact method for binomial distributions.

We used multivariable linear (age, body mass index [BMI], diabetes duration, height) and logistic (sex, smoking status [ever (former + current) vs. never], alcohol consumption) regressions and modelled each patient characteristic as a function of possible DPN and possible painful DPN and an interaction term of DPN and painful DPN, while controlling for age, sex, and diabetes duration. If no significant interaction was observed between DPN and painful DPN, each regression was rerun without the interaction term. The significance level was chosen at <0.05. The cross-sectional study design facilitates an investigation of associations, not of temporal relationships. Therefore, possible DPN and possible painful DPN could be included as the independent variables enabling us to include both DPN and painful DPN simultaneously in the multivariable regression models used to examine associations with patient characteristics. This approach allowed us 1) to investigate the

association of the evaluated patient characteristics and possible DPN defined by DN4 and pain in both feet separately from the association with possible DPN defined by MNSIq, and 2) to handle the fact that some patients had possible painful DPN but had a MNSIq score < 4 (Figure 2), including the evaluation of possible interaction between possible DPN defined by MNSIq and possible painful DPN defined by DN4.

To evaluate the impact of DPN and painful DPN on mental health, we used the same approach as described above, a multivariable linear regression to model QoL and T-scores for sleep, depression, and anxiety as functions of DPN and painful DPN and adjusted for age, sex, diabetes duration, and BMI (model 1). To control for possible confounding by pain other than neuropathic pain in the feet, the regressions were rerun including a variable of the number of pain locations other than extremities (model 2). Because obesity is strongly associated with mental health outcomes, we adjusted for BMI. However, the direction of the association BMI-mental health, could be bidirectional, thus, we performed a sensitivity analysis in which we left out BMI in the regressions. All regressions were first run including an interaction term between DPN and painful DPN and if no interaction was observed, the regressions were rerun without the interaction term. We used a Wald–test to compare the sizes of the associations of DPN and painful DPN with-mental health outcomes in the regression models without interaction term.

Finally, the correlation between mental health and pain intensity in the feet was estimated using spearman's rho.

There were few missing data and all analyses were performed as complete case analyses.

Data were analyzed using STATA version 14.

Results

Patient population

As seen in Figure 1 the number of patients responding to the questionnaire was 5,755 (85.6%). Of these, 225 (3.3%) returned a blank questionnaire (136 (60.4%) patients provided a reason for non-participating) and 16 (0.2%) patients were excluded because they answered the questionnaire multiple times. Of the remaining 5,514 patients (82% of those who initially received a questionnaire), 42.7% were women, mean (±SD) age was 64.1 (10.9) years and median duration of diabetes (IQR) was 4.6 (3.5; 5.7) years. Further patient characteristics are provided in Table 1. Diabetes duration and sex distribution were similar among responders and non-responders (supplementary Table 2), but non-responders were slightly younger than responders (mean age (±SD) 59.6 (12.8) vs. 64.1 (10.9)).

Prevalence

Of the 5,359 patients with valid answers on the MNSIq, 962 had a score ≥4, suggesting a prevalence of possible DPN of 18.0% (95% CI: 16.9%; 19.0%) (Figure 1, supplementary Table 3).

Of the 5,372 patients with valid data to assess painful DPN, 536 reported pain in both feet and had a DN4 score ≥ 3, corresponding to a prevalence of possible painful DPN of 10.0% (95% CI 9.2%; 10.8%) (Figure 1, supplementary Table 3). Of those with painful DPN, 130 (28.0%) did not fulfill the MNSIq criteria for DPN (Table 2).

Prevalence were stable across questionnaire intervals (supplementary Table 4).

Pain: painful diabetic polyneuropathy.

As shown in Table 3 more than 80% of the patients with painful DPN had pain in the feet for more than 1 year. Pain often interfered with daily activities, including household chores and social activities (79.2%) and 60.1% reported concomitant drug treatment for their pain. The average (±SD) pain intensity in the feet was 5.3 (2.1) the last 7 days on a NRS (0 – 10) and 76.2 % had moderate to severe pain intensity (NRS ≥ 4). The most common pain description from the DN4 was burning pain (71.8%), 36.4% reported cold pain and 38.2% had electric shock like pain (data not shown). There was a negative correlation between reported QoL and the intensity of pain (Spearman's rho -0.24, p<001) and a positive but weak correlation between reported symptoms of anxiety, depression and poor sleep and pain in the feet within the last 7 days (Spearman's rho 0.25, 0.23, and 0.26, p<0.001) (data not shown).

The small group of patients with painful DPN that did not fulfill the MNSIq criteria for DPN (N=130) did not differ from those with painful DPN fulfilling the MNSIq criteria (N = 386) regarding age, sex, duration of diabetes, and use of pain medications (Table 2). However, they reported lower mean (±SD) pain intensity (average 7 days: 4.3 (2.1) vs. 5.6 (2.1) (data not shown)). The most common pain descriptors on the MNSIq were in both groups prickling feeling, burning pain, and leg pain (supplementary Figure 1A).

Pain: pain other than painful DPN

A higher proportion of patients with possible DPN and possible painful DPN had complaints of pain in various body sites compared to those with no DPN (Table 2). The proportion of patients reporting pain at 2 or more locations other than the extremities was 24.5 % in those without DPN, 55.4% in those with painful DPN not

fulfilling the MNSIq criteria for DPN, 52.7% in those with DPN not fulfilling the criteria for painful DPN, and 67.6% in those with painful DPN fulfilling the MNSIq criteria for DPN (Table 2).

Association between DPN and painful DPN and patient characteristics

We found no statistically significant interaction between possible DPN defined by MNSIq and possible painful DPN defined by DN4 and pain in both feet, suggesting that the estimates of association between possible DPN and patient characteristics were independent of the presence of possible painful DPN, and vice versa.

After correction for age, sex and painful DPN, DPN was statistically significantly associated with younger age, longer duration of diabetes, higher BMI, female sex, and presence of ever tobacco smoking (Table 4). Associations were generally weaker for painful DPN except for ever tobacco smoking which was statistically significant associated with painful DPN (OR: 1.52 [1.20; 1.93]) (Table 4).

Association between DPN, painful DPN and mental health

Again, we found no statistically significant interaction between possible DPN defined by MNSIq and possible painful DPN defined by DN4 and pain in both feet, suggesting that the estimates of association between possible DPN and mental health outcomes were independent of the estimates of association of possible painful DPN, and vice versa.

Both DPN and painful DPN were independently and additively associated with lower QoL (DPN: -1.16 [-1.31; -1.01], painful DPN: -0.85 [-1.04; -0.667]) and higher T-scores of depression (DPN: 4.18 [3.53; 4.84], painful DPN: 3.35 [2.51; 4.18]), poor

sleep (DPN: 4.65 [4.04; 5.27], painful DPN: 2.22 [1.44; 3.00]), and anxiety (DPN: 3.98~~8~~7 [3.31; 4.64], painful DPN: 2.73 [1.89; 3.58]) after controlling for age, sex, diabetes duration, BMI and DPN or painful DPN status (Table 5). The size of the effect of DPN on mental health outcomes were in general higher than that of painful DPN (Supplementary Table 5, supplementary Figure 2).

Further controlling for pain in other bodily localizations reduced the effect size of the associations, e.g. for depression (DPN: 2.95 [2.30; 3.59], painful DPN: 2.12 [1.30; 2.93]). The total effect of fulfilling both the criteria for DPN and painful DPN on e.g. QoL score (-0.85 + -0.57 = -1.42) was of the same order of magnitude as having pain in three other areas/locations (-1.29) e.g. headache, back pain and stomach pain (Table 5).

Leaving BMI out of the models, resulted in slightly higher DPN and painful DPN estimates for all mental health outcomes, thus not changing any conclusions (supplementary Table 6).

Discussion

In this large study of a nationwide cohort with recently diagnosed type 2 diabetes patients the prevalence of possible DPN was 18% and the prevalence of possible painful DPN was 10%. We found an association between possible DPN and female sex, smoking, longer diabetes duration, lower age, and higher BMI, whereas most relations were weaker for possible painful DPN which was only statistically significant associated with smoking. In contrast, both possible DPN and painful DPN were independently and additively associated with decreased QoL and increased

symptoms of depression, anxiety and poor sleep. Moreover, possible DPN had greater impact on mental health than possible neuropathic pain.

This is the largest questionnaire study to date that examines the prevalence and clinical characteristics of possible DPN and painful DPN in a cohort of recently diagnosed type 2 diabetes patients using validated screening tools. The prevalence of DPN (18%) and painful DPN (10%) found in this study are similar to the prevalence reported in two survey studies using the MNSIq for the diagnosis of DPN and the MNSI in combination with Brief Pain Inventory (BPI) to diagnose painful DPN.[2; 43] In the ADDITION Denmark cohort study consisting of 1445 screenings-detected type 2 diabetes patients the prevalence of DPN at time of diabetes diagnosis was of 13.1%,[2] whereas in a French nationwide cohort study consisting of 1023 type 1 and 2 diabetes patients with a mean duration of diabetes of 15 years, the prevalence of painful DPN was 8% using a MNSIq cutoff of 7 as compared to 4 in our study.[43] In a large UK study of diabetes patients in a community health care setting the duration of diabetes was similar to our study (median 5 years), but the prevalence estimate of painful DPN twice as high or 21%.[1] This difference may be related to painful DPN being defined based on a clinical evaluation in the UK study. Other studies of more longstanding diabetes have likewise reported higher prevalence of both DPN and painful DPN than our study.[1; 3; 25; 31; 37; 38] These differences may be partly explained by the longer diabetes duration, but also by the different diagnostic criteria used for DPN and painful DPN. Thus, our use of questionnaire-based tools to determine DPN and painful DPN in the absence of clinical examination and confirmatory tests reduces the level of certainty of the DPN diagnoses. [14; 17; 33] Moreover, the sensitivity of a MNSIq score ≥ 4 was 40%

compared to clinically defined DPN in a study of younger patients with longstanding type 1 diabetes, thus, we likely also underestimate DPN prevalence in our cohort. We do not know whether this sensitivity can be applied to our type 2 diabetes cohort, but it is likely that the MNSIq also underestimate DPN prevalence in our cohort.

Our associations of female sex, smoking, higher BMI and longer duration of diabetes with DPN in recently diagnosed type 2 diabetes corroborate previous studies of patients with longstanding diabetes.[23; 27; 44] However, in contrast to some previous studies, we only observed an association of painful DPN with smoking status and not with e.g. sex, age, and BMI.[1; 23; 31; 38] An explanation may be our analytical approach which – in comparison with most previous studies - allowed us to disentangle the effect of the risk factor on pain occurrence in DPN independent from that on DPN risk itself.[23; 27; 44] Moreover, power was reduced for painful DPN due to the lower prevalence, however, the estimates were smaller for painful DPN than DPN. We did not observe an association between body height and DPN, although it has been proposed that tall stature is a risk factor for peripheral neuropathy due to increased nerve length and nerve surface area.[9] Surprisingly, we observed that DPN was negatively associated with age. Increasing age is generally a marker of longer diabetes duration, however, the DD2 enrolls type 2 diabetes patients around time of diabetes diagnosis. A younger age at time of diagnosis is a marker of a worse phenotype,[5] which may explain our observation of a negative association of age and DPN. Moreover, non-responders were in general younger and we cannot exclude that part of the age-association may be explained by a responder bias if non-responder have DPN to a lesser extent than responders.

Our observation that painful DPN was associated with lower QoL and symptoms of depression, anxiety, and poor sleep is consistent with previous studies of diabetes.[7; 35; 38] However, we also observed a tendency towards that DPN itself was associated with worse mental health independent of neuropathic pain, which has been observed in some[4; 11] but not all studies,[38] and we even observed that DPN itself (MNSIq-defined) had a stronger association with worse mental health outcomes than neuropathic pain. In accordance, the correlation between pain intensity and mental health outcomes were weak. The effect of DPN and painful DPN on mental health measures was additive, thus, those fulfilling both the DPN and painful DPN criteria had the most severe symptoms, which is in accordance with an Italian study showing more severe depressive symptoms among those with painful DPN as compared to those with non-painful using Beck depression inventory II.[11] In concert with other studies, many of the patients in all three neuropathy groups had complaints of general pain (e.g. back and neck pain, headache and stomachache).[21; 35] The effect size of general pain in 2 bodily localizations on QoL, depression, anxiety and sleep scores was of a similar order of magnitude as that of DPN and painful DNP. Patients with possible DPN and painful DPN more often had pain at other locations than the group without any DPN, also suggesting that positive answers to the MNSIq and pain in the feet could be due to other causes than DPN.

A large proportion (3/4) of the patients with painful DPN reported neuropathic pain of moderate to severe intensity (NRS≥4) and 60.1% reported use of pain medication. This is similar to results published before.[7; 21] Pain intensity was positively correlated to symptoms of anxiety, depression, and sleep disturbance. The fact that

many of the patients had moderate to severe pain intensity despite taking drugs for their pain may indicate either inappropriate treatment or a lack of effective neuropathic drug treatments.[16]

The main strength of this questionnaire study is the large sample size, the high response rate (85.6%) and the low level of missing data. Reassuringly, similar estimates of the prevalence were observed across questionnaire intervals.

The DD2 cohort enrolls patients from primary care and hospital outpatient clinics. Since around half of the patients have been enrolled from hospital outpatient clinics, the DD2 cohort may hold patients with more severe diabetes than the average type 2 diabetes population in Denmark. However, baseline data from the DD2 cohort are similar to data from a cohort of type 2 patients receiving their first glucose-lowering drug indicating that the DD2 cohort is representative of recently diagnosed type 2 diabetes patients in Denmark.[10; 36] The cross-sectional design of this study has some innate limitations including the inability to determine temporal relationships. Lastly, we lack information on other diabetes complications and comorbidity, which can affect QoL related outcome measures.

In conclusion, in this largest questionnaire study of possible DPN in recently diagnosed type 2 diabetes patients, a significant proportion of patients had possible DPN and possible painful DPN. The presence of possible DPN was associated with female sex, longer diabetes duration, higher BMI, and smoking, whereas smoking was the only factor clearly associated with painful DPN. Patients with possible DPN and painful DPN reported lower QoL and more symptoms of anxiety, depression, and poor sleep. Since DPN in recently diagnosed diabetes patients is associated

with modifiable risk factors and has major impact on quality of life, it is important to carefully screen for this early complication in type 2 diabetes.

Conflicts of interest statement

The authors have no financial or other relationships that might lead to a conflict of interest.

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Author contribution: S.S. Gylfadottir designed the study, performed the statistical analyses, drafted the manuscript, contributed to the discussion, and approved the final manuscript. D.H. Christensen collected the data, designed the study, performed the statistical analyses, drafted the manuscript, contributed to the discussion, and approved the final manuscript. S.K. Nicolaisen designed the study, researched the data, revised the manuscript critically, contributed to the discussion, and approved the final manuscript. N.T. Andersen, R.W. Thomsen, N.B. Finnerup, B.C. Callaghan, J.S. Nielsen, S.H. Sindrup, H. Andersen, M. Itani, K.S. Khan, A.G. Kristensen, and T.S. Jensen designed the study, revised the manuscript critically, contributed to the discussion, and approved the final manuscript.

Data availability: More information about the DD2 cohort can be found at the Danish DD2 website www.dd2.nu. The DD2 national advisory forum strongly encourages national and international collaboration and application form can be found via this link https://dd2.nu/media/1264/standard-dd2-protocol_final.doc. Interested researchers can contact DD2 via assistant professor Jens Steen Nielsen at: jsn@rsyd.dk.

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Tables

Table 1: Characteristics of the 5,514 patients who returned a fully or partly completed questionnaire

Variables	
Demographics	
Female, n (%), N = 5,514	2,355 (42.7)
Age, years, mean (SD), N = 5,514	64.1 (10.9)
Diabetes duration, years, median (IQR), N = 5,512	4.6 (3.5; 5.7)
Lifestyle and anthropometric factors	
Height, cm, mean (SD), N = 5,455	172.6 (9.4)
Weight, kg, mean (SD), N = 5,457	91.0 (20.1)
BMI, kg/m², median (IQR), N = 5,412	29.7 (26.4; 33.5)
Smoking, n (%), N = 5,493	
Active smoker	1,078 (19.6)
Daily	849 (15.5)
Occasionally	229 (4.2)
Previous smoker	2,453 (44.7)
Never smoker	1,962 (35.7)
Alcohol consumption[a], >7/14 (female/male), n (%), N = 5,426	856 (15.8)
Physical activity[b], days, median (IQR), N = 5,434	4.0 (2.0;6.0)
Quality of life, sleep, depression and anxiety	
Quality of life, NRS 0-10, median (IQR), N = 5,394	8.0 (6.0; 9.0)
Sleep, PROMIS-29, T-score, mean (SD), N = 4,739	48.2 (7.5)
Anxiety, PROMIS-29, T-score, mean (SD), N = 5,274	50.2 (8.5)
Depression, PROMIS-29, T-score, mean (SD), N = 5,348	48.7 (8.4)
PROMIS-29, T-score categories	
Sleep, n (%)	
Mild impairment	542 (11.4)
Moderate impairment	212 (4.5)
Severe impairment	25 (0.5)
Anxiety, n (%)	
Mild impairment	1,088 (20.6)
Moderate impairment	559 (10.6)
Severe impairment	49 (0.9)
Depression, n (%)	
Mild impairment	806 (15.1)
Moderate impairment	562 (10.5)
Severe impairment	47 (0.9)
General pain	
Pain (Constant or recurrent), n (%), N = 5,439	2,995 (55.1)
Pain location (in the last 3 months), n (%), N = 5,439	

Head or face	1,041 (19.1)
Lower and upper back	2,138 (39.3)
Shoulders	1,376 (25.3)
Hands or arms	1,023 (18.8)
Stomach	590 (10.8)
Legs	1,447 (26.6)
Other	599 (11.0)
Gait instability and falls	
Gait instability, n (%), N = 5,394	1,193 (22.1)
Falls (during last year), n (%), N = 5,455	977 (17.9)

Abbreviations: SD, standard deviation; IQR, interquartile range; MNSIq, Michigan neuropathy screening questionnaire; DN4, Douleur Neuropathique en 4 Questions, BMI: body mass index; NRS, numeric rating scale; PROMIS, Patient-Reported Outcomes Measurement Information System.
Means (SD) were used to describe normally distributed data, and medians (IQR) to describe non-normally distributed variables.
[a]Alcohol units per week. [b]Number of days per week with minimum 30 minutes of physical activity.

Table 2: Characteristics of the 5,249 patients with information on status of *both* possible DPN (defined by MNSIq) *and* possible painful DPN (defined by DN4 and pain location in both feet).

Variables	**MNSIq<4, n=4,311**		**MNSIq≥4, n=938[a]**	
	No pain or DN4<3, n=4,181	**Pain and DN4≥3, n= 130[b]**	**No pain or DN4<3, n=552**	**Pain and DN4≥3, n=386[b]**
Female, n (%), N = 5,249	1,712 (41.0)	58 (44.6)	258 (46.7)	188 (48.7)
Age, years, mean (SD), N = 5,249	64.3 (10.8)	64.1 (10.6)	62.3 (10.8)	63.1 (10.9)
Duration of diabetes, years, median (IQR), N = 5,247	4.5 (3.4; 5.6)	4.8 (3.4; 5.9)	4.7 (3.6; 5.9)	4.9 (3.8; 6.1)
Height, cm, mean (SD), N = 5,197	172,7 (9.3)	172.0 (10.1)	172.6 (10.0)	172.7 (10.0)
BMI, kg/m², median (IQR), N = 5159	29.4 (26.2; 33.1)	29.6 (27.3; 34.8)	31.2 (27.8; 35.5)	31.5 (27.5; 35.7)
Ever smoker, n (%), N = 5,231	2,616 (62.8)	93 (72.1)	378 (68.5)	294 (76.2)
Alcohol consumption, >7/14 (female/male)[c], n (%), N = 5,176	665 (16.1)	19 (14.7)	74 (13.8)	60 (15.8)
Quality of life, NRS 0-10, median (IQR), N = 5,177	8.0 (7.0; 9.0)	7.0 (5.0; 8.0)	7.0 (5.0; 8.0)	6.0 (4.0; 7.0)
PROMIS-29, T-score, mean (SD)				
Sleep, N = 4,591	47.0 (7.0)	49.9 (7.2)	52.2 (7.7)	54.0 (7.5)
Depression, N = 5,147	47.5 (7.8)	51.1 (8.8)	52.3 (9.0)	55.5 (8.9)
Anxiety, N = 5,080	49.0 (8.0)	52.2 (8.8)	53.6 (8.7)	56.1 (8.5)
PROMIS-29, T-score, categories:				
Sleep impairment (mild – severe), n (%)	418 (11.4)	30 (25.9)	157 (33.1)	139 (41.4)
Symptoms of Anxiety (mild – severe), n (%)	1,090 (26.9)	48 (37.2)	253 (48.3)	216 (57.9)
Symptoms of Depression (mild – severe), n (%)	843 (20.5)	50 (38.8)	238 (44.3)	208 (55.2)
Number of other pain locations[d], N=5,235				
0	2,371 (56.9)	30 (23.1)	160 (29.1)	55 (14.3)
1	735 (17.6)	28 (21.5)	99 (18.0)	69 (17.9)
2	588 (14.1)	32 (24.6)	129 (23.5)	94 (24.4)
3	343 (8.2)	27 (20.8)	92 (16.7)	92 (23.9)
4	121 (2.9)	11 (8.5)	61 (11.1)	57 (14.8)
5	12 (0.3)	2 (1.5)	9 (1.6)	18 (4.7)

Abbreviations: MNSIq, Michigan neuropathy screening questionnaire; DN4, Douleur Neuropathique en questions; SD, standard deviation; IQR, interquartile range; BMI: body mass index; NRS, numeric rating scale; PROMIS, Patient-Reported Outcomes Measurement Information System.
Means (SD) were used to describe normally distributed data, and medians (IQR) to describe non-normally distributed variables
[a]938 patients with MNSIq-defined DPN. [b]516 (130+386) patients with DN4-defined painful DPN. [c]Alcohol units per week. [d]Possible pain locations: Head/face, lower or upper back, shoulders, stomach, or “other location” (category capturing locations not listed here). Arms and legs excepted because pain in these locations could be due to diabetic polyneuropathy.
Missing data < 3.2% except for sleep impairment (missing data 12.5%, no difference between neuropathy groups).

T**able 3:** Pain-related characteristics among the 536 patients with possible painful DPN (defined by DN4 and pain location in both feet)

Variables	
Pain in the feet spreads upwards in legs, n (%), N = 529	331 (62.6)
Similar pain in hands or fingers, n (%), N = 527	228 (43.3)
Waking up at night because of pain, n (%), N = 526	264 (50.2)
Pain in the feet, duration, n (%), N = 534	
Less than a month	6 (1.1)
1-3 months	10 (1.9)
3-12 months	71 (13.3)
1-5 years	298 (55.8)
more than 5 years	149 (27.9)
Pain intensity within last 24 hours, NRS (0-10), mean (SD), N = 530	5.2 (2.1)
Pain intensity within last 7 days, NRS (0-10), mean (SD), N = 530	5.3 (2.1)
Drug treatment for pain in the feet, n (%), N = 531	319 (60.1)
Pain interference with daily activities, PROMIS-29, T – score, mean (SD), N = 525	59.1 (7.9)
PROMIS-29, T-score categories	
Mild interference with daily activities	155 (29.5)
Moderate interference with daily activities	232 (44.2)
Severe interference with daily activities	29 (5.5)

Abbreviations: DN4, Douleur Neuropathique en 4 questions, NRS, numeric rating scale; SD, standard deviation, PROMIS, Patient-Reported Outcomes Measurement Information System.

Table 4: The estimates of the association between neuropathy and clinical characteristics among the 5,249 patients with information on status of *both* possible DPN (defined by MNSIq) *and* possible painful DPN (defined by DN4 and pain location in both feet).

	Female	**Smoking[a]**	**Alcohol overconsumption[b]**	**Age, year**	**BMI, kg/m²**	**Diabetes duration, year**	**Height, cm**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Possible DPN[c]	1.24 (1.05; 1.46)*	1.36 (1.14; 1.63)*	0.94 (0.74; 1.18)	-1.90 (-2.78; -1.02)**	1.67 (1.19; 2.14)**	0.25 (0.06; 0.44)*	0.43 (-0.11; 0.96)
Possible painful DPN[d]	1.11 (0.90; 1.37)	1.52 (1.20; 1.93)*	1.09 (0.81; 1.46)	0.45 (-0.68; 1.57)	0.35 (-0.26; 0.95)	0.06 (-0.18; 0.31)	0.21 (-0.47; 0.90)

Abbreviations: MNSIq, Michigan neuropathy screening questionnaire; DPN, diabetic polyneuropathy; DN4, Douleur Neuropathique en 4; BMI: body mass index; OR, odds ratio; CI, confidence interval; β, beta-coefficient. [a]Smoking: Ever smoking (current or former) vs. never smoking. [b]Alcohol overconsumption: > 7/14 units per week (women/men). [c]Multivariable logistic (sex, smoking, alcohol) and linear (age, BMI, diabetes duration, height) regressions adjusted for age, sex, diabetes duration, and possible painful DPN. [d]Multivariable logistic (sex, smoking, alcohol) and linear (age, BMI, diabetes duration, height) regressions adjusted for age, sex, diabetes duration, and possible DPN.
*P-value<0.05, **P-value<0.001

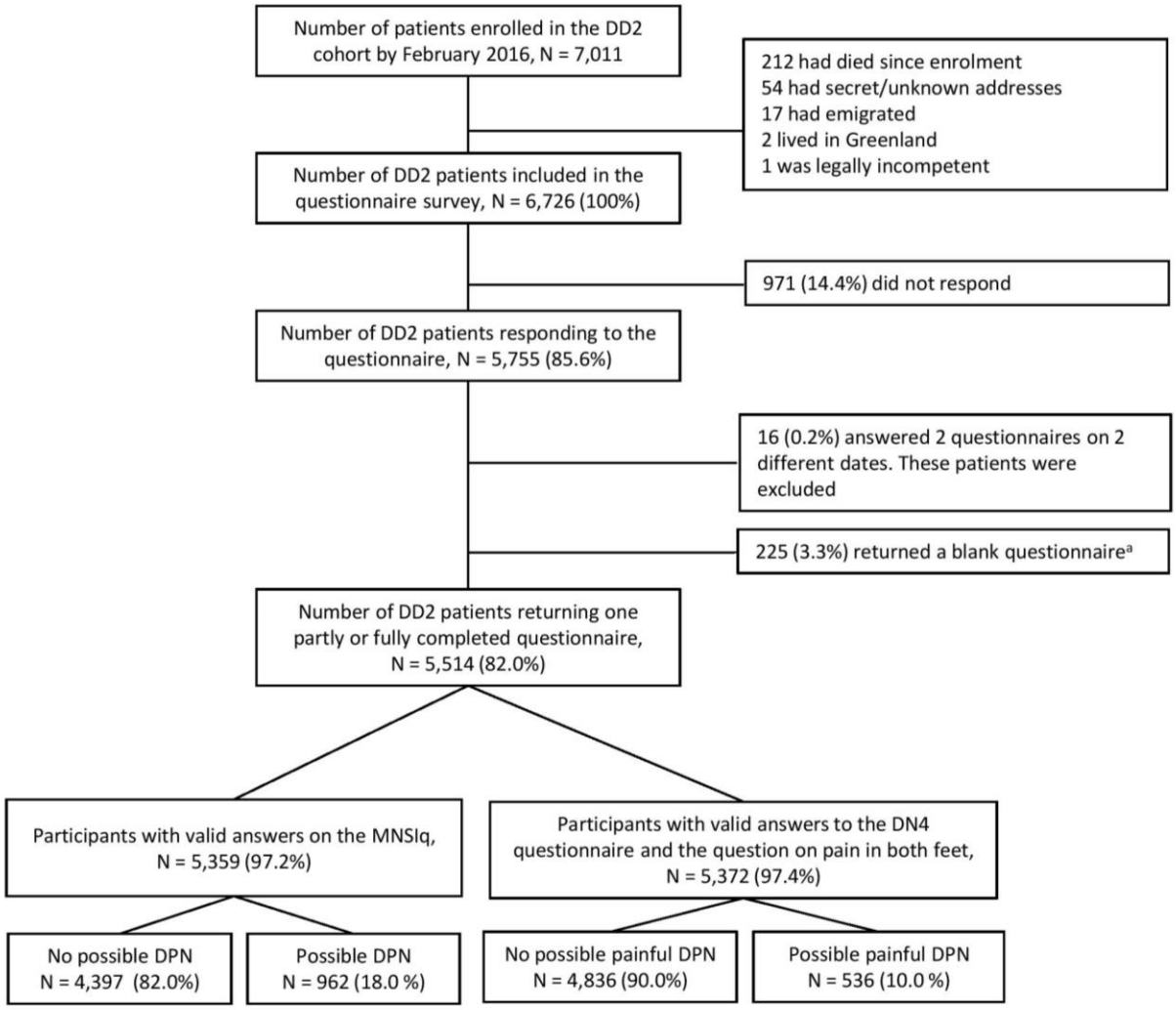
Table 5. The estimates of the association between neuropathy and quality of life, depression, sleep and anxiety among the 5,249 patients with sufficient information to determine status of *both* possible DPN (defined by MNSIq) *and* possible painful DPN (defined by DN4 and pain location in both feet).

