# Prospective Study of Neuropathic Symptoms Preceding Clinically Diagnosed Diabetic Polyneuropathy: ADDITION-Denmark

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## OBJECTIVE

**To evaluate whether diabetic polyneuropathy (DPN) follows the hypothesis for the course of nerve fiber damage reflected by symptoms progressing from pure small through mixed to large nerve fiber symptoms with or without symptoms of loss of function of small nerve fibers.**

## RESEARCH DESIGN AND METHODS

**Repeated assessments of nerve fiber–specific symptoms were obtained in 518 participants of the ADDITION-Denmark study from the time of a screening-based diagnosis of type 2 diabetes using specific items of the Michigan Neuropathy Screening Instrument questionnaire. DPN was clinically assessed 13 years after inclusion. The course of symptoms reflecting dysfunction of specific nerve fibers was evaluated, and the association between symptoms and DPN was estimated using logistic regression models.**

## RESULTS

**An overall stable, yet heterogeneous course of symptoms was seen. According to the hypothesis of symptom progression, 205 (40%) participants remained free of symptoms and 56 (11%) had stable, 114 (23%) progressing, and 132 (26%) improving symptoms. Cross-sectional estimates showed a higher risk of DPN (odds ratios between 2.1 and 4.1) for participants with mixed or large nerve fiber symptoms with or without symptoms of loss of function of small nerve fibers compared with participants without symptoms.**

## CONCLUSIONS

**There was no evidence for a progressive development of nerve fiber damage in DPN reflected by symptoms going from pure small through mixed to large nerve fiber symptoms with or without symptoms of loss of function of small nerve fibers. Yet overall, neuropathic symptoms were prospectively associated with a higher risk of DPN.**


Diabetic polyneuropathy (DPN) is one of the most common complications of type 2 diabetes, with 10–15% of patients having signs of neuropathy already at the time of diabetes diagnosis (1–3). DPN has pivotal consequences because it is associated with a higher morbidity of affected people (1,2). Although previous studies have mainly

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focused on studying the prevalence and incidence of DPN and the risk factors associated with this condition (3,4), little is known about the natural history of DPN, including development of both painful and painless symptoms (5,6). Symptoms in painful DPN have been more extensively studied (7), and a large disparity between painful symptoms and clinical signs of DPN has been reported (5,7,8). Nevertheless, it is widely argued that the heterogeneous nerve fiber damage underlying DPN usually begins with damage of small unmyelinated nerve fibers followed by injury to small myelinated nerve fibers that ultimately leads to damage of large myelinated nerve fibers (6,9–16). This progressive one-way-street hypothesis of the course of nerve fiber damage in DPN is proposed to be reflected initially by symptoms indicative of small nerve fiber damage with subsequent additional symptoms of large nerve fiber damage and, eventually, loss of small nerve fiber–derived symptoms (i.e., pain, prickling feeling) as a result of loss of function of small nerve fibers (5,15). One conceivable explanation for this hypothesized course of nerve fiber damage is a higher vulnerability of small nerve fibers possibly explained by their lack of a myelin sheath (12,16). Moreover, small nerve fibers possess the ability to regenerate (12,13). All in all, these features of small nerve fibers might be in accordance with these nerve fibers representing early-stage DPN in contrast to the irreversible nerve fiber damage seen in large nerve fibers (12,13).

The current model for the course of DPN is mainly pieced together from cross-sectional studies of various groups of people at different stages of the trajectory of metabolic dysfunctions that lead to diabetes (e.g., people with impaired glucose tolerance, people with overt and often long-standing diabetes) (4,6,11,13). No prospective neuropathophysiological studies in larger groups of people with type 2 diabetes exist to support the hypothesis for the course of nerve fiber damage in DPN. Taken together, there is a need to clarify the course of nerve fiber damage in DPN.