	Quality of Life (NRS 0-10)		Depression T-scores		Sleep disturbance T-scores		Anxiety T-scores	
	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Possible DPN	-1.16 (-1.31 ; -1.01)**	-0.85 (-1.00; -0.71)**	4.18 (3.53; 4.84)**	2.95 (2.30; 3.59)**	4.65 (4.04 ; 5.27)**	3.46 (2.86; 4.06)**	3.97 (3.31 ; 4.64)**	2.82 (2.17; 3.48)**
Possible painful DPN	-0.85 (-1.04; -0.67)**	-0.57 (-0.76; -0.39)**	3.35 (2.51; 4.18)**	2.12 (1.30; 2.93)**	2.22 (1.44 ; 3.00)**	1.05 (0.30; 1.81)**	2.73 (1.89; 3.58)**	1.61 (0.78; 2.44)**
Number of other pain locations								
1	-	-0.60 (-0.73; -0.46)	-	1.30 (0.71; 1.89)	-	1.95 (1.40; 2.50)	-	1.28 (0.68; 1.88)
2	-	-0.97 (-1.11; -0.83)	-	3.47 (2.86; 4.09)	-	3.95 (3.37; 4.52)	-	3.37 (2.74; 3.99)
3	-	-1.29 (-1.46; -1.13)	-	5.57 (4.83; 6.31)	-	5.26 (4.57; 5.95)	-	5.20 (4.45; 5.96)
4	-	-1.82 (-2.05; -1.58)	-	7.67 (6.62; 8.72)	-	6.45 (5.49; 7.41)	-	6.86 (5.80; 7.93)
5	-	-1.58 (-2.13; -1.02)	-	8.22 (5.81; 10.62)	-	7.04 (4.78; 9.30)	-	7.42 (4.89; 9.94)

Abbreviations: MNSIq, Michigan neuropathy screening questionnaire; DPN, diabetic polyneuropathy; DN4, Douleur Neuropathique en 4 questions; OR, odds ratio; CI, confidence interval. [a]Model 1: Adjusted for age, sex, diabetes duration, BMI, and DPN or painful DPN, respectively. [b]Model 2: Adjusted for age, sex, diabetes duration, BMI, number of pain locations other than extremities (head/face, lower or upper back, shoulders, stomach, or “other location” [category capturing locations not listed here]), and DPN or painful DPN, respectively.
*P-value < 0.05, **P-value<0.001

Figure legends

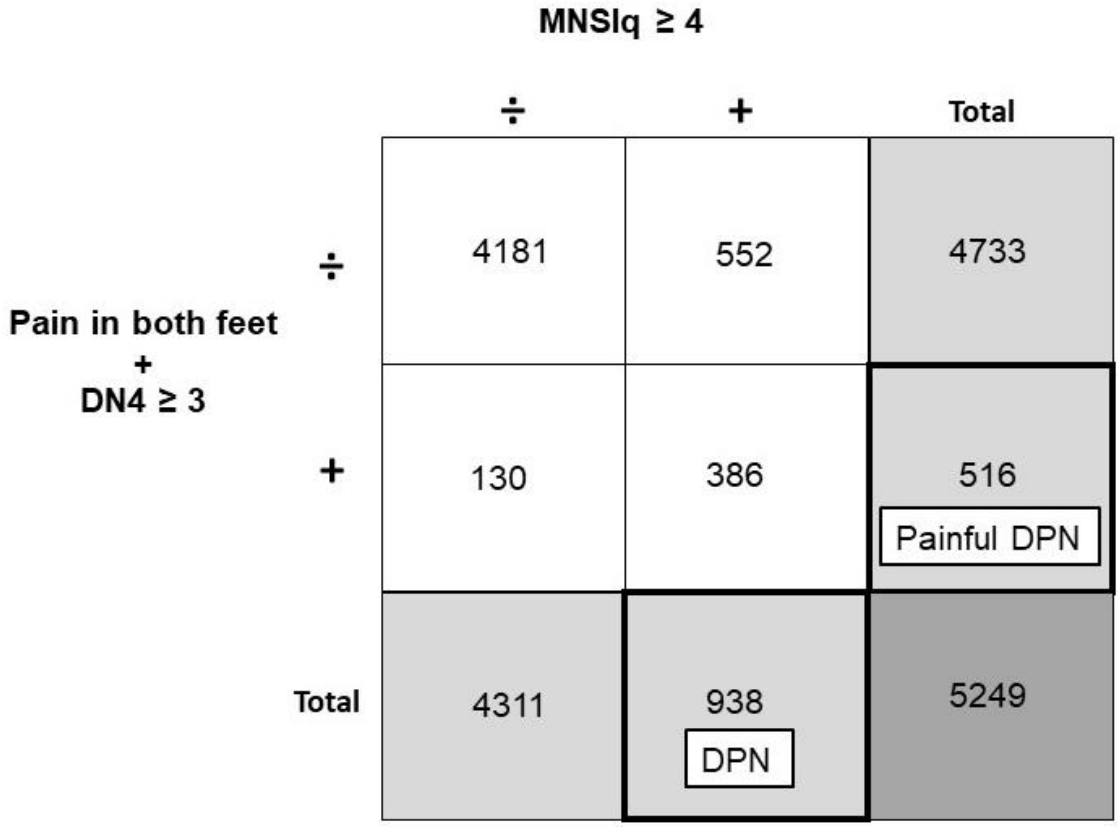
Figure 1: Flowchart of study population.



Abbreviations: DD2, Danish Centre for strategic research in type 2 diabetes; MNSIq: Michigan Neuropathy Screening Instrument questionnaire, DN4: Douleur Neuropathique en 4 questions.

[a]Reason for non-participation: No reason provided: 89 (39.6%), No surplus energy because of other comorbidity: 18 (8.0%), No surplus energy because of death/illness among near relative: 3 (1.3%), Dementia and other conditions hindering adequate answers to the questionnaire: 21 (9.3%), Too busy/no free time: 4 (1.8%), Well-regulated/solely diet-treated thus feeling the questionnaire is not relevant: 25 (11.1%), Mail delivery not possible (invalid address, full or locked mailbox): 31 (13.8%), Died in the time period February to end of questionnaire survey: 9 (4.0%), Other single reasons: 25 (11.1%).

Figure 2: Possible DPN and possible painful DPN definitions.



Abbreviations: MNSIq: Michigan Neuropathy Screening Instrument questionnaire, DN4: Douleur Neuropathique en 4 questions.
The numbers in the figure corresponds to the distribution of patients in the cohort of patients with available data on the criteria for both possible DPN and painful DPN (N=5,249). The numbers are evident from Table 2.

Supplemental Digital Content

Diabetic polyneuropathy and pain, prevalence, and patient characteristics: A cross-sectional questionnaire study of 5,514 patients with early type 2 diabetes.

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Supplementary Digital Content

Supplementary Table 1: *Full version of the questionnaire.*

Supplementary Table 2: *Age, gender, and diabetes duration among non-responders.*

Supplementary Table 3: *Prevalence of possible DPN (defined by MNSIq≥4) and possible painful DPN (defined by DN4≥3 + pain location in both feet) with 95% confidence intervals according to gender and age-groups.*

Supplementary Table 4: *Prevalence of possible DPN (defined by MNSIq≥4) and possible painful DPN (defined by DN4≥3 + pain location in both feet) with 95% confidence intervals in total and according to questionnaire interval.*

Supplementary Table 5. *The difference between the estimates for possible DPN (defined by MNSIq) and possible painful (defined by DN4 and pain location in both feet) with corresponding 95% confidence intervals*.

Supplementary Table 6: *The association between neuropathy and a) quality of life, b) depression, c) sleep and d) anxiety among the 5,249 patients with sufficient information to determine status of both possible DPN (defined by MNSIq) and possible painful DPN (defined by DN4 and pain location in both feet). Sensitivity analysis – without adjustment for BMI.*

Supplementary Figure 1: *Frequency of "yes" responses to the MNSIq by the 4 possible neuropathy groups among the 5,249 patients with information on status of both DPN (defined by MNSIq) and painful DPN (defined by DN4 and pain location in both feet).*

Supplementary Figure 2: *The association of DPN and painful DPN with sleep disturbance – an example.*

Supplementary Table 1: *Questionnaire, full version*

HEIGHT

1. Enter your height in centimetres:	_____ cm

WEIGHT

2. Enter your weight in kilograms:	_____ kg

SMOKING

3. Please tick the relevant item:	☐ Never smoked ☐ Ex-smoker (stopped more than 6 months ago) ☐ Smokes occasionally ☐ Smokes daily

ALCOHOL INTAKE

4. Please tick the relevant item:	☐ Less than 7 units per week (women)/14 units (men) per week ☐ More than 7 units per week (women)/14 units (men) per week

PHYSICAL ACTIVITY

5. How many days a week are you **physically active for at least 30 minutes per day?** One tick only.
(this includes moderate or hard physical activity with increased breathing, muscles exercise and use of strength, eg, recreational sports or competitive sports, heavy gardening, brisk walking, biking at moderate or fast pace or physically strenuous work. Both spare time and work activities are to be included)

Never	1 day	2 days	3 days	4 days	5 days	6 days	7 days
☐	☐	☐	☐	☐	☐	☐	☐

PHYSICAL ACTIVITY - *continued*

6. Do you engage in physical activity in your spare time, or participate in other activities that involve exercise?

☐ Yes ☐ No

7. Physical activity in your spare time in the past year. Tick off the box that best describes your level of activity:

☐ Exercising strenuously and practicing competitive sports regularly and several times a week

☐ Practising recreational sports or doing heavy gardening or similar at least 4 hours a week

☐ Walking, biking or other light exercise at least 4 times per week (Sunday strolls, light gardening and biking/walking to work should also be included)

☐ Reading, watching TV or other sedentary activity

WALKING/FALLS

8. Do you sometimes feel unsteady when walking?

☐ Yes ☐ No

9. Have you fallen in the past year?

☐ Yes ☐ No

If yes, how many times have you fallen in the past year?

☐ Once ☐ 2-4 times ☐ More than 4 times

10. Has your fall/falls made it necessary to contact your general practitioner?

☐ Yes ☐ No

11. Has your fall/falls made it necessary to contact the hospital

☐ Yes ☐ No

QUALITY OF LIFE

12. How will you rate your quality of life in the past 7 days? (one tick only)

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Worst possible quality of life Best possible quality of life

SLEEP

13. How was your sleep quality in **the past 7 days**?
Please respond to each item by marking one box per row.

In the past 7 days…	Very poor	Poor	Fair	Good	Very good
My sleep quality was	☐	☐	☐	☐	☐

In the past 7 days…	Not at all	A little bit	Somewhat	Quite a bit	Very much
I had difficulty falling asleep	☐	☐	☐	☐	☐
My sleep was refreshing	☐	☐	☐	☐	☐
I had a problem with sleep	☐	☐	☐	☐	☐

MENTAL HEALTH

14. How was your mental health **in the past 7 days?**
Please respond to each statement by marking one box per row.

In the past 7 days…	Never	Rarely	Sometimes	Often	Always
I felt uneasy	☐	☐	☐	☐	☐
My worries overwhelmed me	☐	☐	☐	☐	☐
I felt fearful	☐	☐	☐	☐	☐
I found it hard to focus on anything other than my anxiety	☐	☐	☐	☐	☐
I have felt worthless	☐	☐	☐	☐	☐
I have felt helpless	☐	☐	☐	☐	☐
I have felt depressed	☐	☐	☐	☐	☐
I have felt hopeless	☐	☐	☐	☐	☐

FEELING IN YOUR LEGS AND FEET

Please answer the following questions about the feeling in **your legs and feet.**

Check yes or no based on how you usually feel.

	Yes	No
15. Are your legs and/or feet numb?	☐	☐
16. Do you ever have any burning pain in your legs and/or feet?	☐	☐
17. Are your feet too sensitive to touch?	☐	☐
18. Do you get muscle cramps in your legs and/or feet ?	☐	☐
19. Do you ever have any prickling feelings in your legs or feet ?	☐	☐
20. Does it hurt when the bed covers touch your skin?	☐	☐
21. When you get into the tub or shower, are you able to tell the hot water from the cold water?	☐	☐
22. Have you ever had an open sore on your foot?	☐	☐
23. Has your doctor ever told you that you have diabetic neuropathy?	☐	☐
24. Do you feel weak all over most of the time?	☐	☐
25. Are your symptoms worse at night?	☐	☐
26. Do your legs hurt when you walk?	☐	☐
27. Are you able to sense your feet when you walk?	☐	☐
28. Is the skin on your feet so dry that it cracks open ?	☐	☐
29. Have you ever had an amputation?	☐	☐

PAIN

30. Do you have constant or recurring pain?

☐ Yes ☐ No

If yes, have had any of these types of pain in the past 3 months? (tick all that apply)

☐ Headache or facial pain

☐ Back pain, including low back pain and neck pain

☐ Shoulder pain

☐ Pain in the hands/arms

☐ Abdominal pain

☐ Pain in the legs

☐ Other pain (please note what kind of pain)_____

31. Do you have constant or recurring pain **in your feet**

☐ Yes ☐ No

If you have answered YES to question 31, please continue answering the rest of the questionnaire. The remaining questions are about pain in your feet.

If you have answered NO to questions 31, we thank you for your participation and kindly ask you to return the questionnaire in the attached reply envelope.

32. Do you have pain in both feet?

☐ Yes ☐ No

33. Does the pain spread up your legs?

☐ Yes ☐ No

34. Do you have similar pain in your fingers/hands?

☐ Yes ☐ No

35. Do you wake up a nights due to pain in your feet?

☐ Yes ☐ No

36. How long have you had pain in your feet?	Less than a month	1-3 months	More than 3 months, but less than a year	1-5 years	More than 5 years
	☐	☐	☐	☐	☐

37. Please rate your pain intensity by marking the number that best describes your pain on average in your feet **in the past 24 hours**

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

No pain — Worst possible pain

38. Please rate your pain intensity by marking the number that best describes your pain on average in your feet **in the past 7 days**

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

No pain — Worst possible pain

39. Do you take pain medication for the pain in your feet?

☐ Yes, daily ☐ Yes, but not daily ☐ No

If yes, what kind of pain medication (tick more than one box if relevant)

☐ Over-the-counter medicine

☐ Prescription medicine (medicine that has been prescribed by a doctor)

40. The following questions are about how the pain in your feet interferes with your daily life.
Please respond to each question by marking one box per row.

In the past 7 days…	Not at all	A little bit	Somewhat	Quite a bit	Very much
How much did pain interfere with your day to day activities?	☐	☐	☐	☐	☐
How much did pain interfere with work around the house?	☐	☐	☐	☐	☐
How much did pain interfere with your ability to participate in social activities?	☐	☐	☐	☐	☐

How much did pain interfere with your household chores?	☐	☐	☐	☐	☐

41. Please answer the following questions about the characteristics of the pain in your feet by marking one box per row.

Does the pain have one or more of the following characteristics?

	Yes	No
Burning	☐	☐
Painful cold	☐	☐
Electric shocks	☐	☐

Is the pain associated with one or more of the following symptoms in the same area?

	Yes	No
Tingling	☐	☐
Pins and needles	☐	☐
Numbness	☐	☐
Itching	☐	☐

Supplementary Table 2: *Age, gender, and diabetes duration among responders and non-responders*		
	Non-responders[a]	Responders[b]
Age, mean (SD)	59.6 (12.8)	64.1 (10.9)
Female gender, n(%)	495 (40.8)	2,355 (42,7)
Diabetes duration, median (IQR)	4.6 (3.4-5.9)	4.6 (3.5-5.7)
[a]N = 1212: The 971, who never returned a questionnaire + the 225 who returned a blank questionnaire + the 16 who returned multiple questionnaires and were excluded (diabetes duration, n = 1,203) [b]N = 5,514: Those, who returned a fully or partly filled questionnaire		

Supplementary Table 3: *Prevalence of possible DPN (defined by MNSIq≥4) and possible painful DPN (defined by DN4≥3 + pain location in both feet) with 95% confidence intervals in total and stratified according to sex and age among all patients (N = 5,514) who returned a filled out questionnaire.*

	Possible DPN			**Possible Painful DPN**		
	Responses	**Events**	**Prevalence, % (95% CI)**	**Responses**	**Events**	**Prevalence, % (95% CI)**
Total	5,359[a]	962	18.0 (16.9-19.0)	5,372[b]	536	10.0 (9.2-10.8)[c]
Sex						
Female	2,274	503	20.2 (18.6-21.9)	2,278	258	11.3 (10.1-12.7)
Male	3,085	459	16.3 (15.0-17.7)	3,094	278	9.0 (8.0-10.0)
Age, years						
<55	1,129	241	21.3 (19.0-23.9)	1,130	124	11.0 (9.2-12.9)
55 - 65	1,481	194	19.9 (17.8-22.0)	1,478	163	11.0 (9.5-12.7)
65 - 75	1,034	172	16.6 (14.4-19.0)	1,034	93	9.0 (7.3-10.9)
≥ 75	1,715	255	14.9 (13.2-16.6)	1,730	156	9.0 (7.7-10.5)

Abbreviations: MNSIq, Michigan neuropathy screening questionnaire; DPN, diabetic polyneuropathy; DN4, Douleur Neuropathique en 4 Questions, CI, confidence interval.

[a]5,359 persons had sufficient answers to determine MNSIq-defined DPN status.

[b]5,372 persons had sufficient answers to determine DN4-defined painful DPN status.

[c] Including 2.4% (n = 130) with MNSIq<4 and 0.4% (n=20) with unknown MNSIq status

Supplementary Table 4: *Prevalence of possible DPN (defined by MNSIq≥4) and possible painful DPN (defined by DN4≥3 + pain location in both feet) with 95% confidence intervals in total and according to questionnaire interval*

	Possible DPN		**Possible Painful DPN**	
	Distribution of responses	**% (95% CI)**	**Distribution of responses**	**% (95% CI)**
Total	5,359	18.0 (16.9-19.0)	5,372	10.0 (9.2-10.8)
Questionnaire interval[a]				
T1	4,478	17.8 (16.7-19.0)	4,482	10.0 (9.1-10.9)
T2	478	17.8 (14.5-21.5)	488	8.4 (6.1-11.2)
T3	403	19.4 (15.6-23.6)	402	11.7 (8.7-15.2)

Abbreviations: MNSIq, Michigan neuropathy screening questionnaire; DPN, diabetic polyneuropathy; DN4, Douleur Neuropathique en 4 Questions, CI, confidence interval

[a]According to time period: T1: response received in the time interval between first questionnaire and first reminder, T2: response received in the time period from first reminder to second reminder, T3: response received in the time period from second reminder to closure of questionnaire survey.

Supplementary Table 5. *The difference between the estimates for possible DPN (defined by MNSIq) and possible painful (defined by DN4 and pain location in both feet) with corresponding 95% confidence intervals*.

	Quality of Life (NRS 0-10)		**Depression T-scores**		**Sleep disturbance T-scores**		**Anxiety T-scores**	
	Model 1[a]	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Possible DPN	-1.16 (-1.31 ; -1.01)**	-0.85 (-1.00; -0.71)**	4.18 (3.53; 4.84)**	2.95 (2.30; 3.59)**	4.65 (4.04; 5.27)**	3.46 (2.86; 4.06)**	3.97 (3.31 ; 4.64)**	2.82 (2.17; 3.48)**
Possible Painful DPN	-0.85 (-1.04; -0.67)**	-0.57 (-0.76; -0.39)**	3.35 (2.51; 4.18)**	2.12 (1.30; 2.93)**	2.22 (1.44 ; 3.00)**	1.05 (0.30; 1.81)**	2.73 (1.89; 3.58)**	1.61 (0.78; 2.44)**
Difference, 95% CI	-0.30 (-0.59; -0.01)*	-0.28 (-0.56; 0.00)	0.84 (-0.45; 2.13)	0.83 (-0.41; 2.07)	2.43 (1.21; 3.64)**	2.41 (1.25; 3.57)**	1.24 (-0.05; 2.55)	1.21 (-0.05; 2.48)

Abbreviations: MNSIq, Michigan neuropathy screening questionnaire; DPN, diabetic polyneuropathy; DN4, Douleur Neuropathique en 4 questions; OR, odds ratio; CI, confidence interval. [a]Model 1: Adjusted for age, sex, diabetes duration, BMI, and DPN or painful DPN, respectively. [b]Model 2: Adjusted for age, sex, diabetes duration, BMI, number of pain locations other than extremities (head/face, lower or upper back, shoulders, stomach, or "other location" [category capturing locations not listed here]), and DPN or painful DPN, respectively.

*P-value < 0.05, **P-value<0.001

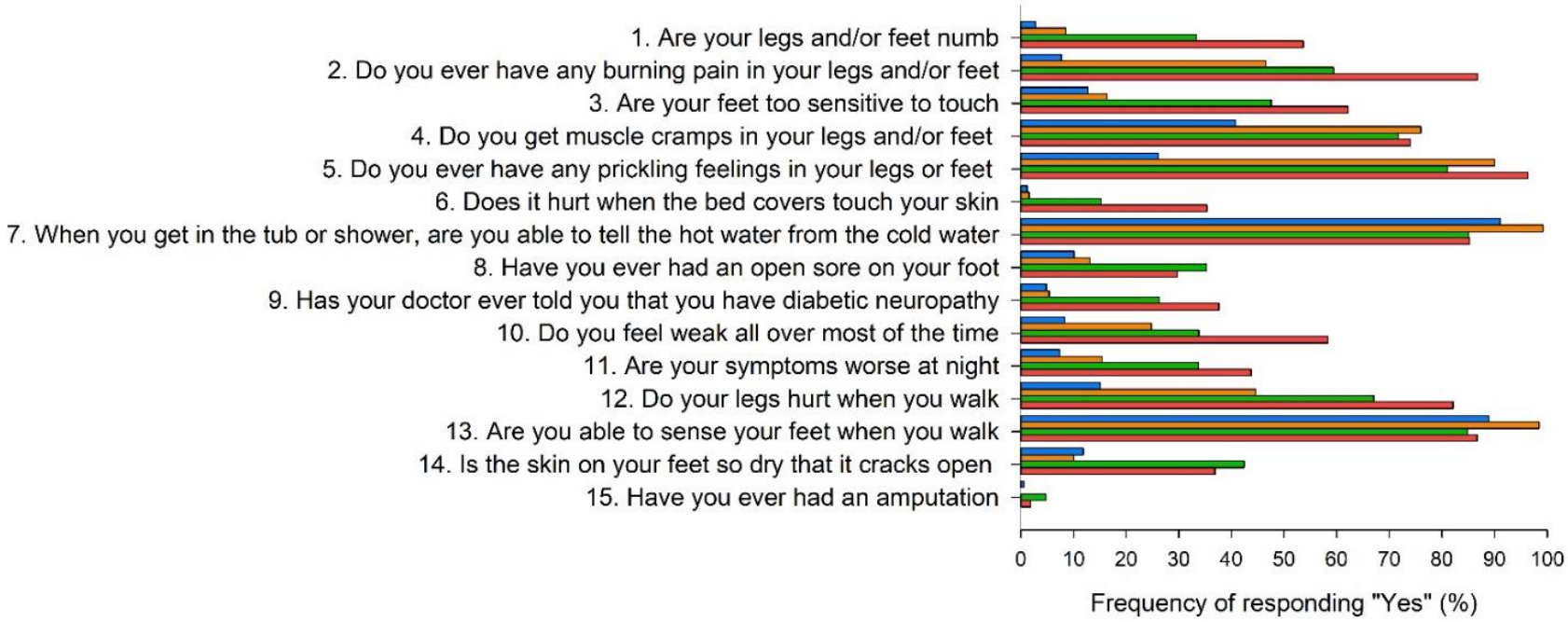
Supplementary Table 6. *The association between neuropathy and a) quality of life, b) depression, c) sleep and d) anxiety among the 5,249 patients with sufficient information to determine status of both possible DPN (defined by MNSIq) and possible painful DPN (defined by DN4 and pain location in both feet). Sensitivity analysis – without adjustment for BMI.*

	Quality of Life (NRS 0-10)		**Depression T-scores**		**Sleep disturbance T-scores**		**Anxiety T-scores**	
	Model 1[a]	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Possible DPN	-1.26 (-1.40 ; -1.11)**	-0.92 (-1.07; -0.78)**	4.42 (3.77; 5.07)**	3.09 (2.46; 3.74)**	4.75 (4.14 ; 5.36)**	3.49 (2.90; 4.07)**	4.04 (3.38 ; 4.70)**	2.83 (2.18; 3.50)**
Possible painful DPN	-0.86 (-1.05 ; -0.67)**	-0.57 (-0.76; -0.39)**	3.37 (2.54; 4.20)**	2.13 (1.32; 2.94)**	2.25 (1.48 ; 3.03)**	1.09 (0.34; 1.85)**	2.79 (1.95 ;3.63)**	1.68 (0.85; 2.50)**
Number of other pain locations								
1	-	-0.62 (-0.75; -0.49)	-	1.39 (0.81; 1.97)	-	1.97 (1.42; 2.51)	-	1.29 (0.70; 1.88)
2	-	-1.02 (-1.15; -0.88)	-	3.61 (3.00; 4.22)	-	3.93 (3.36; 4.51)	-	3.47 (2.84; 4.09)
3	-	-1.35 (-1.52; -1.19)	-	5.66 (4.93; 6.39)	-	5.28 (4.59; 5.96)	-	5.15 (4.40; 5.89)
4	-	-1.86 (-2.09; -1.62)	-	7.74 (6.69; 8.78)	-	6.51 (5.55; 7.46)	-	6.88 (5.82; 7.94)
5	-	-1.60 (-2.16; -1.03)	-	8.21 (5.80; 10.62)	-	7.03 (4.77; 9.29)	-	7.40 (4.87; 9.93)

Abbreviations: MNSIq, Michigan neuropathy screening questionnaire; DPN, diabetic polyneuropathy; DN4, Douleur Neuropathique en 4 questions; OR, odds ratio; CI, confidence interval. [a]Model 1: Adjusted for age, sex, diabetes duration, and DPN or painful DPN, respectively. [b]Model 2: Adjusted for age, sex, diabetes duration, number of pain locations other than extremities (head/face, lower or upper back, shoulders, stomach, or "other location" [category capturing locations not listed here]), and DPN or painful DPN, respectively.