In the Danish arm of the Anglo-Danish-Dutch Study of Intensive Treatment in People With Screen Detected Diabetes in Primary Care (ADDITION-Denmark), information on symptoms indicative of damage to specific nerve fiber types was obtained repeatedly from the time of diabetes diagnosis by screening and during 13 years of follow-up using the Michigan Neuropathy Screening Instrument questionnaire (MNSIq) (17). DPN was clinically diagnosed after 13 years of diabetes using the Toronto criteria for a confirmed diagnosis of DPN, which require abnormal nerve conduction and the presence of clinical signs and/or symptoms of neuropathy (18). The primary aim of this prospective study was to evaluate the course of symptoms indicative of small and large nerve fiber damage from the time of a diagnosis of type 2 diabetes by screening and during 13 years of follow-up. The second aim was to estimate the association between symptoms indicative of specific nerve fiber damage and a clinical diagnosis of DPN 13 years later.

## RESEARCH DESIGN AND METHODS

This nested case-control analysis is based on data from the subgroup of the 585 participants of the ADDITION-Denmark cohort who attended the clinical 13-year follow-up examination (19). The overall aim of ADDITION-Denmark was to evaluate the effect of intensive treatment versus routine care of diabetes on cardiovascular end points in people with screen-detected type 2 diabetes. Study inclusion took place between 2001 and 2006. A clinical 6-year follow-up examination was conducted at trial closure in 2009. Since then, participants have been followed observationally, and a clinical examination for neuropathy was conducted between 2015 and 2016 (i.e., 13 years after baseline). ADDITION and its outcomes have been previously described in detail (19–22).

Baseline characteristics, including height, BMI, systolic and diastolic blood pressure, and metabolic measures ($HbA_{1c}$; total, HDL, and LDL cholesterol; and triglycerides), were assessed according to standardized study protocols (19). Records of alcohol consumption, smoking habits, cohabiting status, and educational level were obtained from self-administered questionnaires. Cohabiting status was dichotomized into living alone or cohabiting, and the level of education was dichotomized into high educational level (degree course, higher education) or low educational level (short education, short technical education, technical education). Comorbidity was assessed by calculating a cumulative Charlson Comorbidity Index (CCI) score using Danish national registers (23). The CCI score was calculated without adjusting for age, and diabetes was excluded from the score. CCI scores were dichotomized into normal (0) or abnormal ($>0$), reflecting the absence or presence of comorbidity, respectively.

Symptoms indicative of specific nerve fiber damage were derived using six items from the MNSIq (17). According to present knowledge, we considered four specific symptoms assessed by the MNSIq indicative of small nerve fiber damage (i.e., burning pain in legs/feet, prickling feelings in legs/feet, inability to discriminate between hot water and cold water, pain when bed covers touch skin) (1,10,24–26). Three of these symptoms were considered indicative of dysfunction of small nerve fibers (i.e., the so-called positive symptoms of burning pain, prickling feelings, allodynia), and one was considered indicative of total loss of function of small nerve fibers (i.e., a so-called negative symptom of an inability to discriminate between hot and cold water) (25). Two other specific symptoms from the MNSIq were considered indicative of large nerve fiber damage (i.e., numbness in legs/feet, inability to sense feet when walking) (1,24). The full MNSIq and selected items for the categorization of symptoms into small, mixed, and large nerve fiber symptoms are shown in Supplementary Table 1.

On the basis of the outlined hypothesis for the course of nerve fiber damage in DPN, we considered an interrelated course of nerve fiber–specific symptoms from no symptoms to small-fiber symptoms only and mixed-fiber symptoms to large-fiber symptoms with or without symptoms of loss of function of small nerve fibers to reflect the likely course of DPN. Accordingly, participants were categorized into four symptom status categories on the basis of their answers to the six MNSIq items mentioned above at the three time points of symptom assessment (baseline and 6- and 13-year follow-up): no symptoms, small-fiber symptoms only (symptoms indicating small nerve fiber damage only), mixed-fiber symptoms (a combination of positive small-fiber symptoms and symptoms of large nerve fiber damage), and large-fiber symptoms with or without symptoms of loss of function of small nerve fibers

(symptoms indicating large nerve fiber damage with or without the one negative small fiber symptom). Additionally, we evaluated the change in symptom status categories of participants from baseline to the 13-year follow-up that complied with the hypothesis for the course of symptoms in DPN: stable without symptoms (no symptoms at baseline and 13-year follow-up), stable with symptoms (symptoms of the same symptom status category at baseline and 13-year follow-up), progressing symptoms (symptoms of a more progressed symptom status category at 13-year follow-up than at baseline), and improving symptoms (symptoms of a less progressed symptom status category at 13-year follow-up than at baseline).

In line with a previous study of DPN in this cohort, participants had DPN clinically diagnosed at 13-year follow-up according to the Toronto criteria for a confirmed diagnosis of DPN (i.e., abnormal nerve conduction together with the presence of clinical signs and/or symptoms of neuropathy) (18,22). Clinical signs of neuropathy were evaluated bilaterally in the feet as outlined in the physical assessment part of the MNSIq (17) and consisted of *1*) activity of ankle reflexes, with reinforcement applied if the reflex did not appear; *2*) vibration sensation at the dorsal aspect of the great toes using a 125-Hz tuning fork and the on-off method; and *3*) light-touch sensation by a 10-g monofilament on the dorsal aspect of the great toes. Signs of DPN were defined as present if at least one of these signs was decreased or absent bilaterally. Participants were interviewed about symptoms indicative of DPN in the feet and/or legs using the MNSIq (17) and the Douleur Neuropathique 4 Questions questionnaire (27). Symptoms were considered present if any of the following symptoms were reported: numbness, burning pain, prickling feeling, tingling feeling, allodynia, abnormal temperature sensation, painful cold, electric shocks, or itching. Sural nerve conduction was assessed using the automated and hand-held DPNCheck device (28). An internal validation of the DPNCheck device against conventional nerve conduction studies has been performed previously in a subset of this study population (29). The results of DPNCheck assessments were considered abnormal by bilateral values below the cutoff levels provided for the device (amplitude $\leq$4 $\mu$V and/or conduction velocity $\leq$40 m/s). Results of DPNCheck assessments were dichotomized into abnormal or normal. In accordance with the Toronto criteria for confirmed DPN, participants were defined as having DPN in the presence of abnormal DPNCheck assessments in combination with symptoms and/or symmetrical signs of DPN.

### Ethics

The study was approved by the Committee on Health Research Ethics in the Central Denmark Region (file nos. 20000183 and 1-10-72-63-15) and the Danish Data Protection Agency (file no. 2005-57-0002, ID185) and was conducted in accordance with the principles of the Declaration of Helsinki version 1996. All study participants gave written informed consent.

### Statistical Analysis

We performed a nested case-control analysis, comparing symptom status categories and the course of symptoms during 13 years between participants who developed DPN at 13-year follow-up (case subjects) and those who did not develop DPN (control subjects). Participant characteristics at baseline were reported by DPN status at 13-year follow-up. In addition, we reported participant characteristics at baseline for the initial cohort by status of participation in the clinical 13-year follow-up examination (deceased, nonassessed for DPN, and assessed for DPN). Data are represented as medians and interquartile ranges (IQRs) for continuous variables and as frequencies and proportions for categorical variables. Covariates were compared using Kruskal-Wallis and $\chi^2$ tests.

The course of DPN, as reflected by change in symptom status categories, was evaluated and illustrated for the participants who completed the relevant items of the MNSIq at all three assessments (baseline and 6- and 13-year follow-up) and were examined for DPN at the 13-year follow-up. Missing responses to the selected MNSIq items at 6-year ($n$ = 25) and 13-year ($n$ = 6) follow-up were imputed by the method of last information carried forward. The course of symptoms was evaluated by the change in symptom status categories between baseline and 13-year follow-up complying with the hypothesis for the course of symptoms in DPN and categorized into the following groups: stable without symptoms, stable with symptoms, progressing symptoms, and improving symptoms. Two separate sensitivity analyses were performed to illustrate the change in symptom status categories of case and control subjects alone.