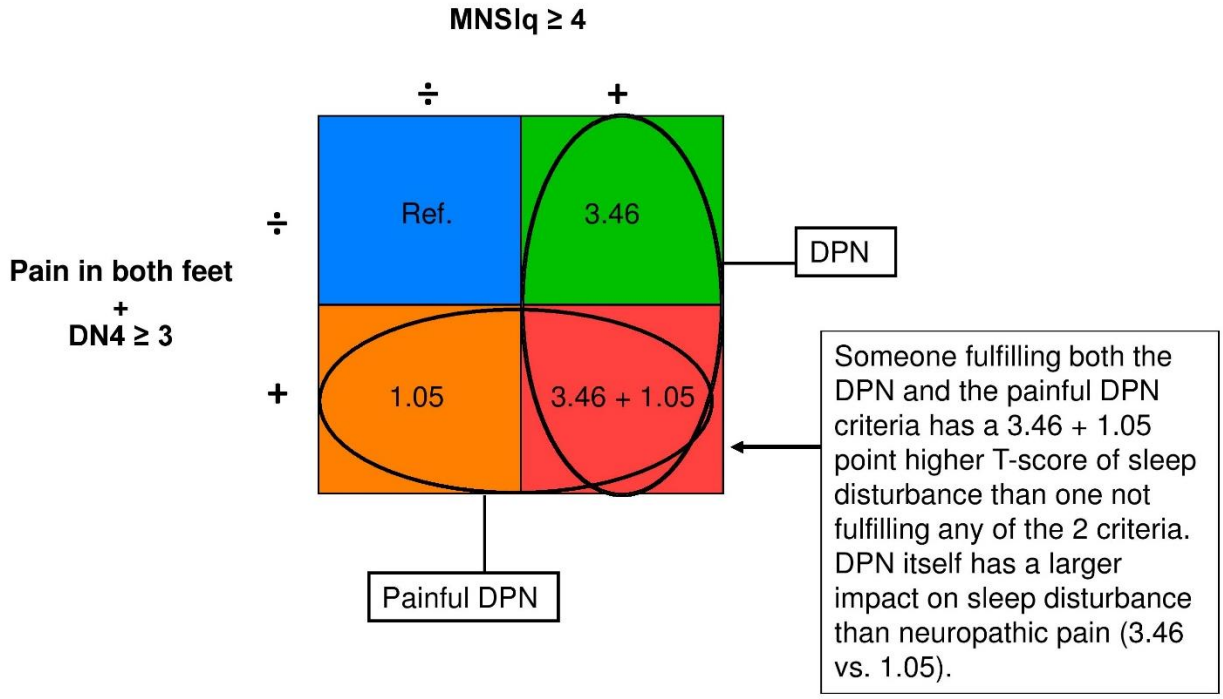
**P-value<0.001

Supplementary Figure 1: *Frequency of "yes" responses to the MNSIq by the 4 neuropathy groups among the 5,249 patients with information on status of both possible DPN (defined by MNSIq) and possible painful DPN (defined by DN4q and pain location in both feet).*



Abbreviations: MNSIq: Michigan Neuropathy Screening Instrument questionnaire, DN4: Douleur Neuropathique en 4 Questions.
Of note: Answering "no" to item 7 and 13 count as 1 point, while answering "yes" to item 1-3, 5-6, 11-12, 14-15 each count as 1 point. Thus, a low frequency of "yes"-responses to item 7 and 13 is associated with a higher likelihood of DPN. Item 4 and 10 are per definition not included in the score.
Blue: MNSIq<4/DN4<3 or no pain, Orange: MNSIq<4/DN4≥3 and pain, Green: MNSIq≥4/DN4<3 or no pain, Red: MNSIq≥4/DN4≥4 and pain

Supplementary Figure 2: *The association of possible DPN and possible painful DPN with sleep disturbance – an example*



Abbreviations: MNSIq: Michigan Neuropathy Screening Instrument questionnaire, DN4: Douleur Neuropathique en 4 Questions.

The estimates are all found in table 5. All estimates are adjusted for age, gender, diabetes duration, BMI, and pain in other locations than extremities.

Appendix III

Title page

ORIGINAL RESEARCH

Running head: Metabolic profile and neuropathy

Christensen DH et al.

Metabolic factors, lifestyle habits, and polyneuropathy in early type 2 diabetes:

A nationwide study of 5,249 patients in the Danish DD2 cohort

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Abstract

Objective: To investigate the association of metabolic and lifestyle factors with diabetic polyneuropathy (DPN) and neuropathic pain in patients with early type 2 diabetes.

Research design and methods: We thoroughly characterized 6,726 patients with recently diagnosed diabetes. After a median of 2.8 years, we sent detailed questionnaires on neuropathy (response rate 78%), including the Michigan Neuropathy Screening Instrument questionnaire (MNSIq) to identify DPN (score ≥4) and the Douleur Neuropathique en 4 Questions (DN4) questionnaire for associated neuropathic pain (pain in both feet + DN4-score ≥3).

Results: Among 5,249 patients, 17.9% (n=938) had DPN, including 7.4% (n=386) with neuropathic pain. In regression analyses, higher BMI and central obesity (waist circumference, waist-hip ratio, and waist-height ratio) were markedly associated with DPN. Important metabolic factors associated with DPN included hypertriglyceridemia ≥1.7 mmol/L: adjusted prevalence ratio (aPR) 1.36 (1.17; 1.59), decreased HDL cholesterol <1.0/1.2 mmol/L (male/female): aPR 1.35 (1.12; 1.62), high-sensitive CRP ≥3.0: aPR 1.66 (1.42; 1.94), c-peptide ≥1,550: aPR 1.72 (1.43; 2.07), HbA1c ≥9.5: aPR 1.38 (1.02; 1.86), and antihypertensive drug use: aPR 1.34 (1.16; 1.55). Smoking at diabetes diagnosis: aPR 1.50 (1.24; 1.81) and lack of physical activity (0 vs ≥3 days/week): aPR 1.61 (1.39; 1.85) were also associated with DPN. Smoking, high alcohol intake, and decreased physical activity after diabetes diagnosis were associated with neuropathic pain.

Conclusions: This large study of patients with early type 2 diabetes provides strong evidence that DPN is associated with metabolic syndrome factors, insulin resistance and inflammation, and modifiable unhealthy lifestyle habits.

Introduction

Diabetic polyneuropathy (DPN) affects 25-50% of patients with type 2 diabetes.[1] DPN increases the risk of falls, foot ulcers, and lower extremity amputations[1] and up to 38% of patients experience neuropathic pain.[1-3] Current preventive measures for DPN are mainly limited to strict glycemic control, which exerts a limited effect against DPN-risk in type 2 diabetes patients.[4]

Increasing evidence supports an association between the degree of obesity and risk of DPN in type 2 diabetes,[5-9] The exact biological mechanisms remain unclear. Visceral fat accumulation associates with metabolic dysfunction e.g. low-grade inflammation, insulin resistance, and dyslipidemia, and individuals, who are obese according to the BMI criteria (i.e. BMI > 30 kg/m^2), which do not hold information on fat distribution, may be metabolically healthy.[10] In accordance, central obesity has been shown to be a stronger predictor of some diabetes complications than – and independently of – BMI.[11 12] It is unknown whether central obesity associates with DPN independently of BMI in type 2 diabetes. Results from studies examining other possible DPN risk factors in diabetes populations such as metabolic syndrome factors including dyslipidemia, hypertension, and hyperglycemia, as well as low-grade inflammation and lifestyle habits like smoking and physical activity are mixed and conflicting.[2 6-8 13-15] Large-scale studies on DPN in type 2 diabetes patients are scarce, and existing DPN studies have often included patients with long-standing diabetes, rather than newly diagnosed diabetes where the potential to prevent complications may be largest. Specifically, little knowledge is available on risk factors that may underlie the presence of neuropathic pain type 2 diabetes.[16]

We therefore conducted a comprehensive study of the association of different existing metabolic and lifestyle factors in type 2 diabetes patients at diagnosis with DPN and neuropathic pain at a median of 2.8 years later. We examined the hypothesis that central obesity markers are strongly – and independently of general obesity – associated with DPN.[12 17 18] We also

investigated whether gradual higher levels of a range of other metabolic and lifestyle risk factors, would associate with gradual increased DPN prevalence. Finally, we explored the hypothesis that distinct metabolic factors associate with painful DPN compared with non-painful DPN.

Research design and methods

Setting

We conducted this cross-sectional study based on the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort. The DD2 cohort is a nationwide cohort of newly or recently diagnosed type 2 diabetes patients (median diabetes duration at enrollment time 1.4 years, interquartile range [IQR] 0.3-3.0 years) enrolled from hospital specialist outpatient clinics and from general practitioners' (GPs) offices in Denmark since November 2010. The enrollment process, implementation, logistics, DD2 biobank, and characteristics of this cohort have previously been described.[19 20] Briefly, interview and clinical examination data for each patient are recorded at the DD2 enrollment date, and fasting blood and urine samples are obtained and stored in the DD2 biobank. The unique civil personal registration (CPR) number assigned to all Danish citizens at birth or upon immigration links the DD2 cohort to other Danish health registries. Thus, complete hospital contact history for each DD2 patient can be obtained from the Danish National Patient Registry (DNPR), individual-level information on filled prescriptions for reimbursable drugs are available from the Danish National Health Service Prescription Database (DNHSP), and information on vital status and migration is available from the Danish Civil Registration System (CRS). For a subcohort of DD2 patients (69%), additional detailed clinical data can be achieved from the nationwide quality-of-care database, the Danish Diabetes Database for Adults (DDDA).[19]

Study population

In June 2016, a median of 2.8 years (IQR: 1.8-3.7 years) after the DD2 enrollment date, a detailed questionnaire on neuropathy and pain was sent out to all 6,726 living DD2 participants enrolled from November 2010 to February 2016.[19, StudyII] The questionnaire included the 15-item Michigan Neuropathy Screening Instrument questionnaire (MNSIq), the 7-item Douleur Neuropathique en 4 Questions (DN4) questionnaire, questions about pain location (e.g. whether the person experienced pain in *both* feet), anthropometric data, and lifestyle factors. Our main study population consisted of the 5,249 (78%) DD2 patients, who provided information on both polyneuropathy and neuropathic pain status from the questionnaire survey (total response rate including incomplete responses was 82% [N = 5,514]). A detailed description of the questionnaire and the primary results has been published.[StudyII, when accepted]

Diabetic polyneuropathy and neuropathic pain - definitions

The MNSIq tool was developed to screen for and identify DPN.[21 22] We used the validated cutoff score of ≥4 (specificity = 92%, sensitivity = 40%) to assess DPN at the level at "possible".[22 23] Neuropathic pain was evaluated according to the International Consensus (NeuroPPIC) for genetic studies[24] and the updated NeuPSIG neuropathic pain grading system[25] defining possible neuropathic pain as i) pain with neuropathic characteristics, ii) an anatomically plausible distribution of the pain (here pain in both feet), and iii) a history of a relevant underlying somatosensory lesion or disease (here diabetes).[25] We used the DN4 questionnaire[26] which has specifically been validated for use in DPN (specificity and sensitivity = 84%),[27] and defined neuropathic pain as the presence of pain in *both* feet together with a DN4 score of ≥3. It was emphasized in the questionnaire that the DN4 questions specifically related to pain in the feet, and should only be answered if there was pain in both feet. Thus, DPN was defined as MNSIq ≥4, painful DPN as MNSIq ≥4 and DN4 ≥3, and non-painful DPN either DN4 <3 or no pain in the feet (Supplementary Figure 1, Panel A)

Obesity measures

We used information on BMI ([weight in kg]/[height in meters x height in meters]) as a measure of general obesity at three different time points; at 20 years of age (based on recall, i.e. self-reported at the time of DD2 enrollment), at time of the DD2 enrollment date between 2010 and 2016 (subcohort: based on DDDA data, i.e. recorded as part of the routine clinical diabetes care[19]), and at time of the questionnaire survey in 2016 (based on self-reported data). We then calculated changes in BMI from age 20 years to the time of questionnaire in 2016.

Waist circumference and hip circumference were measured as part of the DD2 enrollment process and were used to assess central obesity with three different measures; waist circumference, waist-hip-ratio, and waist-height ratio.[11 12]

The timeline of obesity measures, non-obesity metabolic risk factors, lifestyle factors (see below), and DPN-status is shown in Figure 1. For further definitions and categories: see Supplementary Table 1.

Other metabolic and lifestyle factors

Information on other patient characteristics, lifestyle and metabolic factors at time of DD2 enrollment (from here on referred to as *baseline*) were extracted from the DD2 cohort data and linked health registers. Patient characteristics and metabolic factors of particular interest that were available for the entire population included c-peptide level (~insulin resistance), low-grade inflammation assessed by high-sensitive C-reactive protein (hsCRP) (excluding hsCRP values ≥10 mg/L in order to exclude values reflecting ongoing infections),[28] physical activity (days per week with more than 30 minutes of physical activity), alcohol consumption (< or ≥14/21 units per week for females/males, recommended safe dose in 2010 when the DD2 was initiated), hospital-diagnosed macrovascular complications, hospital-diagnosed microvascular renal and ophthalmologic complications, and diabetes duration (at the time of questionnaire survey 2016).

Metabolic risk factors available for the subcohort linked via the DDDA included hemoglobin A1c (HbA1c), lipid levels (total cholesterol, low density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol, and triglycerides), smoking habits, albumin/creatinine ratio, and blood pressure.

Some patients may have had normal lipid levels and blood pressure at baseline due to relevant treatment. Thus, we also retrieved information on lipid-lowering and antihypertensive drug usage within 1 year prior to baseline. Additionally, we retrieved information on glucose-lowering drug usage.

Our main focus was the lifestyle and metabolic risk factor profile at baseline, yet for smoking and physical activity, we also used follow-up data from the neuropathy questionnaire in 2016 to assess the role of risk factor changes.

Statistical analyses

Descriptive data were median (IQR) for continuous variables and proportions (n, [%]) for categorical variables. We examined the proportion of overall DPN, non-painful DPN, and painful DPN. We then calculated prevalence ratios (PRs) with 95% confidence intervals (CIs) of DPN associated with obesity measures and other metabolic and lifestyle factors using log-binomial and Poisson regressions (with robust error variance).[29] Continuous risk factors were investigated both as categorical and continuous variables. All PRs were adjusted for age (continuous variable), biological sex, and diabetes duration (continuous variable). We did not make further adjustments because the obesity measures and other metabolic and lifestyle factors may act as intermediates and clusters in the same incompletely understood pathophysiological pathways. The associations between obesity measures and DPN were also evaluated with the use of restricted cubic spline regressions with 5 knots.[30] To elaborate further on the associations of central obesity measures with DPN, we additionally adjusted for BMI in

these models. The analyses of change of physical activity level were stratified according to baseline physical activity level.

Next, we restricted the cohort to those with DPN (MNSIq ≥4) and calculated the prevalence ratio of painful DPN for each risk factor under study.

In sensitivity analyses, we restricted the population to individuals with a registered diabetes duration <1 year and <½ year at DD2 enrollment in order to focus exclusively on newly diagnosed diabetes and increase the likelihood of incident DPN at assessment a medina of 2.8 years later. Since having diabetes and neuropathic pain (DN4 ≥3) in both feet fulfils the NeuroPPIC/NeuPSIG criteria for possible painful DPN[24 25] despite a MNSIq score <4, we included these patients in the painful DPN group in a sensitivity analysis (Supplementary Figure 1, Panel B). In another sensitivity analysis, we excluded patients with alcohol overconsumption, because peripheral neuropathy may result from DPN, alcoholic polyneuropathy, or a mixture in these patients.

Research ethics and informed consent

The Danish National Committee on Health Research Ethics (record number S-20100082) and the Danish Data Protection Agency (record number 2008-58-0035) approved the DD2 study. All DD2 patients volunteered to participate in the DD2 study and gave written informed consent.

Results

Descriptive data

We included 5,249 patients, of whom 938 (17.9%) had DPN, including 386 (7.4%) with painful DPN (Supplementary Table 2).[StudyII] Median age was 65 years (IQR 57-72), 42% were

female, and median diabetes duration was 4.6 years (IQR 3.5-5.7) at DPN assessment (Supplementary Table 2).

Obesity measures and DPN

Higher BMI at baseline and at the questionnaire date as well as gradual increase in waist circumference, waist-hip ratio and waist-height ratio were all associated with gradual increase in DPN-prevalence (Figure 2). Similar results were observed when the obesity measures were analyzed as continuous variables (Supplementary Table 3).The magnitude of the association with DPN for 1 SD increase was similar for the general and central obesity measures (Supplementary Table 3). Spline regression analyses, yielded approximate linear relations with DPN for general and central obesity measures, except for a J-shaped association observed with BMI at age 20 years and for BMI change since age 20 years (Supplementary Figure 2).

When we additionally adjusted central obesity for BMI, all central obesity measures remained positively associated with DPN (Supplementary Figure 3). For example, for a given BMI, DPN prevalence increased by a factor of 1.86 (95% CI 1.32; 2.61) for individuals with a waist circumference of ≥102/88 cm (male/female) vs. <94/80 cm. In these analyses, BMI persistently associated with DPN.

Other metabolic risk factors, patient characteristics, and DPN

Figure 3 shows risk estimates for baseline non-obesity metabolic risk factors and for lifestyle habits. Metabolic factors markedly associated with DPN included low HDL cholesterol levels (<1.2 mmol/L [male/female], aPR: 1.35 [95% CI 1.12; 1.62]), high triglyceride levels (≥1.7 mmol/L, aPR 1.36 [95% CI 1.17; 1.59]), low-grade inflammation (hsCRP ≥3.0 vs. <1.0 mg/L, aPR 1.66 [95% CI 1.42; 1.94]), higher c-peptide levels (≥1550 vs. <850, aPR 1.72 [95% CI 1.43; 2.07]), and higher HbA1c levels (≥9.5 vs. <6.5%, aPR 1.38 [95% CI 1.02; 1.88]).

Antihypertensive drug treatment (aPR 1.34 [95% CI: 1.16; 1.55]) was associated with DPN, but not systolic and diastolic blood pressure.

Finally, female sex, presence of other diabetes complications (including a high albumin-creatinine ratio), as well as insulin treatment were associated with DPN (Supplementary Table 4-5).

Lifestyle factors and DPN

Lower physical activity (0 days vs. ≥3 days/week, aPR 1.60 [95% CI 1.39; 1.85]) and a current smoker (aPR 1.50 [95% CI 1.24; 1.81]) or former smoker (≥6 months) (aPR 1.39 [95% CI 1.18; 1.64]) status at baseline were clearly associated with DPN. Of note, continued smoking compared with smoking cessation between baseline and questionnaire date was also associated with DPN (aPR 1.24 [95% CI 0.80; 1.92]), yet with limited statistical precision, whereas no clear association was observed with change in physical activity level (Figure 3).

Risk factors and neuropathic pain

Among the group of DPN patients, we did an internal analysis of risk factors associated with neuropathic pain (i.e. factors associated with painful versus non-painful DPN) shown in Table 1 and Supplementary Tables 6-8. Several metabolic risk factors appeared to associate with increased prevalence of painful DPN, yet often with limited statistical precision. These included central obesity (waist circumference [males/females] ≥102/88 vs. <94/85 cm (aPR 1.40 [0.83; 2.37]), waist-hip ratio ≥1.05/0.95 vs. <0.95/0.85 (aPR 1.31 [0.97; 1.76]), high systolic blood pressure (≥ 130 mmHg, aPR 1.16 [0.94; 1.42], high total cholesterol levels (≥ 4.3 mmol/L, aPR 1.25 [0.97; 1.62]), high LDL-cholesterol (≥2.6 vs. <1.8 mmol/L, aPR 1.17 [0.90; 1.52], and high triglycerides (≥ 1.7 mmol/l, aPR 1.17 [0.96; 1.44]). The estimates for the continuous analyses are shown in Supplementary Table 7-8. Statistically significant associations with neuropathic pain were observed for alcohol overconsumption at baseline (aPR 1.31 [1.01; 1.69])

and for current smoking at the questionnaire date (aPR 1.29 [1.03; 1.62]). Increased physical activity from baseline to questionnaire date was associated with lower painful DPN prevalence (aPR 0.82 [0.67; 0.99]).

Sensitivity analyses

Of the 5,249 patients, 130 (2.5%) had pain in both feet and DN4 ≥3 but MNSIq <4.[studyII] For the majority of risk factors, these patients were more similar to patients without DPN than to patients with painful DPN. Including these 130 patients in the painful DPN group therefore marginally reduced most risk estimates for DPN and the occurrence of neuropathic pain in DPN, but did not change any of our conclusions (data not shown).

Restricting the cohort to those with diabetes duration ≤1 year and ≤½ year generally supported the main analyses, but with lower precision (data not shown). However, high systolic blood pressure constituted an exception and was associated with reduced DPN prevalence in these analyses: <1 year diabetes duration (aPR 0.79 [0.61; 1.02]), <½ year diabetes duration (aPR 0.59 [0.43; 0.81]).

Excluding DPN patients with alcohol overconsumption did not change any conclusion (data not shown).

Conclusions

This is the largest study to date to investigate in detail various obesity measures and a wide range of metabolic and lifestyle factors with both DPN and painful DPN in early type 2 diabetes patients. We found that both general and central obesity are strongly - and independently of each other - associated with DPN prevalence. Other metabolic and lifestyle factors clearly associated with DPN prevalence included low-grade inflammation, high triglyceride, c-peptide, and HbA1c levels, low HDL-levels, antihypertensive drug use, tobacco

smoking and low physical activity. Female sex and presence of other diabetes complications also associated with DPN. These findings suggest that controlling metabolic factors through weight loss and medications as well as lifestyle interventions, including smoking cessation and increasing physical activity may potentially reduce the risk of DPN.

Even in this large study, statistical precision was limited for the risk factor analyses of painful DPN. While metabolic syndrome factors (central obesity, increased lipid levels, and hypertension) seemed to associate with the presence of neuropathic pain, these results did not reach statistical significance. Notably, we found clear evidence that high alcohol intake, tobacco smoking, and failure to increase activity after diabetes diagnosis associated with higher prevalence of neuropathic pain in DPN. These results are important as all three of these risk factors are modifiable without the need for medications. Future intervention studies are needed to better understand the impact of alcohol cessation, tobacco cessation, and exercise on painful neuropathy.

It is increasingly accepted that DPN may start to develop already at the prediabetes stage.[31] This highlights the importance of also investigating risk factor-DPN associations also in populations with early diabetes. Our findings corroborate previous observations that the degree of obesity in diabetes strongly correlates with DPN.[5 6 8] Compared to previous studies, we additionally found that central fat distribution is associated with DPN independent of general obesity. In line with this observation, we found increased DPN prevalence with higher low-grade inflammation and c-peptide levels (hyperinsulinemia), and with hypertriglyceridemia, i.e. all metabolic factors that may specifically be caused by central obesity.[18 32] On the contrary, low-grade inflammation and hyperinsulinemia did not seem to associate with neuropathic pain in our study. Doupis et al.[14] previously reported an association of increased CRP in painful DPN versus non-painful DPN, in contrast to our findings. Also, studies of diabetic animal and of nondiabetic human populations have suggested a role of inflammatory markers in painful

polyneuropathy.[33] Future studies of painful DPN in type 2 diabetes should investigate a broader range of inflammatory markers, in addition to hsCRP.

The finding that lower HDL cholesterol associates with DPN was also recently reported in the ADDITION cohort of screen-detected type 2 diabetes[7] and in most studies of longstanding diabetes.[2 8 13] In contrast, lower LDL cholesterol also predicted DPN in the ADDITION cohort[7] and was associated with peripheral nerve damage in another type 2 diabetes study.[34] The authors speculated whether this might result from statin treatment.[7 34] In our study, neither LDL cholesterol nor lipid-lowering drug-use materially affected DPN risk.

In our main analyses, we did not find a clear association of blood pressure with DPN, in line with previous and smaller studies of type 2 diabetes patients.[5 7 8 13 31] Generally, well-controlled blood pressure in our cohort may have hindered identifying an association between DPN and hypertension, which might be supported by the observed higher DPN prevalence among patients receiving antihypertensive drug treatment. However, after restricting the analysis to subjects with the shortest diabetes duration at baseline, i.e. increasing the likelihood of incident DPN, we observed a lower DPN risk with higher systolic blood pressure. The reason for this surprising finding, which persisted after adjusting and stratifying for antihypertensive drug use, is unknown.

Although a meta-analysis have concluded that optimized glycemic control is less successful for reducing DPN risk in type 2 diabetes versus type 1 diabetes patients, hyperglycemia may still contribute to DPN development in type 2 diabetes.[4] Accordingly, we found that higher HbA1c levels associated with DPN risk, but not specifically with neuropathic pain.

As an important finding, we found strong evidence that both current and former smoking associated with DPN and neuropathic pain. Continued smoking versus cessation from baseline to questionnaire associated with both DPN and neuropathic pain, albeit with limited statistical precision. This may possibly reflect detrimental effects from higher cumulative smoke exposure

(dose and time), whereas cessation could possible reverse smoke-induced nerve damage. A recent meta-analysis reported only low-grade evidence for smoking as a DPN risk factor;[35] however, the previous studies often allocated formers smokers to the non-smoker reference-group which may mask any association, as supported by the studies comparing ever-smokers (current and former) with never smokers that generally demonstrated a stronger positive association with DPN.[35] Our results highlight the potential to improve neuropathy and its resulting pain through smoking cessation interventions.

New and promising research advocates physical exercise for preventing and treating DPN,[36] in line with the association of DPN with baseline physical inactivity in our study. We found that increased physical activity after baseline lowered the prevalence of neuropathic pain but did not affect DPN-prevalence. However, if we stratified by baseline physical activity level, we saw that DPN prevalence decreased in patients whose activity was low at baseline but had increased by the time of the questionnaire. Conversely, DPN prevalence increased in patients whose activity was high at baseline but had decreased by the time of the questionnaire. We cannot exclude the possibility of reverse causation, i.e. that DPN symptoms may have led to less physical activity.[37] Future intervention studies are needed to clarify any preventive or therapeutic role of physical activity against DPN.

Our finding that females had a 1.2 fold higher DPN risk than males contrasts with most previous findings.[2 14 38 39] Females may have been more likely to report symptoms than males. However, self-reported neuropathic pain among patients with DPN was not associated with female sex, thus ruling out simple reporting bias as the sole explanation.

Finally, patients with macrovascular or other microvascular complications had a 1.2-1.7-fold higher prevalence of DPN, which may result from shared metabolic risk factors for these complications.[40]

The study's main strengths include the cohort size and the comprehensive and detailed assessment of metabolic and temporal lifestyle factors. Additional, our neuropathy questionnaire

had a remarkable response rate of 82%. Moreover, the study included patients with short diabetes duration, which is relevant when determining correlative features that might factor in as possible preventive features. Finally, the time elapse since patient characteristics were determined at baseline until DPN assessment a median of 2.8 years later implies that cases were a mixture of new incident DPN and prevalent DPN pre-existing at baseline. Our findings from the main analyses were confirmed in newly diagnosed diabetes patients at baseline. If this cohort reported DPN with the questionnaire, it was likelier to be incident DPN, thus strengthening our conclusions and suggesting potential interventional measures. Our study also has limitations. First, DPN and painful DPN assessment relied on the MNSIq and DN4 questionnaires, and not on neurological examinations, nerve conduction studies, or validated small-fiber measures. However, both tools are validated.[22 27] Although MNSIq sensitivity is rather low (40% in a study of longstanding type 1 diabetes patients[22]) for measures of relative risk, a specificity of 100% leads to unbiased comparative results, and high specificity is thus more important than sensitivity.[41] Second, despite the time elapse since patient characteristics were determined at baseline until DPN assessment a median of 2.8 years later, our analyses reflect a cross-sectional study design due to the unknown DPN and pain status at baseline. This leads to intrinsic uncertainty about temporal relationships and the possibility for reverse causality for some associations. Third, self-reported BMI and other factors may be subject to recall errors. There is evidence that self-reported anthropometric data are reasonably accurate and adequate for use in large epidemiological studies.[42] Finally, it is a limitation that some variables were only available for a subcohort of patients.

In conclusion, these data provide evidence that DPN in early type 2 diabetes is closely associated with specific risk factors in addition to hyperglycemia, including metabolic syndrome factors, c-peptide and low-grade inflammation. Moreover, unhealthy lifestyle habits including smoking and physical inactivity are modifiable factors strongly associated with DPN. Pain occurrence in DPN may share some, but not all, of these modifiable risk factors. Future

longitudinal studies should further investigate specific risk factors for DPN and painful DPN, and the clinical effects of improving such factors.

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Author contribution

D.H.C. designed the study, performed the statistical analyses, wrote the first draft, and contributed to the discussion. R.W.T., S.T.K., H.A., N.B.F., B.C.C., and T.S.J. designed the study, contributed to the discussion, and reviewed and edited the manuscript. S.S.G. researched the data, contributed to the discussion, and reviewed and edited the manuscript. J.S.N., H.B-N, and E.L.F. contributed to the discussion and edited and reviewed the manuscript. All authors approved the final manuscript.