The risk of DPN at 13-year follow-up examination by symptom status categories at baseline and 6- and 13-year follow-up was estimated using multivariable logistic regression models. A test for trend with symptom status category as a continuous variable was performed at baseline and 6- and 13-year follow-up. Risk of DPN by course of symptoms was estimated using multivariable logistic regression models. Multivariable logistic regression models were adjusted in steps for sex, age, diabetes duration, trial randomization group, presence of comorbidities, baseline educational level, and baseline cohabiting status. A sensitivity analysis was performed to estimate the risk of DPN, excluding the participants who had DPN diagnosed on the basis of symptoms and abnormal DPNCheck without clinical signs of DPN. Effect modification by sex and other covariates under study was investigated using a Wald test.

## RESULTS

Of the initial 1,533 trial participants in ADDITION-Denmark, 585 (52%) of 1,119 eligible participants attended the clinical 13-year follow-up examination. In this explorative analysis, we excluded 67 participants who did not complete assessment by the DPNCheck device ($n$ = 60) or had no assessment of signs or symptoms of neuropathy ($n$ = 7), which left 518 participants for analysis (Supplementary Fig. 1). In this study sample, median age at baseline was 60.7 years (IQR 55.5; 65.5 years), 873 (57%) participants were men, median follow-up time was 12.8 years (11.8; 13.4 years), and 150 (29%) participants were classified as having DPN (Table 1). Of the 150 cases of DPN, 20 were diagnosed on the basis of symptoms and abnormal DPNCheck, 65 from signs and symptoms together with an abnormal DPNCheck, and 65 from signs and an abnormal DPNCheck. Participants with DPN were significantly older and taller, had a higher BMI and $HbA_{1c}$, and showed a different pattern of symptom status categories, with symptoms

**Table 1—Characteristics of participants at baseline by DPN status at 13-year follow-up: ADDITION-Denmark**

| Characteristic | DPN negative | DPN positive | Number of observations |
|---|---|---|---|
| Participants | 368 (71.0) | 150 (29.0) | 518 |
| Male sex | 226 (61.4) | 106 (70.7) | 518 |
| Age (years) | 57.9 (53.6; 61.9) | 60.8 (56.1; 64.2)* | 518 |
| Randomization group (intensive) | 215 (58.4) | 93 (62.0) | 518 |
| Diabetes duration (years) | 12.7 (11.5; 13.4) | 12.9 (12.1; 13.5) | 518 |
| Symptoms | | * | 507 |
| None | 217 (60.6) | 74 (49.7) | |
| Small fiber only | 77 (21.5) | 27 (18.1) | |
| Mixed fiber | 28 (7.8) | 22 (14.8) | |
| Large fiber | 36 (10.1) | 26 (17.4) | |
| Comorbidity† | 35 (9.5) | 17 (11.3) | 518 |
| Educational level (high)‡ | 273 (77.3) | 114 (80.3) | 495 |
| Cohabiting status (cohabiting) | 293 (82.0) | 122 (81.3) | 512 |
| Height (cm) | 170.9 (164.3; 177.0) | 174.0 (167.9; 179.5)* | 505 |
| BMI (kg/m$^2$) | 29.0 (27.0; 33.0) | 31.0 (28.5; 34.5)* | 505 |
| Systolic blood pressure (mmHg) | 145.7 (134.0; 159.3) | 146.3 (134.7; 158.2) | 505 |
| Diastolic blood pressure (mmHg) | 88.3 (81.0; 95.3) | 86.3 (79.3; 93.5) | 505 |
| $HbA_{1c}$ (%) | 6.3 (6.0; 6.8) | 6.6 (6.1; 7.4)* | 499 |
| $HbA_{1c}$ (mmol/mol) | 45 (42; 51) | 49 (43; 57)* | 499 |
| Total cholesterol (mmol/L) | 5.5 (4.9; 6.4) | 5.6 (4.9; 6.4) | 481 |
| HDL cholesterol (mmol/L) | 1.4 (1.2; 1.6) | 1.3 (1.1; 1.6) | 465 |
| LDL cholesterol (mmol/L) | 3.4 (2.7; 4.0) | 3.4 (2.8; 4.0) | 449 |
| Triglycerides (mmol/L) | 1.6 (1.1; 2.2) | 1.6 (1.2; 2.3) | 470 |
| Alcohol (units/week) | 6.0 (2.0; 14.0) | 7.0 (1.5; 14.5) | 484 |
| Smoking status | | | 515 |
| Nonsmoker | 140 (38.3) | 48 (32.2) | |
| Former smoker | 126 (34.4) | 63 (42.3) | |
| Current smoker | 100 (27.3) | 38 (25.5) | |
| Treatment with lipid-lowering drugs | 54 (15.0) | 18 (12.1) | 508 |
| Treatment with aspirin | 41 (11.4) | 21 (14.1) | 508 |
| Treatment with β-blockers | 59 (16.4) | 25 (16.8) | 508 |
| Treatment with ACE inhibitors | 62 (17.3) | 31 (20.8) | 508 |

Data are *n* (%) or median (IQR) unless otherwise indicated. *$P < 0.05$. †CCI score >0. ‡High educational level corresponds to a degree course or higher education.

overall being more prevalent compared with control participants. Baseline characteristics for the initial cohort ($n$ = 1,533) by status of participation in the clinical 13-year follow-up examination (deceased, nonassessed for DPN, and assessed for DPN) are shown in Supplementary Table 2. Overall, nonassessed and deceased participants were older, shorter, had more comorbidities and a lower educational level, lived more often alone, and comprised fewer nonsmokers and more current smokers compared with the assessed participants of this study sample.

Figure 1 illustrates the flow of participants between symptom status categories from baseline to 6- and 13-year follow-up. Although the prevalence of the symptom status categories was overall stable over time, the individual symptom trajectories were heterogeneous during the 13 years of follow-up. During 13 years, 205 (40%) participants remained negative in symptoms, 56 (11%) showed a stable course of symptoms, 114 (23%) showed a progressing course of symptoms, and 132 (26%) showed an improving course of symptoms when evaluated against the proposed hypothesis for the course of symptoms in DPN. Sensitivity analyses illustrating the flow of participants between symptom status categories of case and control subjects alone showed overall a similar course of symptoms (Supplementary Figs. 2 and 3, respectively).

The risk of clinically confirmed DPN expressed by odds ratios (ORs) by symptom status categories at baseline and 6- and 13-year follow-up is summarized in Table 2. Older age was associated with a higher risk of DPN after 13 years (OR 1.06 [95% CI 1.0; 1.1] per 1 year), whereas none of the other included confounders showed a statistically significant effect on the risk of DPN. When comparing against the symptom status category of no symptoms, a higher risk of DPN was seen for mixed-fiber symptoms (3.0 [1.5; 5.9]) and large-fiber symptoms with or without symptoms of loss of function of small fibers (2.1 [1.2; 3.8]) at baseline. At 13-year follow-up, all three symptom status categories were associated with a higher risk of DPN (2.1 [1.3; 3.5], 4.1 [2.1; 8.3], and 2.5 [1.2; 5.4] for small-, mixed-, and large-fiber symptoms with or without symptoms of loss of function of small fibers, respectively) in models adjusted for sex, age, diabetes duration, and trial randomization group (model 2). Similarly, an overall higher risk of DPN was seen for other symptom status categories at other time points, yet these estimates did not reach