Disclosures

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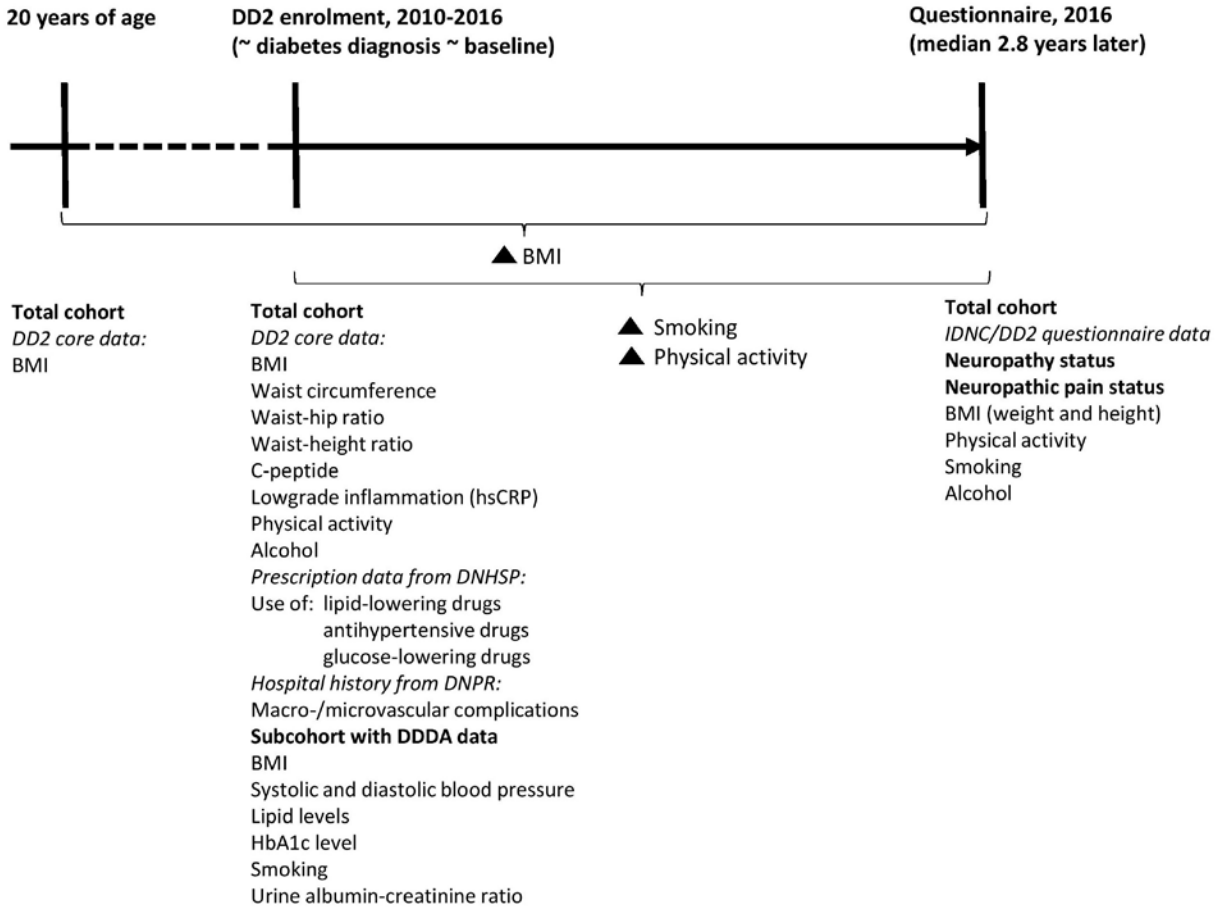
Figures

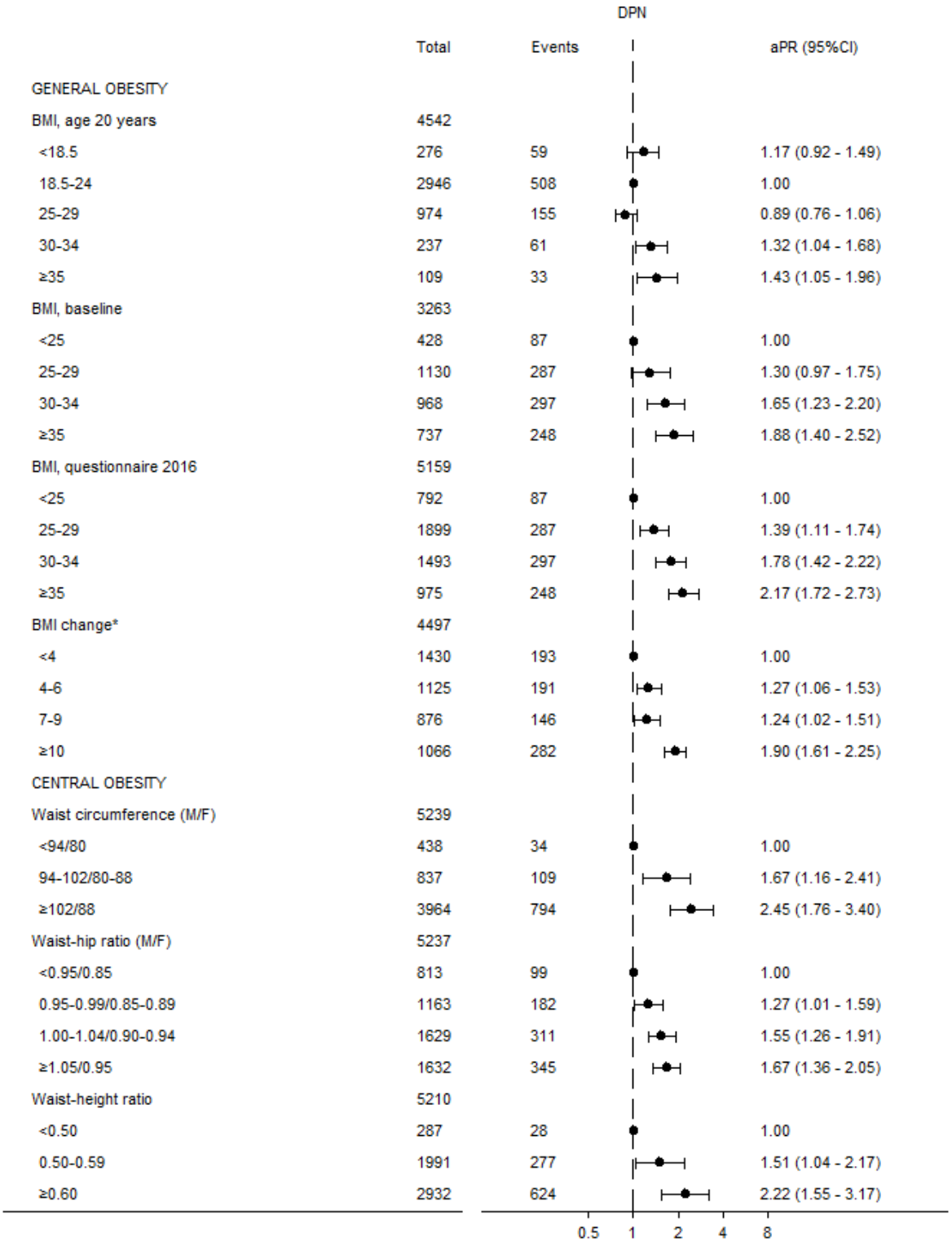
Figure 1. Timeline of assessment of obesity measures, other metabolic and lifestyle factors, and DPN-status.



Abbreviations: DD2; The Danish Centre for Strategic Research in Type 2 Diabetes, BMI; Body mass index, HbA1c; Hemoglobin A1c, hsCRP; high-sensitivity C-reactive protein, DNHSP; Danish National Health Service Prescription Database, DNPR; Danish National Patient Registry, DDDA; Danish Diabetes Database for Adults.

Changes in alcohol consumption from time of DD2 enrollment to questionnaire 2016 was not investigated due to the use of different cut-offs at the two assessments (≥21/14 units vs. ≥14/7 units [female/male]).

Figure 2. Prevalence ratios of DPN associated with general and central obesity measures.

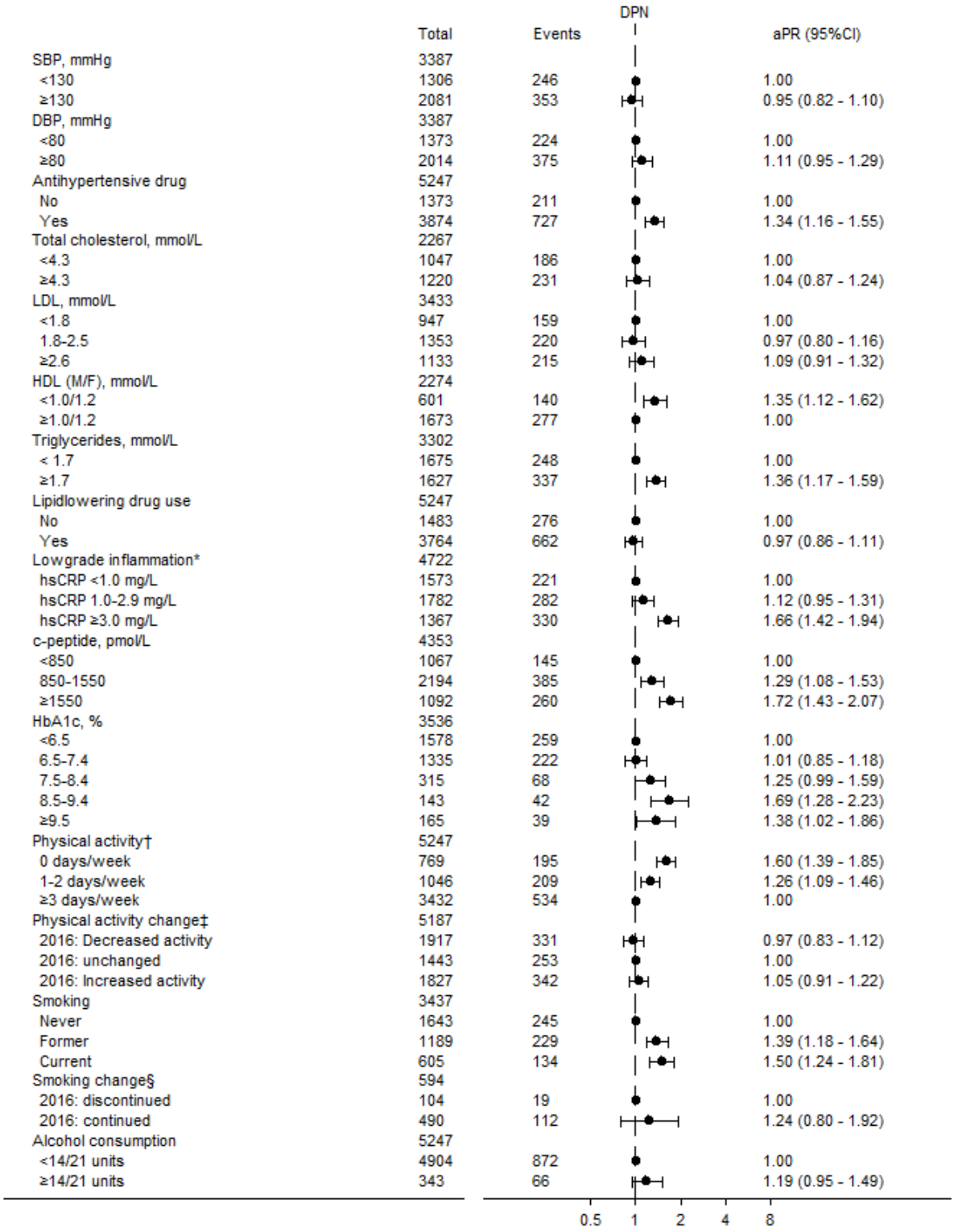


Abbreviations: aPR; adjusted prevalence ratio, DPN; Diabetic polyneuropathy, CI; confidence interval, BMI; body mass index, M/F; male/female.

All estimate are adjusted for age, sex, and diabetes duration.

*BMI change from age 20 to questionnaire 2016.

Figure 3: Prevalence ratios of DPN associated with metabolic risk factors.



Abbreviations: aPR; adjusted prevalence ratio, DPN; Diabetic polyneuropathy, CI; confidence interval, hsCRP; high-sensitivity C-reactive protein, HbA1c; hemoglobin A1c, M/F; male/female.

*hsCRP values above 10 mg/L were excluded in order to exclude values reflecting ongoing infections

†Days per week with minimum 30 minutes of physical activity.

‡Change from baseline (=DD2 enrollment) to questionnaire 2016 in the number of days per week with minimum 30 minutes of physical activity; less active: at least 1 day less per week with more than 30 minutes of physical activity, more active: at least 1 day more per week with minimum 30 minutes of physical activity.

§Among those who were current users at baseline.

All estimates are adjusted for age, sex, and diabetes duration.

Tables

Table 1 Prevalence ratios of neuropathic pain occurrence (pain in both feet + DN4 ≥3) among the 938 patients with DPN

	DPN, Total	Painful DPN n (%)	aPR 95% CI
Total, N (%)	938	386 (41.2)	
OBESITY			
GENERAL OBESITY MEASURES = BMI			
BMI at age 20 years	816		
< 18.5	59	27 (45.8)	1.06 (0.78; 1.43)
18.5 - 24	508	221 (43.5)	1 (ref)
25 - 29	155	58 (37.4)	0.87 (0.69; 1.09)
30 - 34	61	22 (36.1)	0.85 (0.60; 1.22)
≥35	33	11 (33.3)	0.80 (0.48; 1.31)
BMI, baseline	581		
< 25	50	26 (52.0)	1 (ref)
25 - 29	170	66 (38.8)	0.74 (0.54; 1.03)
30 - 34	190	76 (40.0)	0.78 (0.57; 1.07)
≥35	171	66 (38.6)	0.76 (0.55; 1.06)
BMI, questionnaire date	919		
< 25	87	37 (42.5)	1 (ref)
25 - 29	287	115 (40.1)	0.95 (0.72; 1.26)
30 - 34	297	123 (41.4)	1.00 (0.75; 1.32)
≥35	248	107 (43.2)	1.05 (0.79; 1.40)
BMI change from age 20 -> questionnaire date	812		
< 4	193	72 (37.3)	1 (ref)
4 - 6	191	79 (41.4)	1.10 (0.86; 1.41)
7 - 9	146	66 (45.2)	1.21 (0.94; 1.56)
≥10	282	120 (42.6)	1.14 (0.91; 1.44)
CENTRAL OBESITY MEASURES			
Waist circumference (M/F)	937		
< 94/80 cm	34	10 (29.4)	1 (ref)
94-102/80-88 cm	109	46 (42.2)	1.42 (0.81; 2.51)
≥102/88 cm	794	329 (41.4)	1.40 (0.83; 2.37)
Waist-hip ratio (M/F)	937		
< 0.95/0.85	99	34 (34.3)	1 (ref)
0.95 - 0.99/0.85 - 0.89	182	74 (40.7)	1.18 (0.86; 1.64)
1 - 1.04/0.90 - 0.94	311	124 (39.9)	1.17 (0.87; 1.59)
≥1.05/0.95	345	153 (44.3)	1.31 (0.97; 1.76)
Waist-height ratio	929		
< 0.5	28	12 (42.9)	1 (ref)
0.5 - 0.6	277	109 (39.4)	0.92 (0.59; 1.45)
≥0.6	624	262 (42.0)	0.99 (0.64; 1.54)
NON-OBESITY METABOLIC AND LIFESTYLE FACTORS			
Blood pressure (systolic) mmHg	599		
< 130	246	91 (37.0)	1 (ref)
≥130	353	152 (43.1)	1.16 (0.94; 1.42)
Blood pressure (diastolic) mmHg	599		
< 80	224	96 (42.9)	1 (ref)
≥80	375	147 (39.2)	0.94 (0.76; 1.16)
Antihypertensive drug use	938		
No	211	85 (40.3)	1 (ref)
Yes	727	301 (41.4)	1.00 (0.82; 1.21)

Total cholesterol, mmol/L	417		
< 4.3	186	63 (33.9)	1 (ref)
≥4.3	231	97 (42.0)	1.25 (0.97; 1.62)
LDL cholesterol	594		
< 1.8	159	59 (37.1)	1 (ref)
1.8 - 2.6	220	89 (40.5)	1.09 (0.84; 1.42)
≥2.6	215	93 (43.3)	1.17 (0.90; 1.52)
HDL cholesterol (men/women)	417		
< 1.0/1.2	140	53 (37.9)	1 (ref)
≥1.0/1.2	277	108 (39.0)	1.02 (0.79; 1.32)
Triglycerides	585		
< 1.7	248	93 (37.5)	1 (ref)
≥1.7	337	146 (43.3)	1.17 (0.96; 1.44)
Lipid-lowering drug use	938		
No	276	120 (43.5)	1 (ref)
Yes	662	266 (40.2)	0.91 (0.77; 1.08)
Low-grade inflammation (hsCRP),* mg/L	833		
< 1.0	221	89 (40.3)	1 (ref)
1.0-2.9	282	112 (39.7)	0.98 (0.79; 1.22)
≥3.0	330	137 (41.5)	1.03 (0.84; 1.27)
C-peptide	790		
< 850	145	59 (40.7)	1 (ref)
850-1550	385	160 (41.6)	1.03 (0.82; 1.29)
≥1550	260	106 (40.8)	1.00 (0.79; 1.28)
HbA1c, %	630		
< 6.5	259	102 (39.4)	1 (ref)
6.5 – 7.4	222	100 (45.1)	1.15 (0.94; 1.42)
7.5 – 8.4	68	28 (41.2)	1.06 (0.77; 1.47)
8.5 – 9.4	42	13 (31.0)	0.83 (0.51; 1.35)
≥9.5	39	16 (41.0)	1.11 (0.74; 1.68)
Physical activity, baseline†	938		
0	195	83 (42.6)	1.00 (0.83; 1.21)
1 – 2	209	76 (36.4)	0.85 (0.69; 1.04)
≥3	534	227 (42.5)	1 (ref)
Physical activity, change from baseline to questionnaire ‡	926		
Decreased activity	331	139 (42.0)	0.93 (0.77; 1.12)
No change	253	114 (45.1)	1 (ref)
Increased activity	342	126 (36.8)	0.82 (0.67; 0.99)
Smoking, baseline	608		
Never	245	91 (37.1)	1 (ref)
Former	229	101 (44.1)	1.18 (0.95; 1.47)
Current	134	57 (42.5)	1.17 (0.90; 1.51)
Smoking change §	131		
Questionnaire 2016: discontinued	19	7 (36.8)	1 (ref)
Questionnaire 2016: continued	112	49 (43.8)	1.26 (0.67; 2.36)
Alcohol, baseline‖	938		
<14/24	872	352 (40.4)	1 (ref)
≥14/21	66	34 (51.5)	1.31 (1.01; 1.69)

Abbreviations: DPN; Diabetic polyneuropathy, aPR; adjusted prevalence ratio, BMI; body mass index, CI; confidence interval, hsCRP; high-sensitivity C-reactive protein, HbA1c; hemoglobin A1c, M/F; male/female.

All estimates are adjusted for age, sex, and diabetes duration.

*hsCRP values above 10 mg/L were excluded in order to exclude values reflecting ongoing infections.

†Days pr week with minimum 30 minutes of physical activity.

‡Change from baseline to questionnaire 2016 in the number of days per week with minimum 30 minutes of physical activity; Decreased: at least 1 day less per week with more than 30 minutes of physical activity, Increased activity: at least 1 day more per week with minimum 30 minutes of physical activity.

§Among those who were current users at baseline.

||Units of alcohol (women/men), which is the maximum safe amount recommended by the Danish Health Authority, when the DD2 began enrollment.

Supplemental material

Tables:

Supplementary Table 1: *Definitions and codes*

Supplementary Table 2: *Descriptive data*

Supplementary Table 3: *Obesity measures, continuous, including unit = 1 SD*

Supplementary Table 4: *Non-obesity risk factors, categorical, additional variables*

Supplementary Table 5: *Non-obesity risk factors, continuous*

Supplementary Table 6: *Pain occurrence in DPN, non-obesity measures, categorical, additional variables*

Supplementary Table 7: *Pain occurrence in DPN, obesity measures, continuous (clinical relevant units + 1 SD)*

Supplementary Table 8: *Pain occurrence in DPN, non-obesity measures, continuous*

Figures:

Supplementary Figure 1: *DPN definitions*

Supplementary Figure 2: *Spline regressions*

Supplementary Figure 3: *Central obesity measures additionally adjusted for BMI*

TABLES

Supplementary Table 1: Definitions and codes used in this study	
Variable	Definition and codes
DDDA/DD2/IDNC variables	
DDDA variables -Blood pressure -Lipids -HbA1c -Smoking baseline -Micro/macroalbuminuria -BMI (see anthropometric data)	Categories: Systolic blood pressure: </≥130 Diastolic blood pressure: </≥80 Lipids LDL: <1.8/1.8-2.6, ≥2.6 HDL (male/female): <1.0/1.2, ≥1.0/1.2 Triglycerides: </≥1.7 Total cholesterol: </≥4.3 HbA1c: <6.5, 6.5-7.4, 7.5-8.4, 8.5-9.4, ≥9.5 Smoking: Never, former, current (daily + occasionally) Albuminuria: Normal: Albumin-creatinine ratio <30 mg/g, microalbuminuria: Albumin-creatinine ratio 30-300 mg/g, macroalbuminuria: Albumin-creatinine ratio ≥300 mg/g References: https://www.nbv.cardio.dk/dyslipidaemi American Diabetes Association 2003 BMI: see below
DD2 core variables -low-grade inflammation/hsCRP -c-peptide -Physical activity, baseline -Alcohol, baseline -Waist circumference -Waist-hip ratio -Waist-height ratio -BMI age 20 (see anthropometric data)	Low-grade inflammation: excluding measures of hsCRP ≥10mg/L in order to exclude values related to potential ongoing infection. Physical activity: “number of days with minimum 30 minutes of physical activity per week”. Categories Low-grade inflammation: <1.0, 1.0-2.9, ≥3.0 mg/L Physical activity: 0, 1-2, ≥3 days/week Alcohol: </≥14/21 units/week for women/men, which was the recommended safe dose in 2010, where the DD2 began enrollment Waist circumference: see below Waist-hip ratio: see below Waist-height ratio: see below
Questionnaire 2016 variables -Smoking -Alcohol -Physical activity -BMI	Physical activity: “number of days with minimum 30 minutes of physical activity per week”. Categories: Smoking: Never, former, current (daily + occasionally) Alcohol: </≥7/14 units/week for women/men, which was the recommended safe dose in 2016 Physical activity: 0, 1-2, ≥3 days/week BMI: see below
Anthropometric data	
Height	Data on height is available from 3 different sources: DD2 enrollment (2016 onwards), DDDA data (repeated measures), questionnaire data 2016 (self-reported). Regarding DDDA data: a mean height based on all available DDDA heights where calculated for all patients 18 years or older.

	Heights below 130 cm and above 220 were considered outliers and not included in any calculations. See variables below for hierarchically order of the height
BMI age 20 years	Weight: Recalled at DD2 enrollment. Weights below 35 and above 300 kg were considered outliers and not included in the BMI calculation. Height: We do not expect height to change over time among these adults. Thus, we used the available heights in a hierarchically order; height from questionnaire survey in 2016, DD2 enrollment, DDDA. That is, if a patient has a height recorded from the questionnaire, we will use that height. If not, we will use the DD2 enrollment height (measured by health personal, but only a few available measures [not part of the DD2 core data initially]) and if no DD2 enrollment height is available, we will use DDDA height. Categories: BMI age 20 years: <25, 25-29, 30-34, ≥35 kg/m^2
BMI baseline	Weight: If weight recorded as part of the DD2 enrollment process is available (few [not part of the DD2 core data initially]), we used that weight measure, otherwise the DDDA weight. Weights below 35 and above 300 kg were considered outliers and not included in the BMI calculation. Height: We do not expect height to change over time among these adults. Thus, we used the available heights in a hierarchically order; height from questionnaire survey in 2016, DD2 enrollment, DDDA. That is, if a patient has a height recorded from the questionnaire, we will use that height. If not, we will use the DD2 enrollment height (measured by health personal, but only a few available measures [not part of the DD2 core data initially]) and if no DD2 enrollment height is available, we will use DDDA height. Categories: BMI at DD2 enrollment: <25, 25-29, 30-34, ≥35 kg/m^2
BMI questionnaire 2016	Will be based solely on the weight and height data from the 2016 questionnaire survey in order to report the exact same number of missings as in other paper based solely on the neuropathy questionnaire data. (That means 90 missing vs. 73 with if we instead had used the height variable that was used in calculation of the other anthropometric variables) Categories: BMI at DD2 enrollment: <25, 25-29, 30-34, ≥35 kg/m^2
Waist circumference	Categories:

	Male/female: < 94/80 cm, 94-102/80-88 cm, ≥102/88 cm Reference: WHO: World health Organ Tech Rep Ser 2000;894:i-xii, 1-253 IDF: Diabet Med 2006;23:469-480
Waist-hip ratio	Categories: Male/female: < 0.95/0.85 0.95 - 0.99/0.85 - 0.89 1.00 - 1.04/0.90 - 0.94 ≥1.05/0.95 References: We based our categories on often used waist-hip ratio classifications in the scientific literature together with observations of baseline WHR distributions in our cohort.
Waist-height ratio	Categories: <0.5, 0.5-0.6, ≥0.6 Reference: Schneider et al. J Clin Endocrinol Metab 2007;92(2):589-594
Prescription data	For all prescription data the relevant time period is around baseline = DD2 enrollment. Thus, lookback period is 1 year prior to DD2 enrollment date
Lipidlowering drugs	ATC: C10
Statins	ATC: C10AA, C10BA, C10BX
Antihypertensives	ATC: C02, C03, C07, C08, C09
Glucose-lowering drugs	ATC: A10 Categories: No GLD: no A10 prescription redemption Non-insulin only: ≥1 prescription redemption of A10B and NO prescription redemption of A10A Insulin only: ≥1 prescription redemption of A10A and NO prescription redemption of A10B Non-insulin + insulin: ≥1 prescription redemption of A10B and ≥1 prescription redemption of A10A
Danish National Patient register	For all variables, the relevant time period is prior to DD2 enrollment – as a proxy for the history prior to the diabetes diagnosis. Thus, lookback period is from the DD2 enrollment date and all the way back to 1994 (based on international classification of diseases, version 10 diagnosis codes)
Macrovascular complications	Ischemic heart disease DI21, DI23, DI24, DT822A, DT823D, DT823E (acute ischemic heart disease with/without complications), DI20 (angina pectoris), DI25 (chronic ischemic heart disease), KFNA, KFNB, KFNC, KFND, KFNE, KFNF, KFNG, KFNH, KFNW, KFLF (coronary bypass or percutaneous coronary intervention) Cerebrovascular disease DI61 (cerebral bleeding), DI63, DI64, DI65, DI66 (cerebrovascular infarct), DG45 (transient cerebrovascular disease), DI672, DI678, DI679 (unspecified cerebrovascular disease), DI691, DI693, DI694,