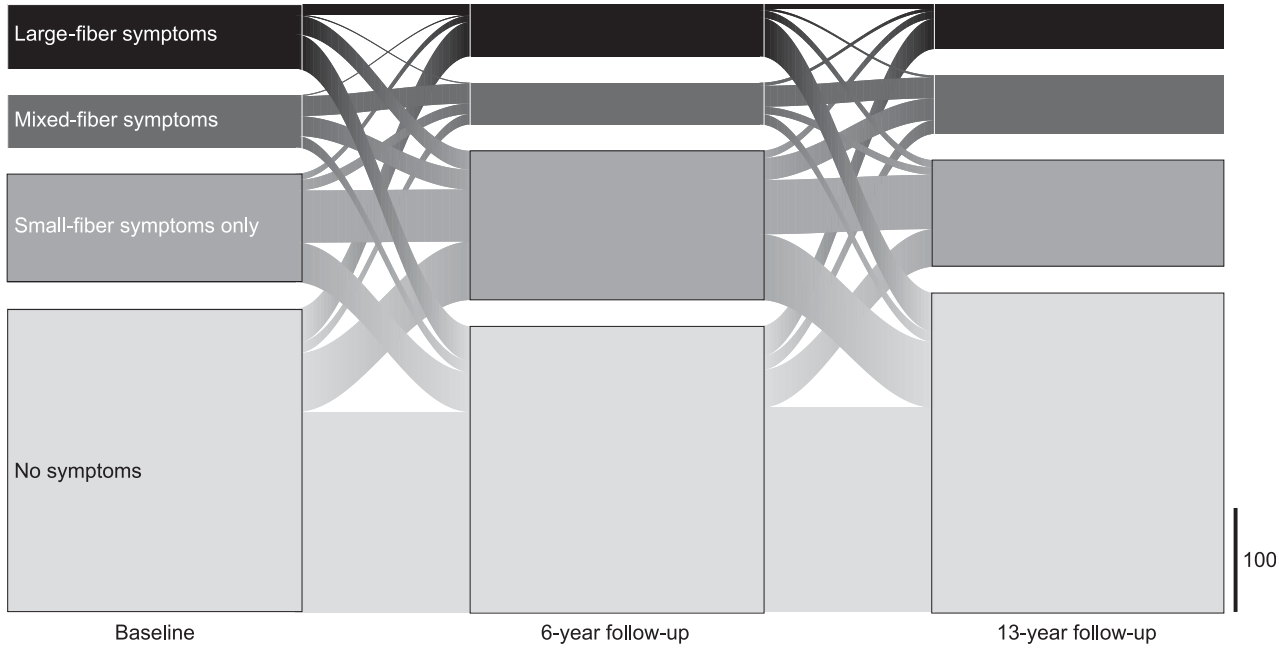


**Figure 1—**The flow of participants between symptom status categories from baseline to 6- and 13-year follow-up: ADDITION-Denmark. The chart shows the magnitude of subgroups of the total study sample ($n = 507$) and changes in symptom status categories: no symptoms, small-fiber symptoms only, mixed-fiber symptoms, and large-fiber symptoms with or without symptoms of loss of function of small fibers. The scale bar corresponds to the width of 100 participants.

statistical significance. Testing for trend with symptom status category as a continuous variable showed an elevated risk of DPN per higher level of symptom status category at baseline (1.4 [1.1; 1.6]) and 13-year follow-up (1.6 [1.2; 2.0]) (e.g., when going from no symptoms to small-fiber symptoms only). Supplementary Table 3 shows the risk of DPN excluding the participants who had DPN diagnosed on the basis of symptoms and abnormal DPNCheck without clinical signs of DPN. Similar associations between symptoms and DPN were seen compared with the results of the main analyses.