	DI695, DI696, DI697, DI698 (previous cerebrovascular disease), KAAL10, KAAL11 (cerebral thrombolyse or thrombendarterektomi) Abdominal- and peripheral vascular disease DE105, DE115, DE125, DE135, DE145 (diabetes with peripheral vascular complications), DI700, DI701, DI702, DI708, DI709, DI739, DI740-DI745, DI748, DI749, DN280, DK550, DK551 (peripheral/abdominal vascular disease) KPAE, KPAF, KPAH, KPAN, KPAP, KPAQ, KPAW99, KPAU74, KPBE, KPBF, KPBH, KPBN, KPBP, KPBQ, KPBW, KPCE, KPCF, KPCH, KPCN, KPCP, KPCQ, KPCW99, KPCW20, KPCU74, KPCU82, KPCU83, KPCU84, KPDE, KPDF, KPDH, KPDN, KPDP, KPDQ, KPDU74, KPDU82, KPDU83, KPDU84, KPDW99, KPDW20, KPEE, KPEF, KPEH, KPEN, KPEP, KPEQ, KPEU74, KPEU82, KPEU83, KPEU84, KPEW, KPFE, KPFH, KPFN, KPFP, KPFQ, KPFU74, KPFU82, KPFU83, KPFU84, KPFW, KPGH10, KPGH20, KPGH21, KPGH22, KPGH23, KPGH30, KPGH31, KPGH40, KPGH99, KPGU74, KPGU83, KPGU84, KPGU99, KPGW, KPWG (vascular surgery)
Renal complication	DE102, DE112, DE122, DE132, DE142 (diabetes with renal complications), DI120, DI131, DI132 (hypertensive nephropathy), DN083 (diabetic glomerular disease), DN06 (renal disease with proteinuria), DN17, DN18, DN19 (chronic and acute renal disease), DR809 (proteinuria), BJFD2, DZ992 (dialysis)
Eye complication	DE103, DE113, DE123, DE133, DE143 (diabetes with ophthalmic complications), DH340, DH341, DH342, DI708A (retinal arterial disease), DH280, DH281, DH282, DH25, DH268, DH269 (cataract), DH330, DH332, DH334, DH335 (amotio retinae), DH430, DH431, DH438, DH439, DH450 (vitreous bleeding or degeneration), DH360 (diabetic retinopathy), DH540, DH541, DH544, DH545 (blindness), DH470 (optic nerve disease), KCKC10, KCKC15 (laser coagulation)

Supplementary Table 2: Patient characteristics of the total cohort of 5,249 DD2 patients and of the subcohort of 3,623 DD2 patients that could be linked to the DDDA

	All, N = 5,249	MNSIq < 4, N = 4,311	MNSI≥4, N = 938	MNSIq≥4	
				No pain or DN4 <3, N = 552	Pain and DN4 ≥3, N = 386
	Total	No DPN	DPN	Non-painful DPN	Painful DPN
Total cohort, N = 5249					
Total, N	5249	4311 (82.1)	938 (17.9)	552 (10.5)	386 (7.4)
Age, questionnaire 2016, N=5249	65.4 (56.6; 71.5)	65.8 (57.2; 71.7)	63.1 (54.9; 70.3)	62.8 (54.9; 70.0)	64.0 (55.3; 70.8)
Female sex, N=5249	2216 (42.2)	1770 (41.1)	446 (47.5)	258 (46.7)	188 (48.7)
Diabetes duration, questionnaire 2016, years, N=5247	4.6 (3.5; 5.7)	4.5 (3.4; 5.7)	4.8 (3.7; 6.0)	4.7 (3.6; 5.9)	4.9 (3.8; 6.1)
Height, N=5220	173 (166; 180)	173 (166; 179)	172 (165; 180)	172 (165; 180)	173 (166; 180)
BMI, age 20 years, kg/m2, N=4542	23.2 (21.0; 25.5)	23.2 (21.0; 25.5)	23.2 (20.9; 25.7)	23.3 (21.1; 25.8)	23.1 (20.5; 25.2)
BMI, questionnaire 2016, kg/m2, N=5159	29.7 (26.4; 33.6)	29.4 (26.2; 33.1)	31.2 (27.7; 35.7)	31.2 (27.8; 35.5)	31.5 (27.5; 35.7)
Waist circumference, com, baseline, N=5239	106 (97; 116)	105 (96; 115)	110 (100; 119)	110 (100; 120)	110 (100; 119)
Waist-hip ratio, baseline, N=5237	0.98 (0.92; 10.4)	0.98 (0.92; 1.04)	0.98 (0.92; 1.04)	0.98 (0.92; 1.04)	0.99 (0.93; 1.04)
Waist-height ratio, N=5210	0.61 (0.56; 0.67)	0.61 (0.56; 0.66)	0.63 (0.58; 0.69)	0.63 (0.58; 0.70)	0.64 (0.58; 0.69)
Low-grade inflammation (hsCRP),* mg/L, N=4722	1.7 (0.8; 3.4)	1.6 (0.7; 3.1)	2.2 (1.0; 4.2)	2.2 (0.9; 4.2)	2.2 (1.0; 4.2)
C-peptide, pmol/L, N=4353	1149 (856; 1553)	1128 (841; 1516)	1266 (958; 1701)	1256 (927; 1691)	1276 (978; 1717)
Physical activity,† baseline, days/week, N=5247	4 (2; 7)	4 (2; 7)	3 (1;7)	3 (1; 7)	3 (1; 7)
Physical activity,† questionnaire 2116, days/week, N=5189	4 (2; 6)	4 (2; 6)	3 (1; 6)	3 (2; 5)	3 (1; 6)
Alcohol, baseline, N=5247					
> 14/21 units/week (women/men)	343 (6.5)	277 (6.4)	66 (7.0)	32 (5.8)	34 (8.8)
Alcohol, questionnaire 2016, N=5176					
> 7/14 units/week (women/men)	818 (15.8)	684 (16.1)	134 (14.6)	74 (13.8)	60 (15.87)
Smoking, questionnaire 2016, N=5231					
Never	1850 (35.4)	1584 (36.9)	266 (28.4)	174 (31.5)	92 (23.8)
Former	2361 (45.1)	1909 (44.5)	452 (48.2)	253 (45.8)	199 (51.6)
Current	1020 (19.5)	800 (18.6)	220 (23.5)	125 (22.6)	95 (24.6)
Antihypertensive drug use, N=5247	3874 (73.8)	3147 (73,0)	727 (77.5)	426 (77.2)	301 (78.0)
Lipid lowering drug use, N=5247	3764 (71,7)	3102 (72.0)	662 (70.6)	396 (71.7)	266 (68.9)
Microvascular complications, N=5247					
Renal complications	136 (2.6)	102 (2.4)	34 (3.6)	22 (4.0)	12 (3.1)
Eye complication	544 (10.4)	430 (10.0)	114 (12.2)	65 (11.8)	49 (12.7)
Macrovascular diabetes complication, N=5247	1222 (23.3)	928 (21.5)	294 (31.3)	173 (31.3)	121 (31.4)
Glucose-lowering drug use, N=5247					
Any glucose-lowering drug	4460 (85.0)	3640 (84.5)	820 (87.4)	477 (86.4)	343 (88.9)
Non-insulin glucose-lowering drug only	4143 (79.0)	3408 (79.1)	735 (78.4)	432 (78.3)	303 (78.5)
Insulin only	53 (1.0)	39 (0.9)	14 (1.5)	7 (1.3)	7 (1.8)

Both insulin + non-insulin glucose-lowering drug	264 (5.0)	193 (4.5)	71 (7.6)	38 (6.9)	33 (8.6)
Subcohort, N = 3,623					
BMI, baseline, kg/m2, N=3263	30.3 (27.1; 34.3)	30.0 (26.8; 34.0)	31.8 (28.1; 36.0)	32.0 (28.4; 36.0)	31.5 (27.7; 36.0)
Systolic blood pressure, mmHg, N=3387	130 (124; 140)	130 (124; 140)	130 (124; 140)	130 (123; 140)	130 (124; 142)
Diastolic blood pressure, mmHg, N=3387	80 (75; 86)	80 (74; 85)	80 (75; 86)	80 (75; 87)	80 (75; 86)
Dyslipidemia					
Total cholesterol, mmol/L, N=2267	4.3 (3.7; 5.1)	4.3 (3.7; 5.1)	4.4 (3.8; 5.1)	4.3 (3.7; 5.0)	4.5 (3.9; 5.3)
HDL cholesterol, mmol/L, N=2274	1.2 (1.0; 1.5)	1.2 (1.0; 1.5)	1.2 (1.0; 1.4)	1.2 (1.0; 1.4)	1.2 (1.0; 1.4)
LDL, mmol/L, N=3433	2.2 (1.7; 2.8)	2.2 (1.7; 2.8)	2.2 (1.7; 2.9)	2.2 (1.7; 2.8)	2.3 (1.8; 2.9)
Triglycerides, mmol/L, N=3302	1.6 (1.1; 2.3)	1.6 (1.1; 2.3)	1.9 (1.3; 2.6)	1.8 (1.2; 2.5)	1.9 (1.3; 2.9)
Glycemic control (HbA1c), N=3536	6.5 (6.1; 7.2)	6.5 (6.1; 7.1)	6.6 (6.1; 7.4)	6.6 (6.1; 7.5)	6.7 (6.2; 7.4)
Smoking, baseline, N=3437					
Never	1643 (47.8)	1398 (49.4)	245 (40.3)	154 (42.9)	91 (36.6)
Former	1189 (34.6)	960 (33.9)	229 (37.7)	128 (35.7)	101 (40.6)
Current	605 (17.6)	471 (16.7)	134 (22.0)	77 (21.5)	57 (22.9)
Albumin/creatinine ratio‡, mg/g, N=3623					
Normal/no albuminuria	2,991 (82.6)	2,478 (83.2)	513 (79.5)	302 (79.3)	211 (79.9)
Microalbuminuria	569 (15.7)	453 (15.2)	116 (18.0)	70 (18.4)	46 (17.4)
Macroalbuminuria	63 (1.7)	47 (1.6)	16 (2.5)	9 (2.4)	7 (2.7)

Abbreviations: MNSIq; Michigan Neuropathy Screening Instrument questionnaire, DN4; Douleur Neuropathique en questions 4, DPN; Diabetic polyneuropathy, DD2; The Danish Centre for Strategic Research in Type 2 Diabetes, DDDA; Danish Diabetes Database for Adults, BMI; body mass index, hsCRP; high-sensitivity C-reactive protein, HbA1c; hemoglobin A1c.

*Of note, CRP value was available for 5111, of which 389 had hsCRP ≥10 mg/L

†Days pr week with minimum 30 minutes of physical activity

‡Normal: Albumin-creatinine ratio <30 mg/g, Microalbuminuria: Albumin-creatinine ratio 30-300 mg/g, Macroalbuminuria: Albumin-creatinine ratio 300 mg/g

Missing data in the total cohort (n = 5249); height (n = 29 missing); BMI at 20 years (n = 707 missing); BMI at questionnaire 2016 (n = 90); waist circumference (n = 10 missing); waist-hip ratio (n = 12 missing), waist-height ratio (n = 39 missing), low-grade inflammation (n = 138 missing hsCRP, 389 with hsCRP ≥10 mg/L); c-peptide (n = 896 missing); physical activity at baseline (n = 2 missing); physical activity at questionnaire 2016 (n = 60), alcohol at baseline (n = 2 missing), alcohol at questionnaire 2016 (n = 70), smoking at questionnaire (n= 18), remaining variables (n = 0 missing)

Missing data in the DDDA-subcohort (n = 3623): BMI at baseline (n = 360); blood pressure (n = 236 missing); total cholesterol (n = 1356 missing); HDL cholesterol (n = 1,349 missing); LDL cholesterol (n = 190 missing); triglycerides (n = 321 missing); HbA1c (n = 87 missing); albumin/creatinine ratio (n = 0 missing), smoking at baseline (n = 186 missing).

Supplementary Table 3: Prevalence ratios of DPN for different obesity measures, continuous data		
		DPN (MNSIq ≥4)
	Total	aPR (95% CI)
General obesity		
BMI, baseline (unit = 2 kg/m^2)	3263	1.07 (1.04; 1.09)
BMI, questionnaire 2016, 2016 (unit = 2 kg/m^2)	5159	1.07 (1.06; 1.09)
Central obesity		
Waist circumference (unit = 5 cm)	5239	1.08 (1.06; 1.10)
Waist-hip ratio (unit = 0.1 cm/cm)	5237	1.16 (1.09; 1.24)
Waist-height ratio (unit = 0.1 cm/cm)	5210	1.29 (1.22; 1.37)
Per 1 SD increase*		
BMI, baseline	3259	1.22 (1.14; 1.30)
Waist circumference	3259	1.27 (1.20; 1.35)
Waist-hip ratio	3259	1.15 (1.07; 1.23)
Waist-height ratio	3259	1.23 (1.16; 1.31)

Abbreviations: DPN; Diabetic polyneuropathy, MNSIq; Michigan Neuropathy Screening Instrument questionnaire, aPR; adjusted prevalence ratio, CI; confidence interval, DD2; The Danish Centre for Strategic Research in Type 2 Diabetes, BMI; body mass index.

*The analyses with a unit of 1 SD were restricted to the 3259 patients with available data on all four obesity measures at baseline = DD2 enrollment. This restriction was applied in order to be able to compare the magnitude of the effect across obesity measures.

All analyses are adjusted for age, sex, and diabetes duration.

Supplementary Table 4: Prevalence ratios of DPN for additional risk factors with 95% CIs, categorical data

		DPN (MNSIq ≥4)	
	Total	N events (%)	aPR (95% CI)
Total, N (%)	5249	938 (17.9)	
Sex	5249		
Male	3033	492(16.2)	1 (ref)
Female	2216	446 (20.1)	1.22 (1.09; 1.38)
Albumin/creatinine ratio, mg/g*	3623		
Normal/no albuminuria	2991	513 (17.2)	1 (ref)
Microalbuminuria	569	116 (20.4)	1.18 (0.99; 1.42)
Macroalbuminuria	63	16 (25.4)	1.47 (0.96; 2.25)
Microvascular complication			
Eye	5249		
No	4705	824 (17.5)	1 (ref)
Yes	544	114 (21.0)	1.25 (1.05; 1.49)
Renal	5249		
No	5113	904 (17.7)	1 (ref)
Yes	136	34 (25.0)	1.45 (1.08; 1.95)
Macrovascular complication	5249		
No	4027	644 (16.0)	1 (ref)
Yes	1222	294 (24.1)	1.70 (1.50; 1.92)
Glucose-lowering drug use	5249		
No	789	118 (15.0)	1 (ref)
Yes	4460	820 (18.4)	1.15 (0.96; 1.38)
Insulin use†			
No	4143	735 (17.7)	1 (ref)
Yes	317	85 (26.8)	1.43 (1.18; 1.75)
Physical activity, questionnaire 2016‡	5189		
0	539	150 (27.8)	1.67 (1.43; 1.96)
1 – 2	1076	195 (18.1)	1.09 (0.94; 1.26)
≥3	3574	581 (16.9)	1 (ref)
Physical activity, change, stratified§			
Baseline, activity level = 0 days	756		
2016: no change	226	76 (33.6)	1 (ref)
2016: more active	530	118 (22.3)	0.67 (0.52; 0.85)
Baseline, activity level = 1-2 days	1035		
2016: less active	223	49 (22.0)	1.08 (0.75; 1.56)
2016: no change	218	44 (20.2)	1 (ref)
2016: more active	594	113 (19.0)	0.94 (0.69; 1.29)
Baseline, activity level ≥3 days	3396		
2016: less active	1694	282 (16.7)	1.20 (1.00; 1.46)
2016: no change	999	133 (13.3)	1 (ref)
2016: more active	703	111 (15.8)	1.14 (0.91; 1.45)
Smoking, questionnaire 2016	5231		
Never	1850	266 (14.4)	1 (ref)
Former	2361	452 (19.1)	1.43 (1.25; 1.65)
Current	1020	220 (21.6)	1.52 (1.30; 1.79)
Alcohol, questionnaire 2016‖	5.176		
<7/14	4.358	782 (17.9)	1 (ref)
≥7/14	818	134 (16.4)	0.98 (0.82; 1.16)

Abbreviations: DPN; Diabetic polyneuropathy, MNSIq; Michigan Neuropathy Screening Instrument questionnaire, DN4; Douleur Neuropathique en questions 4, aPR; adjusted prevalence ratio, CI;

confidence interval, DD2; The Danish Centre for Strategic Research in Type 2 Diabetes, hsCRP; high-sensitivity C-reactive protein, HbA1c; hemoglobin A1c.
All analyses are adjusted for age, and diabetes duration
*Normal: Albumin-creatinine ratio <30 mg/g, Microalbuminuria: Albumin-creatinine ratio 30-300 mg/g), Macroalbuminuria: Albumin-creatinine ratio 300 mg/g
†Among those who use glucose-lowering drugs
‡Days per week with minimum 30 minutes of physical activity
§Stratified according to activity level at baseline = DD2 enrollment
||Units of alcohol [women/men], which is the maximum safe amount recommended by the Danish Health Authority in 2016

Supplementary Table 5: Prevalence ratios of DPN for non-obesity risk factors, continuous data

		DPN
		MNSIq ≥4
	Total	aPR(95% CI)
Systolic blood pressure (unit = 10 mmHg)	3387	1.00 (0.95; 1.05)
Diastolic blood pressure (unit = 5 mmHg)	3387	1.02 (0.98; 1.05)
Total cholesterol (unit = 0.5 mmol/l)	2267	0.99 (0.95; 1.04)
LDL cholesterol (unit = 0.25 mmol/l)	3433	1.01 (0.99; 1.03)
HDL cholesterol (unit = 0.25 mmol/l)	2274	0.92 (0.86; 0.98)
Triglycerides (unit = 0.5 mmol/l)	3302	1.04 (1.02; 1.06)
Low-grade inflammation, hsCRP (unit = 1)	4722	1.08 (1.06; 1.11)
C-peptide (Unit = 25 pmol/l)	4353	1.01 (1.00; 1.01)
HbA1c (unit = 1%)	3536	1.06 (1.02; 1.11)
Physical activity, baseline (unit = 1 day)	5247	0.95 (0.93; 0.97)

Abbreviations: DPN; Diabetic polyneuropathy, MNSIq; Michigan Neuropathy Screening Instrument questionnaire, aPR; adjusted prevalence ratio, CI; confidence interval, hsCRP; high-sensitivity C-reactive protein, HbA1c; hemoglobin A1c.

All analyses are adjusted for age, sex, and diabetes duration.

Supplementary Table 6: Prevalence ratios of neuropathic pain occurrence (pain in both feet + DN4 ≥3) among the 938 patients with DPN defined as MNSIq ≥4: additional non-obesity risk factors

		Painful DPN	
		MNSIq ≥4 and pain in feet + DN4 ≥3	
	Total	N events (%)	aPR (95% CI)
Total, N (%)	938	386 (41.2)	
Sex	938		
Male	492	198 (40.2)	1 (ref)
Female	446	188 (42.2)	1.05 (0.90; 1.22)
Albumin/creatinine ratio, mg/g*	645		
Normal/no albuminuria	513	211 (41.1)	1 (ref)
Microalbuminuria	116	46 (39.7)	0.97 (0.75; 1.24)
Macroalbuminuria	16	7 (43.8)	1.11 (0.63; 1.96)
Microvascular complication			
Eye	938		
No	824	337 (40.9)	1 (ref)
Yes	114	49 (43.0)	1.02 (0.81; 1.29)
Renal	938		
No	904	374 (41.4)	1 (ref)
Yes	34	12 (35.3)	0.85 (0.53; 1.35)
Macrovascular complication	938		
No	644	265 (41.2)	1 (ref)
Yes	294	121 (41.2)	0.98 (0.82; 1.16)
Glucose-lowering drug use	938		
No	118	43 (36.4)	1 (ref)
Yes	820	343 (41.8)	1.15 (0.90; 1.49)
Insulin use†	820		
No	735	303 (41.2)	1 (ref)
Yes	85	40 (47.1)	1.18 (0.92; 1.53)
Physical activity, questionnaire 2016‡	926		
0	150	68 (45.3)	1.12 (0.91; 1.37)
1 - 2	195	76 (39.0)	0.96 (0.78; 1.17)
≥3	581	235 (40.5)	1 (ref)
Physical activity, change, stratified[d]			
Baseline, activity level = 0 days	194		
2016: no change	76	36 (47.4)	1 (ref)
2016: more active	118	46 (39.0)	0.83 (0.60; 1.15)
Baseline, activity level = 1-2 days	206		
2016: less active	49	20 (40.8)	1.19 (0.70; 2.02)
2016: no change	44	15 (34.1)	1 (ref)
2016: more active	113	39 (34.5)	1.01 (0.62; 1.64)
Baseline, activity level ≥3 days	526		
2016: less active	282	119 (42.2)	0.89 (0.71; 1.11)
2016: no change	133	63(47.4)	1 (ref)
2016: more active	111	41 (36.9)	0.78 (0.57; 1.05)
Smoking, questionnaire 2016	938		
Never	266	92 (34.6)	1 (ref)
Former	452	199 (44.0)	1.28 (1.05; 1.56)
Current	220	95 (43.2)	1.29 (1.03; 1.62)
Alcohol, questionnaire 2016[e]	916		
<7/14	782	320 (40.9)	1 (ref)
≥7/14	134	60 (44.8)	1.11 (0.90; 1.37)

Abbreviations: DPN; Diabetic polyneuropathy, MNSIq; Michigan Neuropathy Screening Instrument questionnaire, DN4; Douleur Neuropathique en questions 4, aPR; adjusted prevalence ratio, CI; confidence interval, hsCRP; high-sensitivity C-reactive protein, HbA1c; hemoglobin A1c.
All analyses are adjusted for age, and diabetes duration
*Normal: Albumin-creatinine ratio <30 mg/g, Microalbuminuria: Albumin-creatinine ratio 30-300 mg/g), Macroalbuminuria: Albumin-creatinine ratio 300 mg/g
†Among those who use glucose-lowering drugs
‡Days pr week with minimum 30 minutes of physical activity
§Stratified according to activity level at baseline = DD enrollment date
||Units of alcohol [women/men], which is the maximum safe amount recommended by the Danish Health Authority in 2016

Supplementary Table 7: Prevalence ratios of neuropathic pain occurrence (pain in both feet + DN4 ≥3) among the 938 patients with DPN defined as MNSIq ≥4: continuous obesity data

		Painful DPN
		MNSIq ≥4 and pain in feet + DN4 ≥3
General obesity	Total	aPR (95% CI)
BMI, age 20 years (unit = 2 kg/m^2)	816	0.97 (0.94; 1.01)
BMI, baseline (unit = 2 kg/m^2)	581	0.98 (0.95; 1.01)
BMI, questionnaire, 2016 (unit = 2 kg/m^2)	919	1.00 (0.98; 1.03)
BMI change from age 20 -> questionnaire 2016 (unit = 2 kg/m^2)	812	1.02 (0.99; 1.04)
Central obesity		
Waist circumference (unit = 5 cm)	937	1.01 (0.98; 1.03)
Waist-hip ratio (unit = 0.1 cm/cm)	937	1.09 (0.98; 1.21)
Waist-height ratio (unit = 0.1 cm/cm)	929	1.02 (0.93; 1.12)
Per 1 SD increase*		
BMI, baseline	581	0.95 (086; 1.04)
Waist circumference	581	1.01 (0.91; 1.10)
Waist-hip ratio	581	1.09 (0.97; 1.23)
Waist-height ratio	581	0.98 (0.89; 1.08)

Abbreviations: DPN; Diabetic polyneuropathy, MNSIq; Michigan Neuropathy Screening Instrument questionnaire, DN4; Douleur Neuropathique en questions 4, aPR; adjusted prevalence ratio, CI; confidence interval BMI; body mass index.

*All analyses are adjusted for age, sex, and diabetes duration[a]For the analyses with a unit of 1 SD, we restricted the population to the 581 patients that had available obesity data at time of enrollment in order to be able to compare the magnitude of the effect across obesity measures.

Supplementary Table 8: Prevalence ratios of neuropathic pain occurrence (pain in both feet + DN4 ≥3) for different metabolic risk factors measures among the 938 patients with DPN defined as MNSIq≥4, continuous data

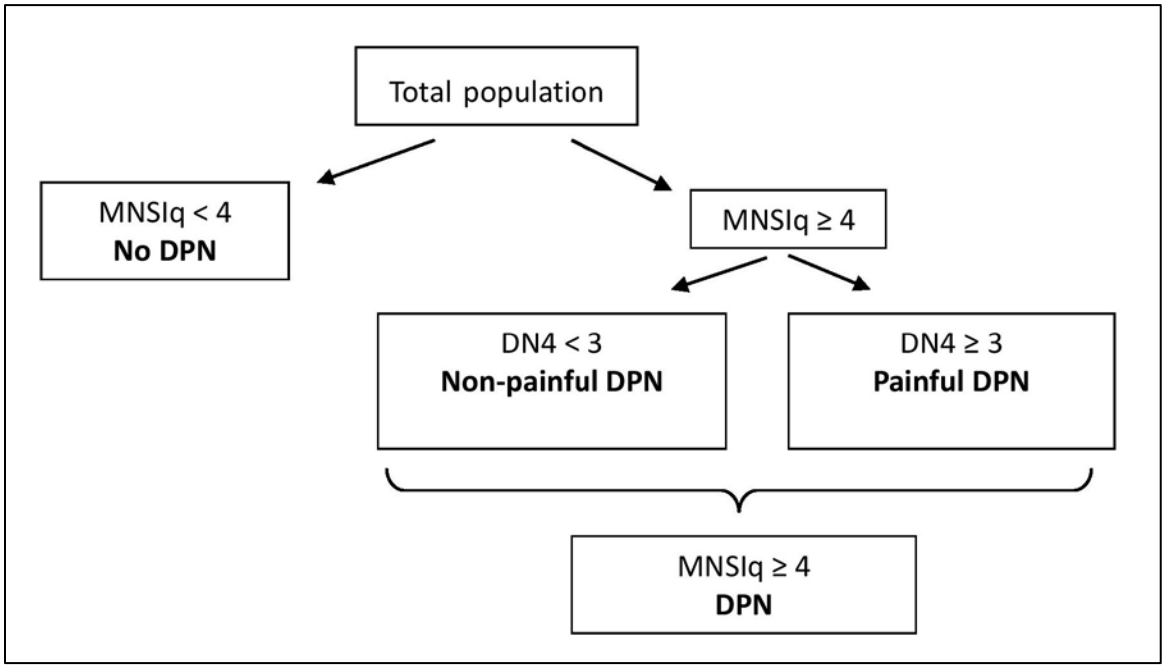
	Painful DPN	
	MNSIq ≥4 and pain in feet + DN4 ≥3	
	Total	aPR (95% CI)
Systolic blood pressure (unit = 10 mmHg)	599	1.03 (0.97; 1.09)
Diastolic blood pressure (Unit 5 mmHg)	599	0.98 (0.94; 1.03)
Total cholesterol (unit 0.5 mmol/l)	417	1.07 (1.01; 1.13)
LDL cholesterol (unit = 0.25 mmol/l)	594	1.02 (0.99; 1.04)
HDL cholesterol (unit = 0.25 mmol/l)	417	1.01 (0.93; 1.10)
Triglycerides (unit = 0.5 mmol/l)	585	1.03 (1.00; 1.06)
Low-grade inflammation, hsCRP (unit = 1)	833	1.01 (0.97; 1.04)
C-peptide (unit = 25 pmol/l?)	790	1.00 (1.00; 1.00)
HbA1c (unit = 1%)	630	1.01 (0.94; 1.08)
Physical activity, baseline	938	1.01 (0.98; 1.04)

Abbreviations: DPN; Diabetic polyneuropathy, MNSIq; Michigan Neuropathy Screening Instrument questionnaire, DN4; Douleur Neuropathique en questions 4, aPR; adjusted prevalence ratio, CI; confidence interval, hsCRP; high-sensitivity C-reactive protein, HbA1c; hemoglobin A1c.
All analyses are adjusted for age, sex, and diabetes duration.

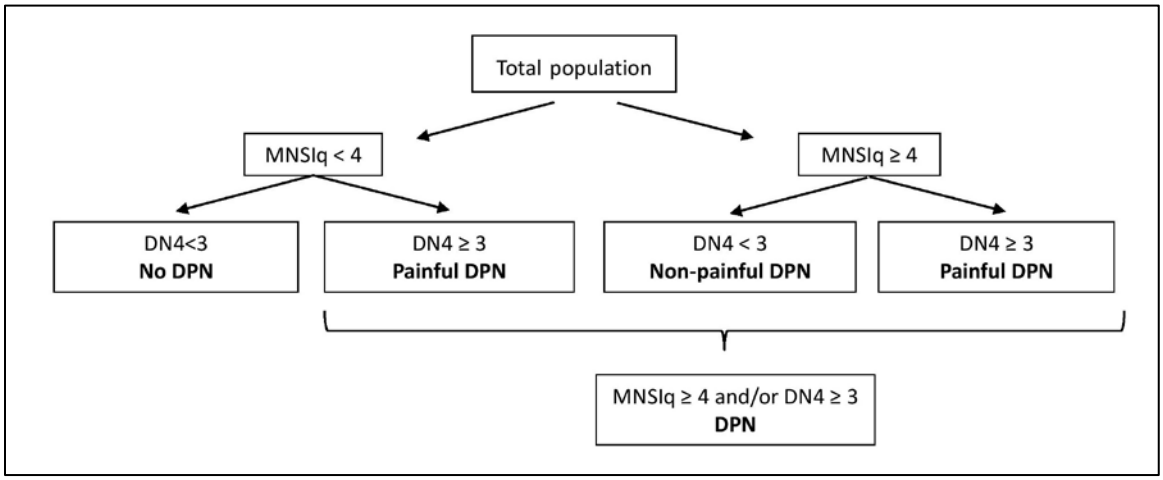
Figures:

Supplementary Figure 1, title/legend: Schematic overview of the definition of DPN and the division into non-painful DPN and painful DPN in A) main analyses and B) sensitivity analyses. Of note, the DN4 score related specifically to pain in both feet and was only filled if pain in feet were present. Thus, DN4 <3 means either no pain in feet or pain in both feet but DN4 <3.

A)

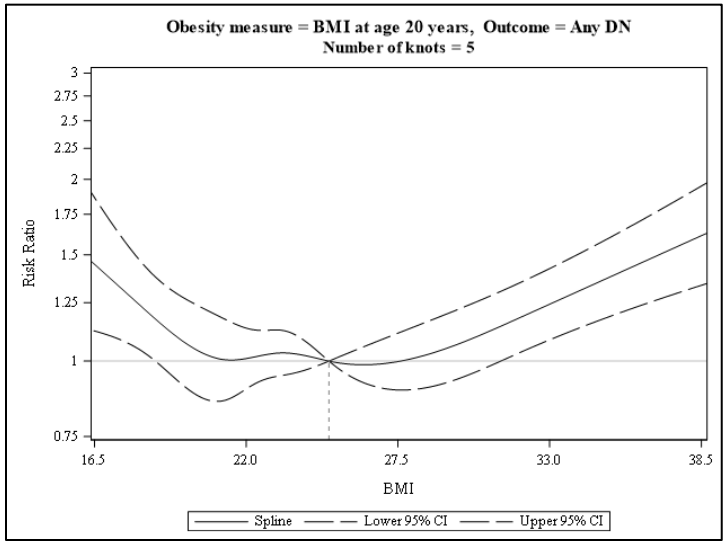


B)

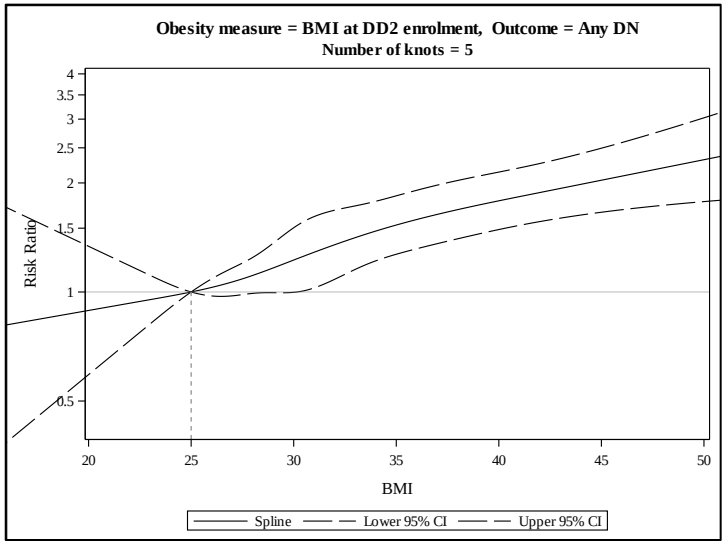


Supplemental Figure 2, title/legend: Restricted cubic spline regression of DPN for central and general obesity measures.

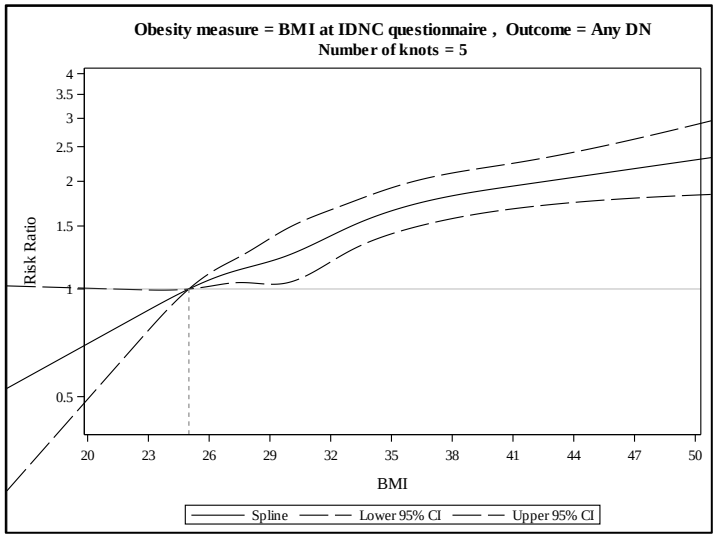
Panel A: BMI, 20 years



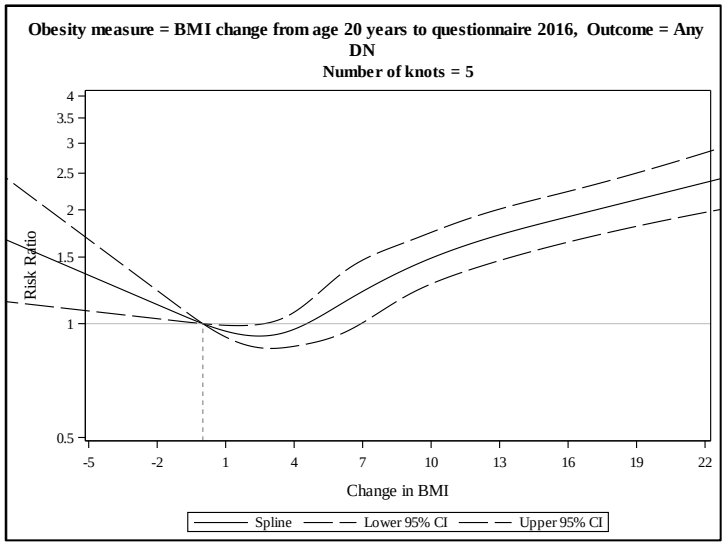
Panel B: BMI, baseline



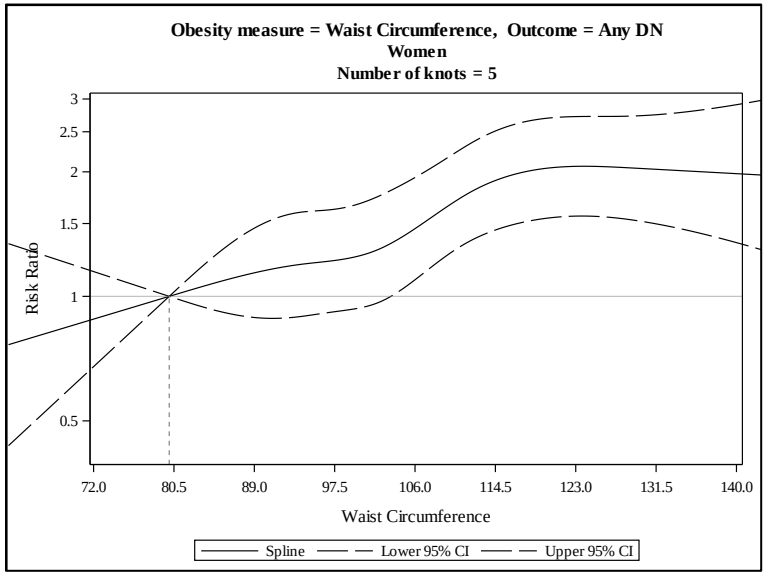
Panel C: BMI, questionnaire date



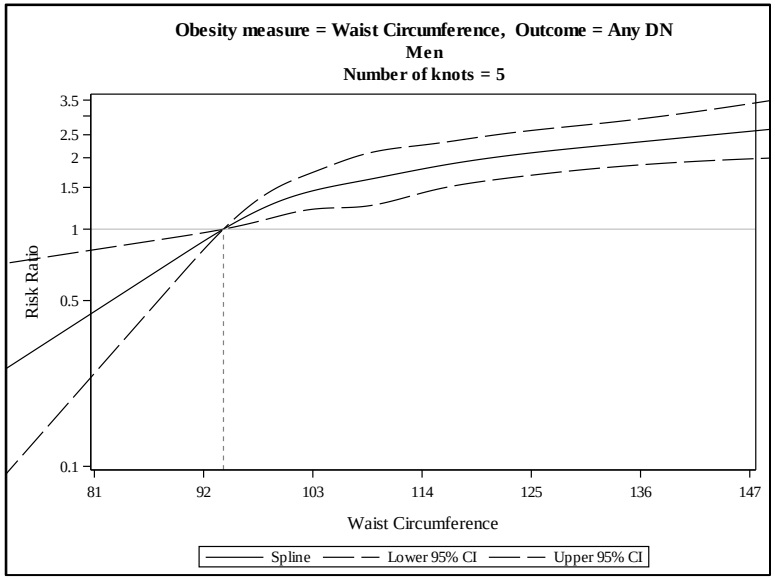
Panel D: BMI change (baseline -> questioannire)



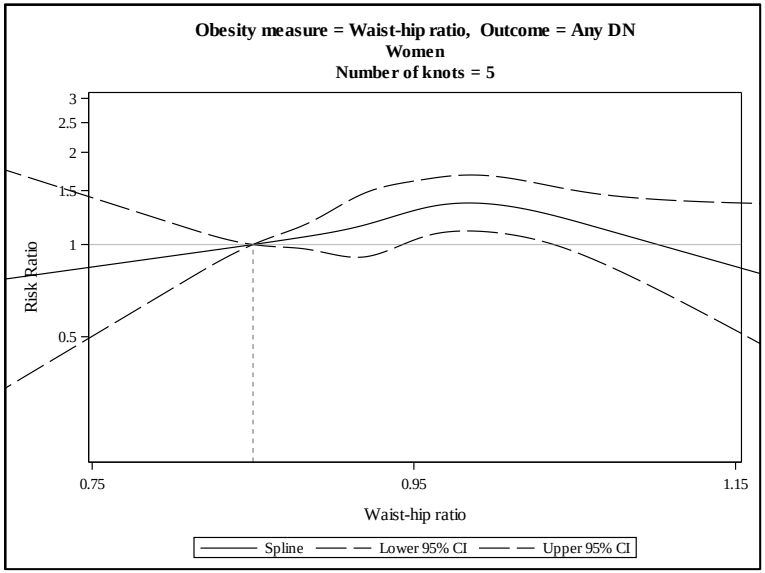
Panel E: Waist circumference, female



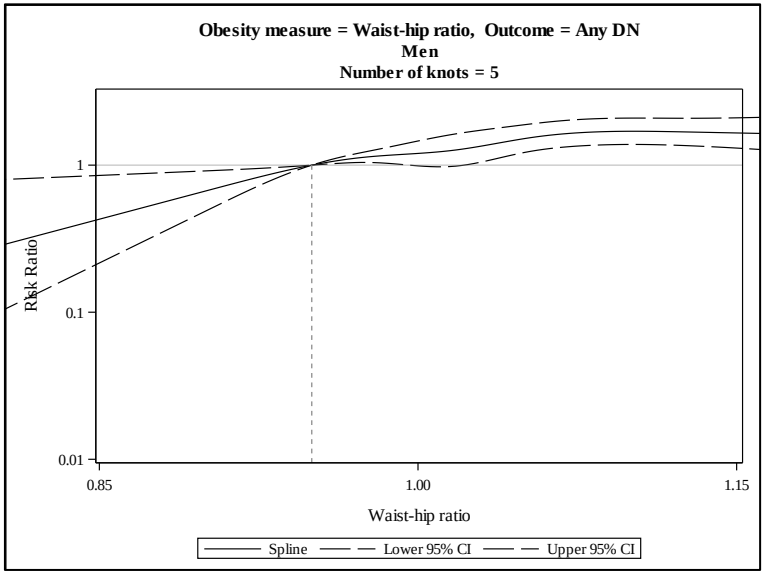
Panel F: Waist circumference, male



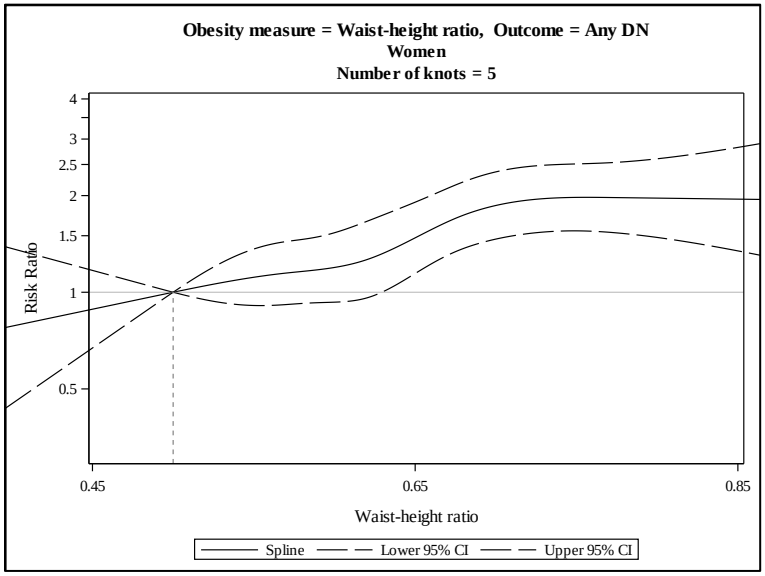
Panel G: Waist-hip ratio, female



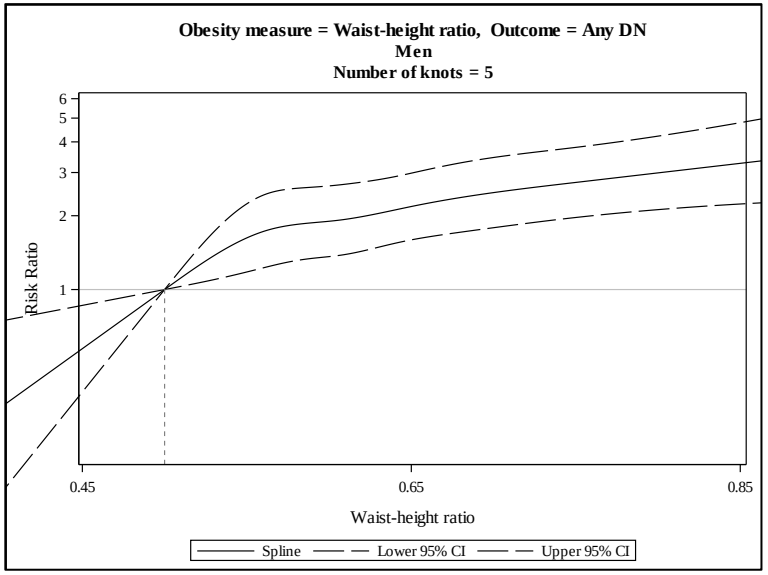
Panel H: waist-hip ratio, male



Panel I: waist-heigth ratio, female



Panel J: Waist-heigth ratio ratio, male



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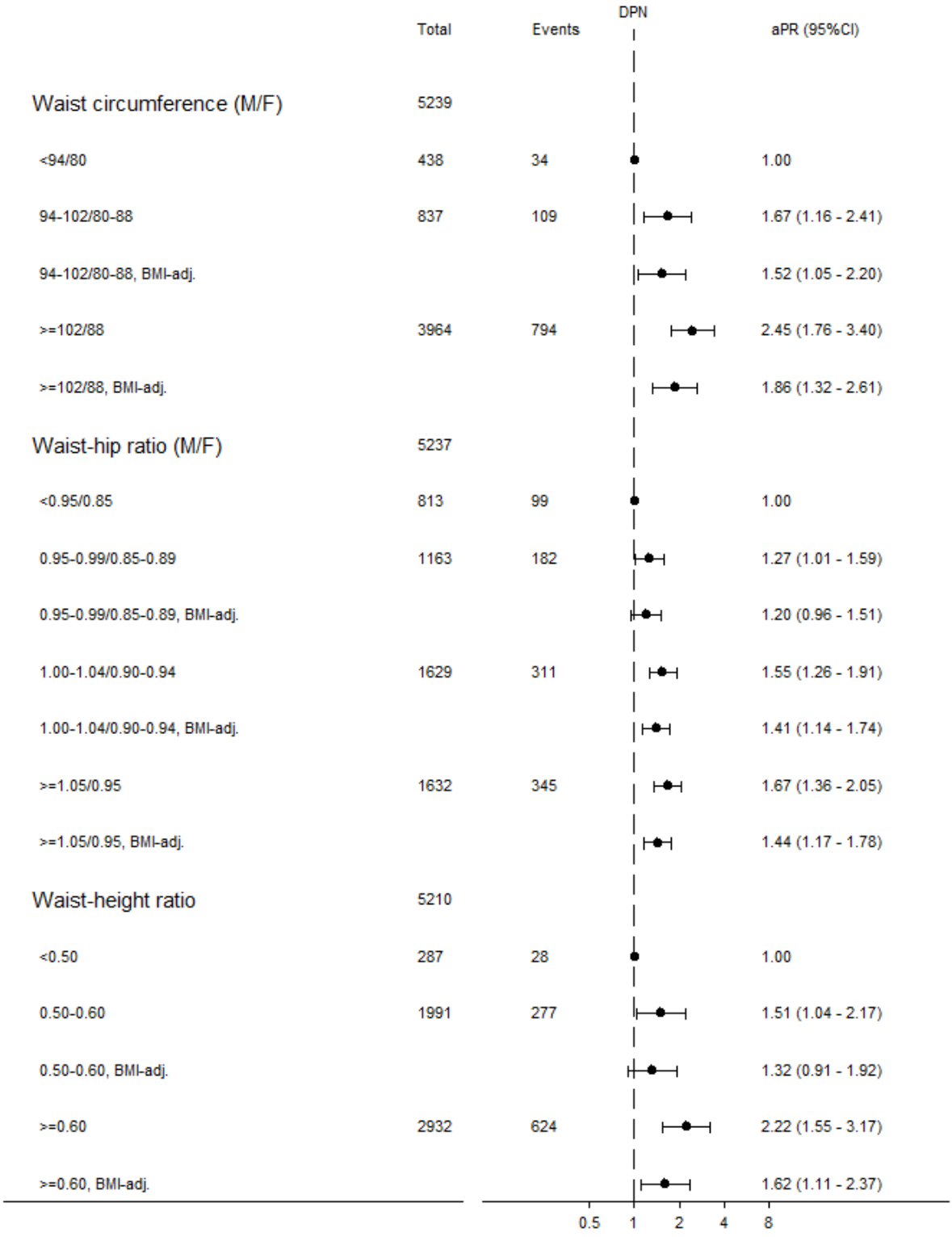
Supplementary Figure 3, title/legend: Prevalence ratios of DPN for different obesity measures, without and with additional adjustment for BMI.



Abbreviations: aPR; adjusted prevalence ratio, DPN; Diabetic polyneuropathy, CI; confidence interval; BMI; body mass index.
Of note: BMI from questionnaire 2016 is used for adjustment because this BMI measure is available for N=5159, whereas BMI at baseline = DD2 enrollment is available only for N=3263. Only a minor difference in BMI was observed between these two time points (median change: -0.4 kg/m [IQR: -1.6; 0.6]) and central obesity measures were also associated with DPN independent of BMI if adjusted for BMI at baseline instead of BMI at questionnaire.
All estimates are adjusted for age, sex, and diabetes duration.

Appendix IV

Clinical Epidemiology

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ORIGINAL RESEARCH

Can diabetic polyneuropathy and foot ulcers in patients with type 2 diabetes be accurately identified based on ICD-10 hospital diagnoses and drug prescriptions?

This article was published in the following Dove Press journal:
Clinical Epidemiology

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Purpose: We examined whether diabetic polyneuropathy (DPN) and diabetic foot ulcers in type 2 diabetes can be accurately identified using International Classification of Diseases, 10th revision discharge diagnosis codes, surgery codes, and drug prescription codes.

Methods: We identified all type 2 diabetes patients in the Central Denmark region, 2009–2016, who had ≥1 primary/secondary diagnosis code of "diabetes with neurological complication" (E10.4-E14.4), "diabetic polyneuropathy" (G63.2), or "polyneuropathy, unspecified" (G62.9). Patients with potential painful DPN and non-painful DPN were identified based on prescription history for serotonin–norepinephrine reuptake inhibitors, tricyclic antidepressants, or gabapentinoids. Likewise, type 2 diabetes patients with potential foot ulcers were identified based on diagnosis or surgery codes. We used medical record review as the reference standard and calculated positive predictive values (PPVs).

Results: Of 53 randomly selected patients with potential painful DPN, 38 were classified as having DPN when validated against medical records; of these, 18 also had neuropathic pain, yielding a PPV of 72% (95% CI: 58–83%) for DPN and 34% (95% CI: 22–48%) for painful DPN. Likewise, among 54 randomly selected patients with potential non-painful DPN, 30 had DPN based on medical record data; of these, 27 had non-painful DPN, yielding PPVs of 56% (95% CI: 41–69%) and 50% (95% CI: 36–64%), respectively. Secondary E-chapter codes often denoted stroke or mononeuropathies, rather than DPN. Excluding secondary E-chapter codes from the algorithm increased the PPV for DPN to 78% (95% CI: 63–89%) for the painful DPN cohort and to 74% (95% CI: 56–87%) for the non-painful DPN cohort. Of 53 randomly selected patients with potential diabetic foot ulcer, only 18 diagnoses were confirmed; PPV=34% (95% CI: 22–48%).

Conclusion: G-chapter and primary E-chapter diagnosis codes can detect type 2 diabetes patients with hospital-diagnosed DPN, and may be useful in epidemiological research. In contrast, our diabetic foot ulcer algorithm did not perform well.

Keywords: positive predictive value, epidemiology, registries, diabetic polyneuropathy, diabetic foot ulcer, type 2 diabetes

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Introduction

Diabetic polyneuropathy (DPN) is a common and serious diabetes complication.[1] One-fifth of patients with DPN may develop debilitating neuropathic pain.[2] Moreover, patients with DPN may suffer from a number of complications including

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diabetic foot ulcers, lower extremity amputations, and death.[1] The etiology and pathogenesis behind painful and non-painful DPN, especially in type 2 diabetes,[3] are still not fully understood, which hinders effective prevention and improved treatment of DPN.

There may be a great potential in using large medical registries and administrative databases to study risk and prognosis of DPN in type 2 diabetes, if diagnosis codes of DPN and its complications are valid. A high validity would be expected for codes of well-defined conditions like death and extremity amputations,[4–8] whereas this may not be true for DPN and diabetic foot ulcers. Only a few studies have examined the potential of using diagnosis or procedure codes to identify patients with documented painful and non-painful DPN or diabetic foot ulcer. In a US study, an algorithm for painful diabetic peripheral neuropathy consisting of International Classification of Diseases (ICD) version 9 diagnosis codes was developed and validated against medical records in a diabetes registry.[9] The authors reported a positive predictive value (PPV) of 79% of the final algorithm.[9] Another US study found a PPV >90% of the specific ICD-9 code for "polyneuropathy in diabetes" (357.2) when compared with medical records,[10] whereas a third US study validated 5 different ways to identify diabetic foot ulcers using ICD-9 diagnosis codes and Current Procedural Terminology procedure codes and found PPVs between 55% and 88%.[11] These results are all from the US exclusively and based on ICD-9 codes. To our knowledge, the potential of using ICD-10 codes together with drug prescription registries to identify patients with painful DPN, non-painful DPN, or diabetic foot ulcer has not previously been studied.

Therefore, we examined whether hospital-diagnosed DPN, including painful DPN and non-painful DPN, and diabetic foot ulcers in patients with type 2 diabetes can be accurately identified using diagnosis codes, surgery codes, and drug prescription codes in Danish registries.

Materials and methods

Design and setting

This cross-sectional validation study is based on data from Danish medical registries and was conducted in the Central Region of Denmark (N≈1.3 million inhabitants), one of the five Danish administrative regions. The Danish National Health Service provides universal tax-supported health care for the entire Danish population including free access to general practitioners and hospitals in Denmark and partial reimbursement for prescribed drugs.[12] Since 1968, the Danish Civil Registration System has assigned a unique 10-digit civil personal registration number (the CPR-number) to all Danish residents at birth or immigration.[4] The CPR-number is used in all Danish Registries and allows accurate and unambiguous individual-level linkage across the registries.[4]

Health registries

We used ICD-10 codes to identify type 2 diabetes patients with hospital-diagnosed DPN and diabetic foot ulcers in the Danish National Patient Registry (DNPR).[13] The DNPR holds information on all admissions at non-psychiatric hospitals since 1977, on non-psychiatric hospital outpatient and emergency room visits since 1995 and on all psychiatric hospital contacts (inpatient, outpatient, and emergency room) since 1995. From 1994 onwards, all diagnoses have been coded according to the ICD-10, whereas since 1996 all surgery has been coded according to the Nordic Medico-Statistical Committee classification of surgical procedures.[13] We used the National Health Service Prescription Database (NHSPD) to obtain complete information on prescriptions on glucose-lowering drugs and neuropathic pain medications in our patients.[12] The NHSPD has recorded data on redemption of reimbursed prescriptions from outpatient pharmacies since 2004. The recorded data include the amount and type of drug prescribed according to the Anatomical Therapeutic Chemical (ATC) classification system, and the date on which the drug was dispensed.[12]

Identification of the type 2 diabetes population

We defined eligible type 2 diabetes patients as those who had at least one in- or outpatient hospital discharge code of "diabetes mellitus" E10-14, "diabetic retinopathy" H36.0, "diabetes mellitus in pregnancy" O24 (excluding "gestational diabetes mellitus" O24.4), or "diabetic polyneuropathy" G63.2 at any hospital in Denmark, or at least one prescription redemption of a glucose-lowering drug, ATC-codes A10 between January 1, 1994, and July 10, 2016, N=436,402. This algorithm has previously been validated; the PPV of diagnosis codes for identifying patients with diabetes is 97% and the sensitivity 64%, whereas the PPV of the glucose-lowering drug prescription codes is 95% and sensitivity 72%.[14] To avoid inclusion of

patients treated with metformin for polycystic ovary syndrome, we did not include females aged 20–39 treated with metformin monotherapy who did not have a diabetes discharge code. We included other diabetes codes than the type 2 diabetes codes (E11), because type 1 diabetes, type 2 diabetes and other types of diabetes cannot be completely differentiated based on diagnosis codes E10-14 alone.[15] In order to minimize misclassification of patients with other types of diabetes than type 2 diabetes, we excluded patients younger than 30 years at diabetes diagnosis treated with insulin monotherapy (Table 1 and Figure 1).

Identification of DPN

Any DPN population

From the population of type 2 diabetes patients, we identified those who had an in- or outpatient hospital diagnosis code that was indicative of DPN: the potential DPN population, N=35,490. Codes indicative of DPN were "polyneuropathy, unspecified" G62.9, "diabetic polyneuropathy" G63.2, or "diabetes with neurological complication" E10.4, E11.4, E12.4, E13.4, E14.4 (excluding among the latter patients who also had a diagnosis code of G73.0 "amyotrophy", G99.0 "autonomic neuropathy", or G59.0 "mononeuropathy"). We included both primary (first-listed, ie the primary cause for the hospital contact) and secondary diagnosis codes. Only patients with a DPN code given on the same date or later than a first type 2 diabetes registration (diagnosis or prescription) were included in the DPN population.