The risk of clinically confirmed DPN expressed by ORs by course of symptoms from baseline to 13-year follow-up is summarized in Table 3. A higher risk of DPN was seen for participants showing a progressing course of symptoms (OR 2.9 [95% CI 1.6; 5.0]) or an improving course of symptoms (1.8 [1.1; 3.0]) in models adjusted for sex, age, diabetes duration, and trial randomization group (model 2) compared with participants without any symptoms between baseline and 13-year follow-up. No effect modification by sex was found.

**Table 2—Risk of clinically confirmed DPN by symptom status category at baseline and 6- and 13-year follow-up: ADDITION-Denmark**

| | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | OR | 95% CI | OR | 95% CI |
| Baseline | | | | | | |
| Small-fiber symptoms only | 1.1 | 0.7; 1.9 | 1.1 | 0.7; 1.9 | 1.2 | 0.7; 2.1 |
| Mixed-fiber symptoms | 3.0* | 1.5; 5.8 | 3.0* | 1.5; 5.9 | 2.9* | 1.4; 5.8 |
| Large-fiber symptoms | 2.1* | 1.2; 3.8 | 2.1* | 1.2; 3.8 | 2.3* | 1.3; 4.3 |
| 6-year follow-up | | | | | | |
| Small-fiber symptoms only | 1.3 | 0.8; 2.0 | 1.3 | 0.8; 2.1 | 1.3 | 0.8; 2.1 |
| Mixed-fiber symptoms | 1.7 | 0.8; 3.7 | 1.7 | 0.8; 3.5 | 1.7 | 0.8; 3.9 |
| Large-fiber symptoms | 1.3 | 0.6; 2.5 | 1.2 | 0.6; 2.6 | 1.1 | 0.5; 2.5 |
| 13-year follow-up | | | | | | |
| Small-fiber symptoms only | 2.1* | 1.3; 3.5 | 2.1* | 1.3; 3.5 | 2.0* | 1.2; 3.4 |
| Mixed-fiber symptoms | 4.2* | 2.3; 7.8 | 4.1* | 2.1; 8.3 | 3.8* | 1.9; 7.9 |
| Large-fiber symptoms | 2.5* | 1.3; 5.1 | 2.5* | 1.2; 5.4 | 2.6* | 1.2; 5.4 |

The risk of DPN is expressed by multivariable logistic regression modeling per symptom status category against the symptom status category of no symptoms. Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, diabetes duration, and trial randomization group. Model 3: adjusted for sex, age, diabetes duration, trial randomization group, comorbidity, educational level, and cohabiting status. *$P < 0.05$.

## CONCLUSIONS

To our knowledge, this prospective cohort study in people with type 2 diabetes is the first to evaluate the course of symptoms indicative of specific nerve fiber damage during the course of DPN from the onset of diabetes diagnosed by screening. The findings from this study do not provide clear support for the hypothesis of the course of symptoms in DPN. However, the study showed a higher risk of clinically confirmed DPN among participants with neuropathic symptoms, with a stepwise higher risk of DPN by progressing symptom status according to the hypothesis for the course of symptoms in DPN.

The novel nature of this study prevents a direct comparison of our findings with previous reports. Our finding of an overall stable, yet heterogeneous phenotype for

**Table 3—Risk of clinically confirmed DPN by course of symptoms between baseline and 13-year follow-up: ADDITION-Denmark**

| | | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|---|
| Course of symptoms | *n* | OR | 95% CI | OR | 95% CI | OR | 95% CI |
| Stable with symptoms | 56 | 1.8* | 0.9; 3.5 | 1.7 | 0.8; 3.4 | 1.7 | 0.8; 3.4 |
| Progressing symptoms | 114 | 2.9* | 1.7; 4.9 | 2.9* | 1.6; 5.0 | 2.9* | 1.6, 5.0 |
| Improving symptoms | 132 | 1.9* | 1.2; 3.2 | 1.8* | 1.1; 3.0 | 1.9* | 1.1; 3.2 |