Painful DPN population

Next, we combined the DPN-algorithm with prescription data on medications used for the treatment of neuropathic pain in order to define an algorithm to identify patients with potential painful DPN: the painful DPN population, N=6,978. A patient was considered to have painful DPN if that patient had a minimum of one prescription redemption of an anti-epileptic medicine; N03AX09, N03AX12, N03AX16, N03AG01, N03F01, N03F02, or a serotonin–norepinephrine reuptake inhibitor (SNRI)/tricyclic antidepressant (TCA); N06AX16, N06AX21, N06AA02, N06AA04, N06AA09, N06AA10, N06AA21. Prescriptions had to be redeemed within 1 year prior to and a half year after a DPN diagnosis and patients had to have no registration of a relevant exclusion diagnosis in DNPR from 1994 onwards. Exclusion diagnoses were epilepsy (G40, G41) for those with anti-epileptic medicine prescription redemption and depression/anxiety (F30-F34, F40-42, F48.8+F48.9) for those with SNRI/TCA prescription redemption. We did not include NSAIDs and opioids in our algorithm since these drugs are prescribed for a wider and more unspecific range of diseases and conditions.

Table 1 Algorithms of in- and outpatient discharge codes and prescription codes used to identify patients with painful and non-painful DPN and diabetic foot ulcer

Type 2 diabetes[a] ≥1 diabetes discharge code (E10-E14, H36.6, O24 [except O24.4], G63.2) OR ≥1 prescription of a glucose-lowering drug (ATC: A10)[b]
Potential DPN: Type 2 diabetes plus ≥1 discharge code for "diabetes with neurological complication" (E10.4, E11.4, E12.4, E13.4, E14.4)[c] OR Type 2 diabetes plus ≥1 discharge code for "diabetic polyneuropathy" (G63.2) OR Type 2 diabetes plus ≥1 discharge code for "polyneuropathy, unspecified" (G62.9)
Potential painful DPN: DPN plus ≥1 prescription code for antiepileptic drugs *minus* an epilepsy discharge code (G40+G41) OR DPN plus ≥1 prescription code for antidepressants (SNRI/TCA) *minus* a depression/anxiety discharge code (F30-F34, F40-42, F48.8 +F48.9)
Potential non-painful DPN: DPN patients that do not fulfil the criteria for painful DPN
Potential diabetic foot ulcer: Type 2 diabetes plus ≥1 discharge code for "diabetes with peripheral vascular complication" (E10.5, E11.5, E12.5, E13.5, E14.5) OR Type 2 diabetes plus ≥1 discharge code for "ulcer" (L97, L98.4, R02) OR Type 2 diabetes plus ≥1 discharge code for "osteomyelitis" (M86) OR Type 2 diabetes plus ≥1 surgery code for surgery of lower extremity (KQDA, KQDB, KQDG)

Notes: [a]All patients younger than 30 years at diagnosis treated with insulin monotherapy were excluded in order to minimize misclassification of type 1 diabetes patients. [b]Except females aged 20–39 prescribed metformin exclusively in order to minimize misclassification of patients with polycystic ovarian syndrome. [c]Excluding patients with ICD-10 codes for G73.0 amyotrophy, G99.0 autonomic neuropathy, G59.0 mononeuropathy.

Abbreviations: DPN, diabetic polyneuropathy; ICD-10, International Classification of Diseases; version 10; SNRI, serotonin–noradrenalin reuptake inhibitors; TCA, tricyclic antidepressants.

Non-painful DPN population

DPN-patients who did not fulfill the criteria for painful DPN were considered to have potential non-painful DPN,

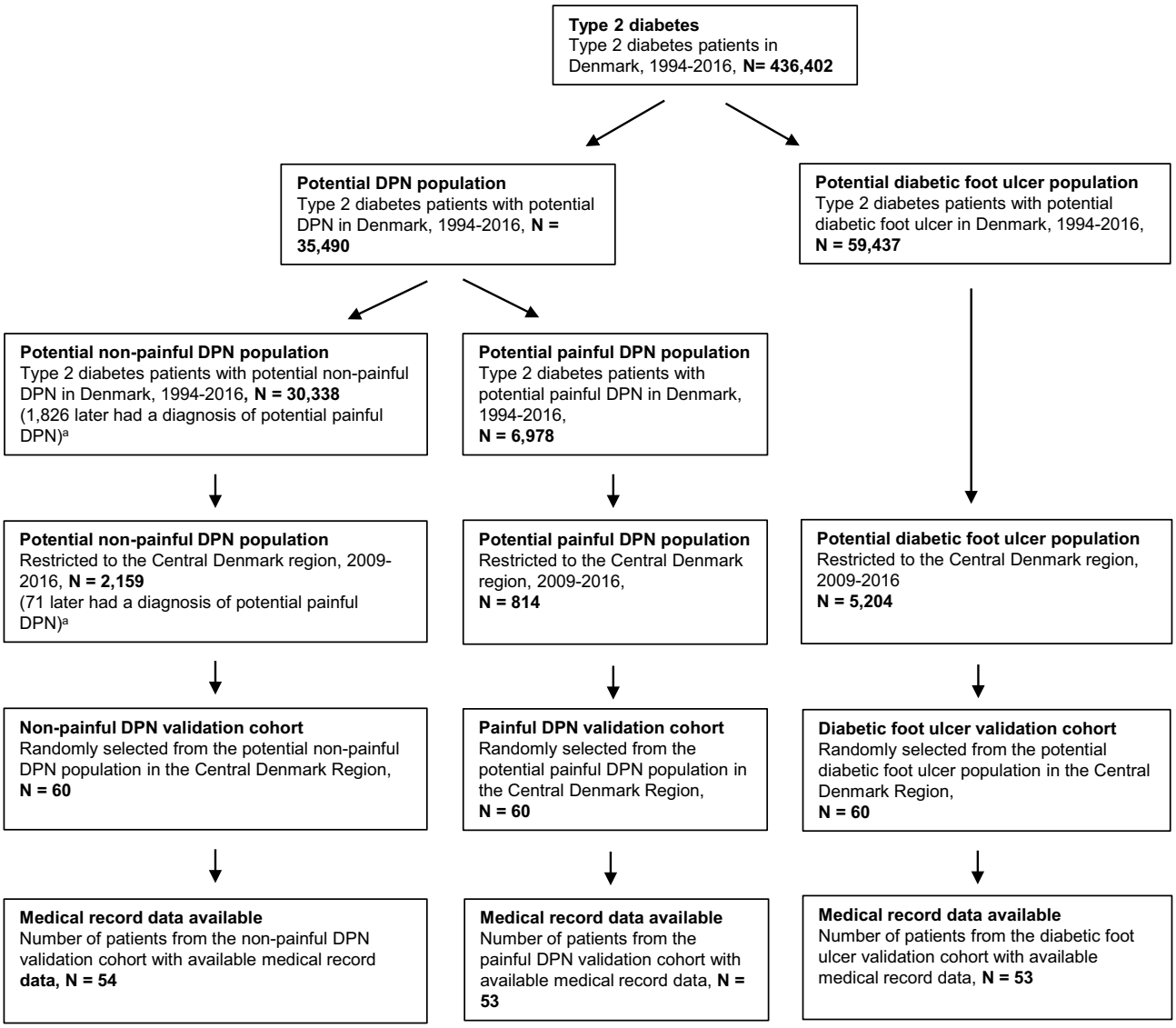

Figure 1 Flowchart of study population. Overview of patient selection. [a]Of the 30,338 patients with potential non-painful DPN, 1826 later fulfilled the criteria for potential painful DPN. Thus, these patients are included in both the non-painful and painful DPN populations at two distinct time points. Likewise, after restricting to the Central Denmark region, 2009–2016.
Abbreviation: DPN, diabetic polyneuropathy.

N=30,338. Of these, 1.826 patients fulfilled the criteria for non-painful DPN and at a later point of time fulfilled the criteria for painful DPN. Thus, they were included in both the non-painful DPN and the painful DPN population with two distinct DPN hospital contacts at two distinct time points.

Diabetic foot ulcer population

We identified all patients from the type 2 diabetes cohort who had at least one hospital diagnosis code or surgery code that was suggestive of diabetic foot ulcer. We used the following codes: "diabetes with peripheral vascular complication" E10.5-E14.5, "ulcer at lower extremity" L97, "chronic ulcer" L98.4, "gangrene" R02, "osteomyelitis" M86, "treatment of ulcer at lower extremity" KQDB, "operations for chronic ulcer/fistula at lower extremity" KQDG, "puncture, incisions and local destructions of pathological tissue in the skin at the lower extremity" KQDA, N=59,437.

The painful DPN validation cohort, the non-painful DPN validation cohort, and the diabetic foot ulcer validation cohort

Next, we restricted the painful DPN population, the non-painful DPN population and the diabetic foot ulcer population to those with a diagnosis in the Central Denmark Region between January 1, 2009 and July 10, 2016 (N=814,

N=2,159, and N=5,204, respectively). Patients who had been seen at any department of neurology/neurophysiology, mixed internal medicine, endocrinology, dermatology, vascular surgery, plastic surgery, or orthopedic surgery, at one university hospital and four regional hospitals were randomly listed in each population (not taking into account age, gender, calendar year, specific diagnosis code, etc.) and the 60 first-listed individuals in each population constituted the painful DPN validation cohort, the non-painful DPN validation cohort, and the diabetic foot ulcer validation cohort, respectively.

Medical chart review

We attained permission to access medical record data on the 180 randomly selected patients from the Danish Health and Medicine Authorities. One physician (DHC) performed the medical record reviews. All cases with an uncertain diagnosis based on the available information were discussed with a specialist physician in diabetology (STK) and diagnoses were made according to consensus among the reviewing and specialist physician. We used a predefined checklist of symptoms, signs, and diagnostic test results described in the medical record as the gold standard (see Table 2 for details). We categorized patients from the painful DPN and non-painful DPN validation cohorts as "having DPN" if they fulfilled one of the following four criteria: 1) positive nerve conduction test supporting DPN; 2) ≥one symptom of polyneuropathy in feet (including neuropathic pain), eg numbness, prickling/tingling, shooting pain, stabbing pain; 3) ≥one sign of polyneuropathy, eg abnormal vibration, abnormal light touch, abnormal pinprick; or 4) physician notes documenting presence of polyneuropathy (eg noted in the medical record: "This T2D patient who has late complications including polyneuropathy, nephropathy…"). Patients, who did not fulfill one of these criteria, were categorized as "not having DPN". Moreover, if a patient had another more likely and significant cause of polyneuropathy (eg cancer, chemotherapy treatment, sarcoidosis, hereditary, and inflammatory polyneuropathy) the patient was also classified as "not having DPN". For alcohol overuse and vitamin B12 deficiency, severity and duration were often vaguely described,[16] and the diagnosis given by the treating physician was most often DPN despite alcohol overuse/B12 deficiency description in the medical records. Thus, only if it was unequivocally stated in the medical record that polyneuropathy was caused by these conditions, the patient was categorized as "not having DPN". For all patients, it was noted whether neuropathic pain was described in the medical record.

Table 2 Descriptions of symptoms and signs in both feet, and diagnostic test results used to verify DPN in the medical records

Use of the following descriptions/terms of symptoms and signs in both feet and test results in the medical record were used to verify DPN
Numbness
Prickling/tingling
Paresthesia
Hypoesthesia
Hypalgesia
Hyperalgesia/allodynia
Dysesthesia
Self-reported insensitivity or decreased sensitivity (eg "the patient reports decreased sensitivity in her feet")
Self-reported description of inability to differentiate between warm/cold
Abnormal prick-sensation/abnormal pinprick
Abnormal temperature
Abnormal vibration
Abnormal light touch
Abnormal position
Physician described "decreased sensitivity"
Positive nerve conduction test (by physician interpretation/ conclusion)
Physician documented diabetic polyneuropathy (eg "T2D patient with known complications including diabetic polyneuropathy, retinopathy.")
Neuropathic pain (described below)
Neuropathic pain – use of the following descriptions for pain in both feet the medical record were used to verify painful DPN
Burning pain
Pins/needles or stabbing pain
Shooting pain
Squeezing pain
Prickling/tingling described painful
Other neuropathic pain (to capture less frequents used descriptions/terms)
Hyperalgesia/allodynia

Abbreviation: DPN, diabetic polyneuropathy.

We classified patients with explicitly noted "diabetic foot ulcer" in the medical record or with ≥one ulcer on toes/feet and no other pathogenesis to foot ulcer than diabetes (eg, trauma, gout) as "having diabetic foot ulcer". All other patients in the diabetic foot ulcer validation cohort were categorized as "not having diabetic foot ulcer".

Statistical analyses

Our study outcome was the PPV of the three algorithms defined as the proportion of painful DPN, non-painful

DPN and diabetic foot ulcer patients identified by the algorithms, which could be classified as having the disease when validated against the medical records. We provide 95% CIs as the exact binomial CI. For the painful DPN algorithm, we calculated both the PPV for having DPN (painful or non-painful) and the PPV for having painful DPN. Likewise, for the non-painful DPN algorithm, we calculated a PPV for having DPN (painful or non-painful) and non-painful DPN.

We stratified the PPVs according to hospital type, department type, admission type, diagnosis type, and diagnosis/surgery code. Moreover, we investigated different combinations of the diagnosis codes, eg, we separately investigated the PPV of the ICD-10 G-codes and the E-codes.

Research ethics and informed consent

This study was approved by the Danish Data Protection Agency (record number KEA-2015-13 and KEA-2015-4). Permission to access information from medical records without individually informed patient consent was granted by the Danish Health and Medicine Authorities (record number 3-3013-1479/1 and 3-3013-1479/2) in accordance with Danish law. Since this study was non-experimental and used only existing registry data, additional ethical committee approval was not required.

Results

Descriptive data

We were able to retrieve medical record data for 53 of 60 (88%) patients in the painful DPN validation cohort, 54 of 60 (90%) patients in the non-painful DPN validation cohort, and 53 of 60 (88%) patients in the diabetic foot ulcer validation cohort. Table 3 shows characteristics of the included patients. In all three cohorts most patients were diagnosed in the hospital outpatient clinic setting (painful DPN: n=44 [83%], non-painful DPN: n=41 [76%], diabetic foot ulcer: n=37 [70%]) versus inpatient setting. For both DPN validation cohorts, most patients were diagnosed in the departments of neurophysiology (painful DPN: n=22 [42%], non-painful DPN: n=18 [33%]), neurology (painful DPN: n=9 [17%], non-painful DPN: n=15 [28%]), or internal medicine (painful DPN: n=13 [25%], non-painful DPN: n=12 [22%]). In the diabetic foot ulcer validation cohort, 49% (n=26) had diagnosis codes only, 34% (n=18) had surgery codes only, and 17% (n=9) had both. The most frequent surgery code was "treatment of ulcer at lower extremity" KQDB accounting for 86% (n=24) of all surgery codes (45% of patients in the diabetic foot ulcer validation cohort), whereas "diabetes with peripheral vascular complication" E10.5-E14.5 were the most used diagnosis codes accounting for 72% (n=26) of all diagnosis codes (49% of patients in the diabetic foot ulcer validation cohort). Most patients in the diabetic foot ulcer validation cohort were diagnosed in the departments of orthopaedic surgery (n=21 [40%]), vascular surgery (n=14 [26%]), or internal medicine (n=12 [23%]).

Positive predictive values

Of the 53 patients with potential painful DPN, 38 were classified as having DPN when validated against medical record data; of these, 19 had neuropathic pain, corresponding to a PPV of 72% (95% CI: 58–83) for hospital-diagnosed DPN and 36% (95% CI: 23–50) for painful DPN (Table 4). Among the 54 patients with potential non-painful DPN, 30 had DPN when validated against the medical records; of these, 27 had non-painful DPN, corresponding to PPVs of 56% (95% CI: 41–69) for hospital-diagnosed DPN and 50% (95% CI: 36–64) for non-painful DPN, respectively. E-chapter codes, especially when listed as a secondary diagnosis (Tables S1 and S2), were often used for other neurological conditions than DPN, such as stroke and mononeuropathies, in particularly at neurological departments. Restricting the algorithm to primary and secondary G-chapter codes and primary E-chapter codes yielded a PPV of 78% (95% CI: 63–89) for DPN and 40% (95% CI: 24–54) for painful DPN in the painful DPN validation cohort (N=45) and a PPV of 74% (95% CI: 56–87) for DPN and 65% (95% CI: 46–80) for non-painful DPN in the non-painful DPN validation cohort (N=34) (Table 4). Further restricting the algorithm to only G-chapter codes increased the PPV for DPN to 86% (95% CI: 70–95) and the PPV for painful DPN to 43% (95% CI: 26–61) among those with potential painful DPN (N=35). Among those with potential non-painful DPN (N=24), the PPVs for DPN remained unchanged (DPN: PPV =71% [95% CI: 49–87], non-painful DPN: PPV 67% [96% CI: 45–85]).

Among the 53 patients with potential diabetic foot ulcer, only 18 patients had diabetic foot ulcer based on the medical record data corresponding to a PPV of 34% (95% CI: 22–48). The PPVs for E10.5-E14.5 (N=26) and KQDB (N = 24), that constituted the most frequent diagnosis and surgery codes in the diabetic foot ulcer

Table 3 Descriptive data of hospital contacts of potential painful and non-painful DPN and potential diabetic foot ulcer identified using ICD-10 hospital codes and/or surgery in the DNPR and prescription codes in the NHSPD from 2009 to 2016

	Painful DPN, N=53	Non-painful DPN, N=54	Diabetic foot ulcer, N=53
Sex			
Male	34 (64)	40 (74)	34 (64)
Female	19 (36)	14 (26)	19 (36)
Age, years			
Median (quartiles)	64 (53–69)	67 (61–74)	74 (62–83)
Hospital type			
University hospital	22 (42)	20 (37)	19 (36)
Regional hospital	31 (58)	34 (63)	34 (64)
Department type			
Internal medicine	13 (25)	12 (22)	12 (23)
Neurological	9 (17)	15 (28)	1 (2)
Neurophysiological	22 (42)	18 (33)	0 (0)
Orthopaedic surgery	5 (9)	5 (9)	21 (40)
Vascular surgery	5 (8)	4 (7)	14 (26)
Dermatological	0 (0)	0 (0)	4 (8)
Plastic surgery	0 (0)	0 (0)	1 (2)
Admission type			
Inpatient	9 (17)	13 (24)	16 (30)
Outpatient	44 (83)	41 (76)	37 (70)
Diabetic foot ulcer code type			
Diagnosis code, only	N/A	N/A	26 (49)
Surgery code, only	N/A	N/A	18 (34)
Diagnosis+surgery code	N/A	N/A	9 (17)
Diagnosis code type[a]			
Primary diagnosis code	38 (72)	28 (52)	16 (30)
Secondary diagnosis code	16 (30)	26 (48)	19 (36)
Polyneuropathy – diagnosis code[b]			
E10.4-E14.4 (diabetes with neurological complication)	19 (36)	30 (56)	N/A
E10.4	2 (4)	8 (15)	N/A
E11.4	11 (21)	15 (28)	N/A
E12.4	0 (0)	0 (0)	N/A
E13.4	2 (4)	2 (4)	N/A
E14.4	4 (8)	5 (9)	N/A
DG62.9 (polyneuropathy, unspecified)	24 (45)	19 (35)	N/A
DG63.2 (diabetic polyneuropathy)	11 (21)	5 (9)	N/A
Type of neuropathic analgesics[c]			
Antiepileptic medicine			
Gabapentin	21 (40)	0 (0)	N/A
Pregabalin	14 (26)	0 (0)	N/A
Lamotrigine	2 (4)	0 (0)	N/A
Valproic acid	1 (2)	0 (0)	N/A
Carbamazepine	2 (4)	0 (0)	N/A
Oxcarbazepine	0 (0)	0 (0)	N/A

(*Continued*)

Table 3 (Continued).

	Painful DPN, N=53	Non-painful DPN, N=54	Diabetic foot ulcer, N=53
Serotonin–noradrenalin reuptake inhibitors			
Venlafaxine	1 (2)	0 (0)	N/A
Duloxetine	0 (0)	0 (0)	N/A
Tricyclic antidepressants		0 (0)	N/A
Imipramine	2 (4)	0 (0)	N/A
Clomipramine	0 (0)	0 (0)	N/A
Amitriptyline	23 (43)	0 (0)	N/A
Nortriptyline	6 (11)	0 (0)	N/A
Maprotiline	1 (2)	0 (0)	N/A
Diabetic foot ulcer – diagnosis and surgery codes[d]			
E10.5-E14.5 (DM with peripheral vascular complication)	N/A	N/A	26 (49)
L97 (ulcer of lower limb)	N/A	N/A	4 (8)
L98.4 (chronic skin ulcer, non specified)	N/A	N/A	5 (9)
R02 (gangrene)	N/A	N/A	0 (0)
M86 (osteomyelitis)	N/A	N/A	1 (2)
KQDA (puncture, incisions and local destructions of pathological tissue in the skin at the lower extremity)	N/A	N/A	2 (4)
KQDB (treatment of ulcer at lower extremity)	N/A	N/A	24 (45)
KQDG (operations for chronic ulcer/fistula at lower extremity)	N/A	N/A	2 (4)

Notes: [a]One patient was discharged with an A- and a B-diagnosis that were both included in the polyneuropathy algorithm. Thus the percentage does not sum up to 100%. [b]One patient was discharged with 2 different codes, that were both included in the in polyneuropathy algorithm. [c]39 patients had redeemed prescriptions of one type of ATC-code, 10 patients had redeemed prescriptions of two types of ATC-codes, 3 patents had redeemed prescriptions of three types of ATC-codes and 1 had redeemed prescriptions of five different types of ATC-codes. [d]Each patient may have been given one or more diagnosis codes and/or one or more surgery codes on the diabetic foot ulcer date.

Abbreviations: DPN; Diabetic polyneuropathy, ICD-10; International classification of diseases, version 10, DNPR; Danish National Patient Register, DHSPR; Danish Health Service Prescription Register, N/A; Not applicable, e.g. diabetic foot ulcer-defining codes not relevant for polyneuropathy groups and neuropathic pain treatment codes and polyneuropathy-defining codes not relevant for diabetic foot ulcer group.

validation cohort were 46% (95% CI: 27–67) and 29% (95% CI: 13–51) (Table S3). Around half of the E10.5-E14.5 codes represented peripheral ischemia rather than ulcer, and the remaining a mixture of conditions like Charcot foot, callosities, and clavus. The KQDB procedure code was often used for ulcers above malleoli level, ulcers in relation to gout, and debridement of callosities.

Discussion

The main finding of this study was that ICD-10 diagnosis codes for "diabetic polyneuropathy" G63.2, "polyneuropathy, unspecified" G62.9, and primary diagnosis codes for "diabetes with neurological complication" E10.4-E14.4 can be used to identify type 2 diabetes patients with hospital-diagnosed DPN in health care registers, whereas the secondary E-chapter codes often represented diseases like stroke or mononeuropathies. Patients with painful versus non-painful DPN could not be accurately distinguished based on prescription redemption of neuropathic pain treatment when validated against medical records. Finally, our algorithm for diabetic foot ulcer did not perform well for identification of diabetic foot ulcer patients.

Validated against medical record data, Hartsfield et al[9] reported a PPV of 79% of an ICD-9 diagnosis code-based algorithm to identify patients with painful diabetic peripheral neuropathy (including other types of peripheral neuropathy, eg, mononeuropathies, autonomic peripheral neuropathy). In their initial algorithm, they found – like us – that prescription codes for neuropathic pain treatment did not perform well in identifying patients with painful diabetic neuropathy. There are several explanations for our low PPV for the presence of pain. First, even if a person has true neuropathic pain this may not necessarily be described in the medical record if the main reason for the hospital contact is unrelated to polyneuropathy, thus falsely underestimating the PPV for painful DPN. Second, we did not have data on possibly milder cases of treated depression/anxiety diagnosed by general practitioners. However, half of the painful DPN validation cohort patients with verified DPN and missing pain description

Table 4 Numbers and positive predictive values of potential DPN and diabetic foot ulcer

	Potential painful DPN, N=53					Potential non-painful DPN, N=54					Potential diabetic foot ulcer, N=53		
	Medical record review, conclusion			PPV (95% CI)		Medical record review, conclusion			PPV (95% CI)		Medical record review, conclusion		PPV (95% CI)
	Non-painful DPN	Painful DPN	Not DPN[a]	DPN	Painful DPN	Non-painful DPN	Painful DPN	Not DPN[a]	DPN	Non-painful DPN	Diabetic foot ulcer	Not diabetic foot ulcer	
Total	19	19	15	72 (58–83)	36 (23–50)	27	3	24	56 (41–69)	50 (36–64)	18	35	34 (22–48)
All G-codes and primary E-codes[b]	17	18	10	78 (63–89)	40 (24–54)	22	3	9	74 (56–87)	65 (47–80)	-	-	-
All G-codes	15	15	5	86 (70–95)	43 (26–61)	16	1	7	71 (49–87)	67 (45–84)	-	-	-

Notes: [a]Either not polyneuropathy or polyneuropathy is likely caused by other diseases. [b]Secondary E-codes often denoted stroke or mononeuropathies.
Abbreviations: DPN, diabetic polyneuropathy; PPV, positive predictive value.

in the medical record data were prescribed gabapentinoids. These drugs are primarily used for either hospital specialist diagnosed epilepsy (which we excluded) or neuropathic pain, suggesting that it was missing descriptions of true pain that led to falsely low PPVs.

Hoffman et al[10] evaluated the validity of different polyneuropathy codes among a general population and reported a PPV for DPN of 91% for the ICD-9 code "polyneuropathy in diabetes" 357.2 (N=105), which is similar to our result for the ICD-10 code "diabetic polyneuropathy" G63.2 (Table S1). The most frequently inaccurate coding was idiopathic polyneuropathy; 9% of validated patients coded with idiopathic polyneuropathy had diabetic polyneuropathy according to medical chart data. Likewise, another American study[17] found that diagnosis codes for idiopathic polyneuropathy were frequently used in patients with diabetic polyneuropathy. We did not include codes for idiopathic polyneuropathy in our algorithm. However, these codes were infrequently used in type 2 diabetes patients (including the codes would result in only 71 additional DPN patients, 0.2% of our total potential DPN population).

Sohn et al[11] evaluated one newly developed and four previously used diabetic foot ulcer algorithms against medical records. These algorithms varied in complexity. The algorithm most similar to ours – the Holzer algorithm defining diabetic foot ulcer by the use of at least one diagnosis or one procedure code – had a PPV of 72%, compared to our 34%. The remaining four algorithms had PPVs of 61–82%. Opposite to the algorithms validated by Sohn et al we included the frequently used "DM with peripheral vascular complication" E10.5-E14.5 codes, which also cover "Diabetes with foot ulcer" E10.5B-E14.5B. However, these codes turned out to have a low predictive value for diabetic foot ulcer, and as they had been given to half of the diabetic foot ulcer validation cohort, they diminished the overall PPV of our algorithm. A PPV of 82–89% has been reported for the ICD-9 code "ulcer of lower limbs, except decubitus" 707.1x,[6,11] corresponding to the ICD-10 diagnosis code L97 in our algorithm (our PPV: 75%, N=4). The L97 code may be valid in Danish registers as well; however, this needs to be investigated in a larger study.

A number of limitations need to be considered when interpreting our results.

First, we used medical record data as the reference standard, which may falsely lower the PPV due to incomplete information as described above. On contrary,

our criteria for verifying polyneuropathy were less stringent than those suggested by the Toronto Consensus Panels on DPN implying a risk of overestimation of the PPV.[18] Also, determination of intraepidermal nerve fiber density for the diagnosis of small-fiber polyneuropathy is not part of the everyday clinical examination for polyneuropathy and thus was not included in our criteria used to verify DPN based on the medical record data. However, since neuropathic pain in feet was a DPN verifying criteria in our study, we were also able to verify the DPN diagnosis among patients with small-fiber polyneuropathy. Second, we evaluated only the PPV and no other measures of validity, eg, sensitivity, specificity, and negative predictive value. The importance of different validity measures depends on the study question. A high PPV is important when identifying patient cohorts for studies of the prognosis of a given disease. Moreover, the PPV is a good approximation for the specificity when disease-prevalence is low, and even with low sensitivity, a high specificity will lead to unmeasured relative risks,[19] eg, in studies of DPN-risk factors. On contrary, low sensitivity may compromise studies of incidence and surveillance. Since we did not examine the sensitivity, cautious interpretation of DPN incidence and surveillance in studies based on the evaluated codes is necessary. Third, the study was conducted only in the Central Denmark region. However, the Danish health care system is uniform in its structure and practice; thus, our results are most likely generalizable to other parts of our country and countries with similar structure. Fourth, only a single reviewer evaluated most of the medical record data, and reviewers were not blinded to the registered discharge diagnosis codes, since a DPN- or diabetic foot ulcer-indicative diagnosis per definition had been given to all evaluated patients. Moreover, if discharge summaries or surgery descriptions were available (with the specific discharge diagnosis codes listed) they were included in the reviewed data. Finally, our validation sample sizes were small and a compromise between expected statistical power and practical feasibility, because we depended on health professionals at all involved departments to identify medical records for our study.

Conclusion

Our data suggest that G-chapter and primary E-chapter discharge diagnosis codes can detect patients with hospital-diagnosed DPN, and thus may be useful in epidemiological research. Our algorithm for diabetic foot ulcer did not perform well in identifying persons with diabetic foot ulcer, and a larger validation study to determine ways of identifying diabetic foot ulcers in Danish registers is warranted.

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SUPPORTING INFORMATION

Can diabetic polyneuropathy and foot ulcers in patients with type 2 diabetes be validly identified based on ICD-10 hospital diagnoses and drug prescriptions?

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Supplemental tables:

Supplemental table A1: *Numbers and positive predictive values for potential DPN stratified according to relevant covariates*

Supplemental table A2: *Positive predictive values for potential DPN for E-codes and G-codes separated, in total and stratified according to relevant covariates*

Supplemental table A3: *Numbers and positive predictive values for potential diabetic foot ulcers in total and stratified according to relevant covariates*

Table A1: Numbers and positive predictive values of potential DPN stratified according to relevant covariates

	Potential painful DPN, N=53					Potential non-painful DPN, N=54				
	Medical record review, conclusion			PPV (95% CI)		Medical record review, conclusion			PPV (95% CI)	
	Non-painful DPN	Painful DPN	Not DPN [a]	DPN	Painful DPN	Non-painful DPN	Painful DPN	Not DPN [a]	DPN	Non-painful DPN
Total	19	19	15	72 (58-83)	36 (23-50)	27	3	24	56 (41-69)	50 (36-64)
Type of hospital										
University hospital	9	11	2	91 (71-99)	50 (28-72)	9	1	10	50 (27-73)	45 (23-68)
Regional	10	8	13	58 (39-75)	26 (12-45)	18	2	14	59 (41-75)	53 (35-70)
Type of department										
Internal medicine	2	7	4	69 (39-91)	54 (25-81)	7	1	4	67 (35-90)	58 (28-85)
Neurological	2	5	2	78 (40-97)	56 (21-86)	2	0	13	13 (2-40)	13 (2-40)
Neurophysiological	12	7	4	86 (65-97)	32 (14-55)	12	1	5	72 (47-90)	67 (41-87)
Orthopaedic surgery	3	0	2	60 (15-95)	0 (0-52)*	4	1	0	100 (48-100)*	80 (28-99
Vascular surgery	0	0	4	0 (0-60)*	0 (0-60)*	2	0	2	50 (7-93)	50 (7-93)
Dermatology	0	0	0	-	-	0	0	0	-	-
Plastic surgery	0	0	0	-	-	0	0	0	-	-
Admission type										
Inpatient	0	3	6	33 (8-70)	33 (8-70)	3	0	10	23 (5-54)	23 (5-54)
Outpatient	19	16	9	80 (65-90)	36 (22-52)	24	3	13	66 (49-80)	59 (42-74)
Diagnosis type [b]										
Primary code	15	16	7	82 (66-92)	42 (26-59)	17	3	8	71 (51-87)	61 (41-78)
Secondary code	4	4	8	50 (25-75)	25 (7-52)	10	0	16	38 (20-59)	38 (20-59)
ICD-10 codes [c]										
E chapter codes										

E10.4-E14.4	4	5	10	47 (24-71)	26 (9-51)	11	2	17	43 (25-63)	37 (20-56)
E10.4	0	1	1	50 (1-99)	50 (1-99)	3	0	5	38 (9-76)	38 (9-76)
E11.4	3	3	5	55 (23-83)	27 (6-61)	4	2	9	40 (16-68)	27 (8-55)
E12.4	0	0	0	-	-	0	0	0	-	-
E13.4	1	1	0	100 (16-100)*	50 (1-99)	1	0	1	50 (1-99)	50 (1-99)
E14.4	0	0	4	0 (0-60)*	0 (0-60)*	3	0	2	60 (15-95)	60 (15-95)
G chapter codes										
G62.9 + G63.2	15	15	5	86 (70-95)	43 (26-61)	16	1	7	71 (49-87)	67 (45-84)
G62.9	8	12	4	83 (63-95)	50 (29-71)	13	0	6	68 (43-87)	68 (43-87)
G63.2	7	3	1	91 (59-100)	27 (6-61)	3	1	1	80.0 (28-99)	60 (15-95)

Notes: [a]Either not polyneuropathy or polyneuropathy is likely caused by other diseases. [b]One patient in the pDPN validation cohort was discharged with an E1x.4 and a G62.9 diagnosis on the same date. Thus, the total number sum up to 54 instead of 53 in the analyses stratified for ICD-10 diagnosis. [c]One patient in the pDPN validation cohort was discharged with an A- and a B-diagnosis that were both included in the polyneuropathy algorithm. Thus, the total number sum up to 54 instead of 53 in the analyses stratified for A/B diagnosis type, because that person is represented in both the A- and the B-diagnosis group.

*One-sided 97% confidence interval

Abbreviations: DPN; Diabetic polyneuropathy, PPV; Positive predictive value, CI; Confidence interval, ICD-10; International classification of diseases, version 10

Supplemental Table A2: Positive predictive values for potential DPN for E-codes and G-codes separated, in total and stratified according to relevant covariates

	Potential painful DPN, N=53			Potential non-painful DPN, N=54		
	N	Medical record review, PPV (95% CI)		N	Medical record review, PPV (95% CI)	
		DPN	Painful DPN		DPN	Non-painful DPN
TOTAL	**53**	**72 (58-83)**	**36 (23-50)**	**54**	**56 (41-69)**	**50.0 (36-64)**
ICD-10 code: E10.4-E14.4[1]	**19**	**47 (24-71)**	**26 (9-51)**	**30**	**43 (25-63)**	**37 (20-56)**
Type of hospital						
University hospital	4	100 (40-100)*	100 (40-100)*	7	14 (0-58)	14 (0-58)
Regional	15	33 (12-62)	7 (0-32)	23	52 (31-73)	43 (23-66)
Admission type						
Inpatient	5	20 (0-72)	20 (0-72)	12	17 (2-48)	17 (2-48)
Outpatient	14	57 (29-82)	29 (8-58)	18	61 (36-83)	50 (26-74)
Diagnosis type						
Primary code	10	50 (19-81)	30 (7-65)	10	80 (44-98)	60 (26-88)
Secondary code	9	44 (14-79)	22 (3-60)	20	25 (9-49)	25 (9-49)
ICD-10 code: G62.9 + G63.2[1]	**35**	**86 (70-95)**	**43 (26-61)**	**24**	**71 (49-87)**	**67 (45-85)**
Type of hospital						
University hospital	19	89 (67-99)	42 (20-67)	13	69 (39-90)	62 (32-86)
Regional	16	81 (54-96)	43 (19-70)	11	73 (39-94)	73 (39-94)
Admission type						
Inpatient	4	50 (7-93)	50 (7-93)	1	100 (3-100)*	100 (3-100)*
Outpatient	31	90 (74-98)	42 (24-61)	23	70 (47-87)	65 (43-84)
Diagnosis type						
Primary code	28	93 (76-99)	46 (28-66)	18	67 (41-87)	61 (36-83)
Secondary code	7	57 (18-90)	29 (4-71)	6	83 (36-100)	83 (36-100)

All G-codes and primary E-codes	**45**	**78 (63-89)**	**40 (24-54)**	**34**	**74 (56-87)**	**65 (47-80)**

[1]One patient in the painful DPN validation cohort was discharged with an E1x.4 and a G62.9 diagnosis on the same date. Thus, the total number sum up to 54 instead of 53.

*one-sided, 97.5 confidence interval

Abbreviations: DPN; Diabetic polyneuropathy, PPV; Positive predictive value, CI; Confidence interval, ICD-10; International classification of diseases, version 10

Table A3: Numbers and positive predictive values of potential diabetic foot ulcers in total and stratified according to relevant covariates

	Medical record review conclusion		PPV
	Diabetic foot ulcer	Not diabetic foot ulcer	(95% CI)
Total	18	35	34 (22-48)
Type of hospital			
University hospital	3	16	16 (3-40)
Regional	15	19	44 (27-62)
Type of department			
Internal medicine	4	8	33 (10-65)
Neurological	0	1	0 (0-97.5)*
Neurophysiological	0	0	-
Orthopaedic surgery	9	12	43 (22-66)
Vascular surgery	5	9	36 (13-65)
Dermatology	0	4	0 (0-60)*
Plastic surgery	0	1	0 (0-97.5)*
Admission type			
Inpatient	7	9	44 (20-70)
Outpatient	11	26	30 (16-47)
Code type			
Diagnosis code, only	9	17	35 (17-56)
Surgery code, only	3	15	17 (4-41)
Diagnosis + surgery code	6	3	67 (30-93)
ICD-10 and NOMESCO codes			
E10.5-E14.5	12	14	46 (27-67)
L97	3	1	75 (19-99)
L98.4	1	4	20 (0-72)
R02	-	-	-
M86	0	1	0 (0-97.5)*
KQDA	1	1	50 (1-99)
KQDB	7	17	29 (13-51)
KQDG	1	1	50 (1-99)
Diagnosis type			
Primary code	8	8	50 (25-75)
Secondary code	7	12	37 (16-62)

Notes: *one-sided, 97.5 confidence interval

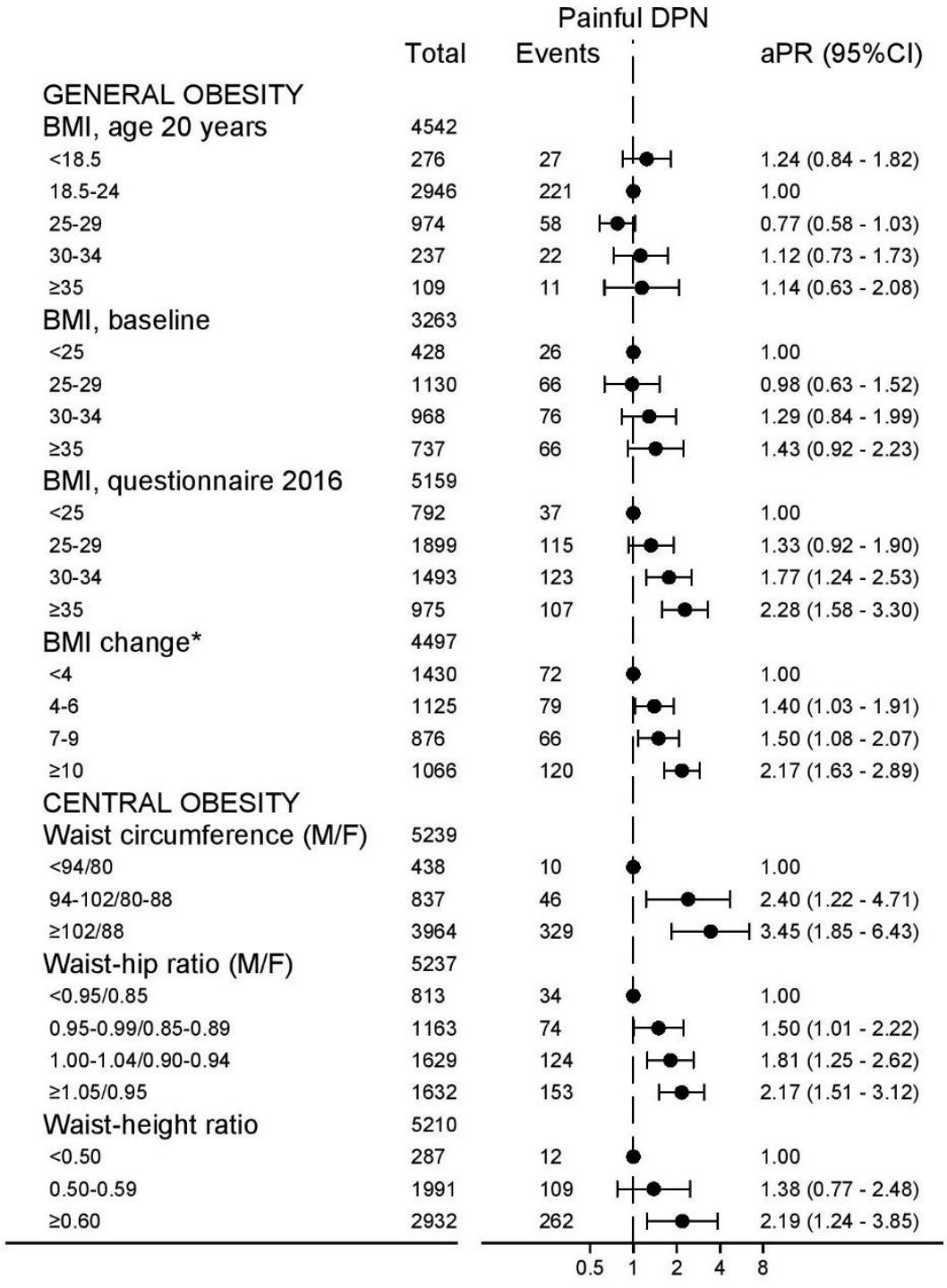
Abbreviations: PPV; Positive predictive value, CI; Confidence interval, ICD-10; International classification of diseases, version 10, ICD-10; ICD-10; International classification of diseases, version 10, NOMESCO; Nordic Medico-Statistical Committee

Appendix V

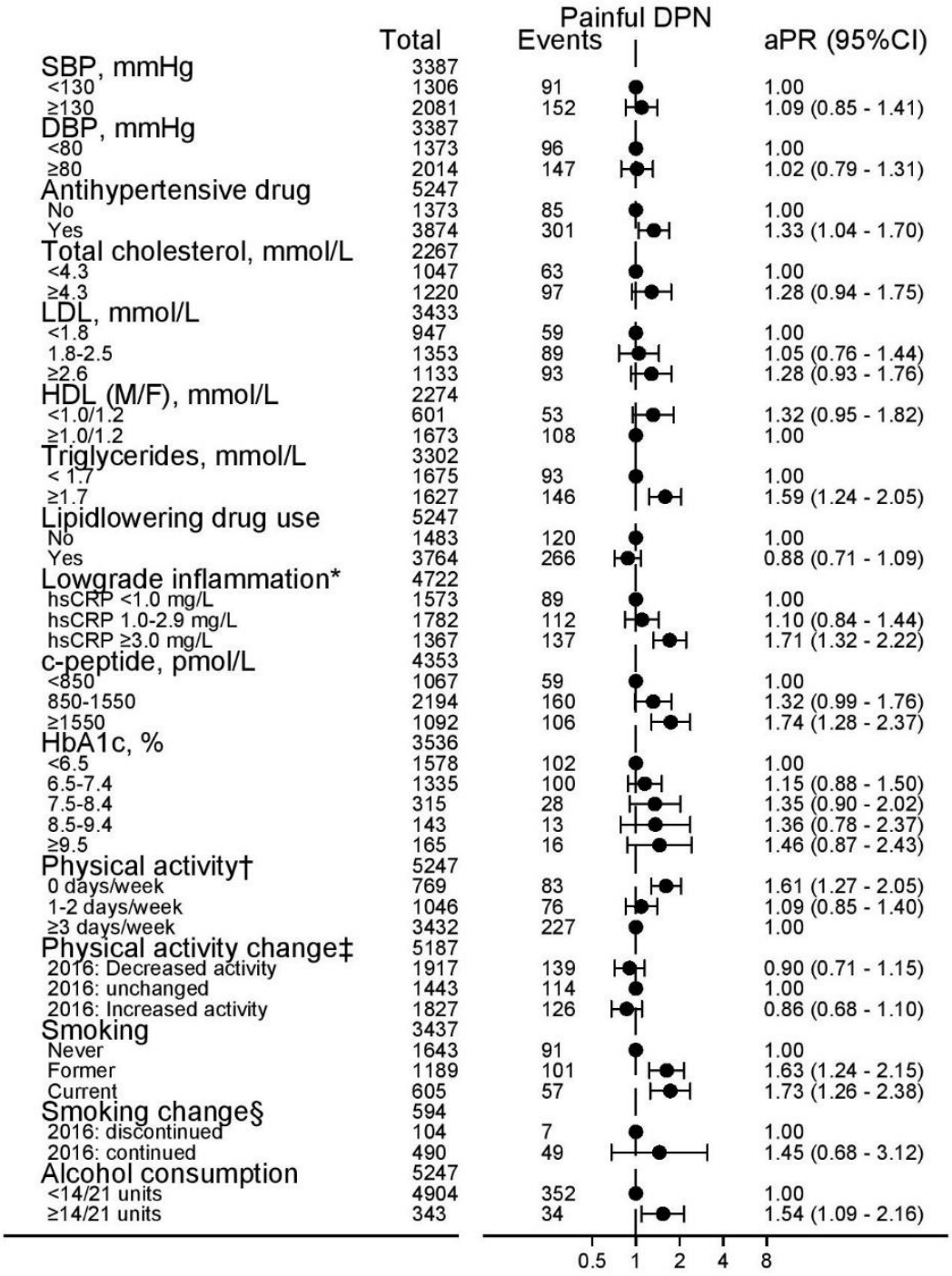
Appendix V

This figure shows the prevalence ratios of painful DPN associated with obesity (panel a) and metabolic and lifestyle factors (panel b) in the whole cohort, i.e. not internal among those with DPN. Thus, these prevalence ratios do not provide information on whether the associations are driven by DPN itself, neuropathic pain, or both conditions.

Panel a.



Panel b.


Painful DPN
Total
Events
aPR (95%CI)
SBP, mmHg 3387
<130 1306 91 1.00
≥130 2081 152 1.09 (0.85 - 1.41)
DBP, mmHg 3387
<80 1373 96 1.00
≥80 2014 147 1.02 (0.79 - 1.31)
Antihypertensive drug 5247
No 1373 85 1.00
Yes 3874 301 1.33 (1.04 - 1.70)
Total cholesterol, mmol/L 2267
<4.3 1047 63 1.00
≥4.3 1220 97 1.28 (0.94 - 1.75)
LDL, mmol/L 3433
<1.8 947 59 1.00
1.8-2.5 1353 89 1.05 (0.76 - 1.44)
≥2.6 1133 93 1.28 (0.93 - 1.76)
HDL (M/F), mmol/L 2274
<1.0/1.2 601 53 1.32 (0.95 - 1.82)
≥1.0/1.2 1673 108 1.00
Triglycerides, mmol/L 3302
< 1.7 1675 93 1.00
≥1.7 1627 146 1.59 (1.24 - 2.05)
Lipidlowering drug use 5247
No 1483 120 1.00
Yes 3764 266 0.88 (0.71 - 1.09)
Lowgrade inflammation* 4722
hsCRP <1.0 mg/L 1573 89 1.00
hsCRP 1.0-2.9 mg/L 1782 112 1.10 (0.84 - 1.44)
hsCRP ≥3.0 mg/L 1367 137 1.71 (1.32 - 2.22)
c-peptide, pmol/L 4353
<850 1067 59 1.00
850-1550 2194 160 1.32 (0.99 - 1.76)
≥1550 1092 106 1.74 (1.28 - 2.37)
HbA1c, % 3536
<6.5 1578 102 1.00
6.5-7.4 1335 100 1.15 (0.88 - 1.50)
7.5-8.4 315 28 1.35 (0.90 - 2.02)
8.5-9.4 143 13 1.36 (0.78 - 2.37)
≥9.5 165 16 1.46 (0.87 - 2.43)
Physical activity† 5247
0 days/week 769 83 1.61 (1.27 - 2.05)
1-2 days/week 1046 76 1.09 (0.85 - 1.40)
≥3 days/week 3432 227 1.00
Physical activity change‡ 5187
2016: Decreased activity 1917 139 0.90 (0.71 - 1.15)
2016: unchanged 1443 114 1.00
2016: Increased activity 1827 126 0.86 (0.68 - 1.10)
Smoking 3437
Never 1643 91 1.00
Former 1189 101 1.63 (1.24 - 2.15)
Current 605 57 1.73 (1.26 - 2.38)
Smoking change§ 594
2016: discontinued 104 7 1.00
2016: continued 490 49 1.45 (0.68 - 3.12)
Alcohol consumption 5247
<14/21 units 4904 352 1.00
≥14/21 units 343 34 1.54 (1.09 - 2.16)
0.5
1
2
4
8

Appendix VI

Appendix VI

Baseline data of those with valid data on MNSIq, pain in both feet and DN4, and of those who received a questionnaire, but either did not respond or did not provide data on MNSIq, pain in both feet and DN4.
Presented as n(%) or median (IQR).

	Patients receiving a questionnaire, N = 6.726	
TOTAL COHORT	With available DPN and neuropathic pain data, N = 5,249 (78%)	Without available DPN or neuropathic pain data, N = 1,477 (22%)
Age, questionnaire 2016	65.4 (56.6; 71.5)	61.2 (52.4; 70.1)
Female sex	2216 (42.2)	634 (42.9)
Diabetes duration, questionnaire 2016	4.6 (3.5; 5.7)	4.6 (3.4; 5.9)
BMI, age 20 years, kg/m2	23.3 (21.2; 25.6)	23.8 821.4; 26.3)
Waist circumference, cm, baseline	106 (97; 116)	107 (97; 118)
Waist-hip ratio, baseline	0.98 (0.92; 1.04)	0.98 (0.92; 1.04)
Waist-height ratio	0.61 (0.56; 0.67)	0.62 (0.56; 0.68)
Low-grade inflammation (hsCRP), mg/L	1.7 (0.8; 3.4)	1.9 (0.8; 3.8)
C-peptide, pmol/l	1149 (856; 1553)	1177 (859; 1605)
Physical activity, baseline, days/week	4 (2; 7)	3 (1; 7)
	Mean (SD): 3.82 (2.52)	Mean (SD): 3.53 (2.64)
Alcohol, baseline		
> 14/21 units/week (women/men)	343 (6.5)	103 (7.0)
Antihypertensive drug use	3874 (73.8)	1024 (69.3)
Lipid lowering drug use	3764 (71.7)	987 (66.8)
Microvascular complications		
Renal complications	136 (2.6)	53 (3.6)
Eye complication	544 (10.4)	134 (9.01)
Macrovascular diabetes complication	1222 (23.3)	359 (24.3)
Glucose-lowering drug use		
Any glucose-lowering drug	4460 (85.0)	1243 (84.2)
Non-insulin glucose-lowering drug only	4143 (78.9)	1114 (75.4)
Insulin only	53 (1.0)	22 (1.5)
Both insulin + non-insulin glucose-lowering drug	264 (5.0)	107 (7.2)
DDDA SUBCOHORT	3,623 (78%)	997 (22%)
BMI, baseline, kg/m2	30.4 (27.1; 34.5)	31.2 (27.3; 35.8)
Systolic blood pressure, mmHg	130 (124; 140)	130 (122; 140)
Diastolic blood pressure, mmHg	80 (75; 86)	80 (75; 86)
Dyslipidemia		
Total cholesterol, mmol/L	4.3 (3.7; 5.1)	4.4 (3.8; 5.2)
HDL cholesterol, mmol/L	1.2 (1.0; 1.5)	1.2 (1.0; 1.4)
LDL cholesterol, mmol/L	2.2 (1.7; 2.8)	2.4 (1.8; 3.0)
Triglycerides, mmol/L	1.6 (1.1; 2.3)	1.7 (1.2; 2.5)
Glycemic control (HbA1c), %	6.5 (6.1; 7.2)	6.7 (6.2; 7.5)
Smoking, baseline		
Never	1643 (47.8)	465 (49.3)
Former	1189 (34.6)	258 (27.3)
Current	605 (17.6)	221 (23.4)
Albumin/creatinine ratio		
Normal/no albuminuria	2991 (82.6)	796 (79.8)
Microalbuminuria	569 (15.7)	171 (17.2)
Macroalbuminuria	63 (1.7)	30 (3.0)

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Declaration of co-authorship concerning article for PhD dissertations

Full name of the PhD student: Diana Hedevang Nielsen

This declaration concerns the following article/manuscript:

Title:	Cohort Profile: The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) Project Cohort of Newly Diagnosed Type 2 Diabetes Patients
Authors:	Diana Hedevang Christensen, Sia Kromann Nicolaisen, Klára Berencsi, Henning Beck-Nielsen, Jørgen Rungby, Søren Friborg, Ivan Brandslund, Jens Sandahl Christiansen, Allan Vaag, Henrik Toft Sørensen, Jens Steen Nielsen, Reimar Wernich Thomsen

The article/manuscript is: Published x Accepted ☐ Submitted ☐ In preparation ☐

If published, state full reference: BMJ Open 2018;8(4): e017273 doi: 10.1136/bmjopen-2017-017273

If accepted or submitted, state journal:

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No x Yes ☐ If yes, give details:

The PhD student has contributed to the elements of this article/manuscript as follows:

A. Has essentially done all the work
B. Has done most of the work (67-90 %)
C. Has contributed considerably (34-66 %)
D. Has contributed (10-33 %)
E. No or little contribution
F. N/A

Element	Extent (A-F)
1. Formulation/identification of the scientific problem	C
2. Development of the method	C
3. Planning of the experiments and methodology design and development	C
4. Involvement in the experimental work/clinical studies/data collection/obtaining access to data	B
5. Development of analysis plan and preparation of data for analysis	C
6. Planning and conducting the analysis of data	C
7. Interpretation of the results	B
8. Writing of the first draft of the manuscript	A
9. Finalization of the manuscript and submission	B

Signatures of first- and last author, and main supervisor

Date	Name	Signature

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02.10.19	Diana Hedevang Christensen	
02.10.19	Reimar Wernich Thomsen	

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Declaration of co-authorship concerning article for PhD dissertations

Full name of the PhD student: Diana Hedevang Christensen

This declaration concerns the following article/manuscript:

Title:	Diabetic polyneuropathy and pain, prevalence, and patient characteristics: A cross-sectional questionnaire study of 5,514 patients with recently diagnosed type 2 diabetes.
Authors:	Sandra S. Gylfadottir,* Diana H. Christensen,* Sia K. Nicolaisen, Henning Andersen, Brian C. Callaghan, Mustapha Itani, Karolina S. Khan, Alexander G. Kristensen, Jens S. Nielsen, Søren H. Sindrup, Niels T. Andersen, Troels S. Jensen, Reimar W. Thomsen, Nanna B. Finnerup (*shared first authors)

The article/manuscript is: Published ☐ Accepted ☐ Submitted X In preparation ☐

If published, state full reference:

If accepted or submitted, state journal: Pain

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

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This declaration concerns the following article/manuscript:

Title:	Metabolic factors, lifestyle habits, and polyneuropathy in early type 2 diabetes: A nationwide study of 5,249 patients in the Danish DD2 cohort
Authors:	Diana H. Christensen, Søren T. Knudsen, Sandra S. Gylfadottir, Lotte B. Christensen, Jens S. Nielsen, Henning Beck-Nielsen, Henrik T. Sørensen, Henning Andersen, Brian C. Callaghan, Eva L. Feldman, Nanna B. Finnerup, Troels S. Jensen, Reimar W. Thomsen

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Title:	Can diabetic polyneuropathy and foot ulcers in patients with type 2 diabetes be accurately identified based on ICD-10 hospital diagnoses and drug prescriptions?
Authors:	Diana H. Christensen, Søren T. Knudsen, Sia K. Nicolaisen, Henning Andersen, Brian C. Callaghan, Nanna B. Finnerup, Troels S. Jensen, Reimar W. Thomsen

The article/manuscript is: Published X Accepted ☐ Submitted ☐ In preparation ☐

If published, state full reference: Clin Epidemiol. 2019;11:311-321. doi: 10.2147/CLEP.S197474

If accepted or submitted, state journal:

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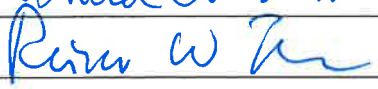
02.10.19	Reimar Wernich Thomsen	

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