The risk of DPN is expressed by multivariable logistic regression modeling. ORs expressed per course of symptoms against a course of stable without symptoms ($n = 205$). Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, diabetes duration, and trial randomization group. Model 3: adjusted for sex, age, diabetes duration, trial randomization group, comorbidity, educational level, and cohabiting status. *$P < 0.05$.

the course of neuropathic symptoms during 13 years, with similar findings in sensitivity analyses of DPN case and control subjects alone, might speak against the proposed progressive one-way street for the hypothesis of the course of nerve fiber damage in DPN. There are a number of possible explanations for our findings. First, there is a risk of misclassification of participants because the selected symptoms as assessed by the MNSIq are not validated to reflect the specific nerve fiber involvements proposed in this study (17,30). In general, the classification of neuropathic symptoms as either small or large fiber derived is challenging. For example, the sensation of touch has been suggested to involve both large and small nerve fiber function (31). In this study, we considered allodynia to indicate small-fiber dysfunction, but other studies have proposed allodynia to be large fiber derived and possibly explained by abnormal activity in regenerating large nerve fibers (i.e., Aβ fibers) (25). However, post hoc analyses categorizing allodynia to indicate large-fiber dysfunction instead of small-fiber dysfunction revealed a course of symptoms and associations between symptoms and DPN similar to those in the main analyses (data not shown). In addition, a study showed that 56% of people with idiopathic small-fiber neuropathy report numbness—conventionally regarded as a large nerve fiber symptom (32). Moreover, people might interpret the various symptoms assessed by the MNSIq ambiguously (e.g., numbness might be interpreted either as a spontaneous unpleasant sensation of numbness, such as asleep numbness, or as a deficit of sensation) (8,33,34). This might lead to underreporting of numbness as a deficit of sensation and overall to an underestimation of the true presence of symptoms by the MNSIq. On the basis of present knowledge, we consider the proposed categorization of nerve fiber–specific symptoms the most optimal (1,10,24–26). In addition, the relatively long time intervals between assessments of symptoms could hinder observation of the true course of symptoms in DPN. However, DPN is regarded as a slowly progressing disease, and thus, we consider the time intervals reasonable to detect the expected course of nerve fiber–specific symptoms. Finally, the multifactorial treatment of diabetes provided in this study might have had both a protective and an improving effect on nerve fiber damage, which might explain the overall stable course of symptoms seen during 13 years (19,35). However, we did not show a different course of symptoms for DPN case subjects versus control subjects, which speaks against such a possible masking effect of treatment on the true course of symptoms in our study.

Of note, a relatively large proportion of participants had mixed- or large-fiber symptoms with or without symptoms of loss of function of small fibers present already at the screening-based diagnosis of diabetes. This may question the hypothesis for the course of nerve fiber damage. However, participants with mixed- or large-fiber symptoms may have had small-fiber symptoms before baseline not assessed in this study. In line with our findings, other studies have shown large nerve fiber involvement in early stages of DPN, such as in people with impaired glucose tolerance (13,36) and in people with diabetes but without clinically confirmed DPN (37,38). However, most of these studies demonstrated subclinical large nerve fiber damage by abnormal nerve conduction study, which is likely present at an earlier time point than clinical signs or symptoms of small or large nerve fiber damage assessed in this study. In conclusion, our findings on the course of symptoms in DPN can neither confirm nor refute the hypothesis for the progressive course of nerve fiber damage in DPN starting with small nerve fiber damage and ending with large nerve fiber damage.

This study demonstrates that the presence of neuropathic symptoms of any category is associated with a higher risk of DPN after 13 years compared with the risk of DPN in participants without symptoms. There was a trend for an elevated risk of DPN per higher level of symptom status category according to the hypothesis for the course of symptoms, which could be in accordance with the proposed hypothesis for the course of nerve fiber damage in DPN. However, the higher risk of DPN seen by mixed-fiber symptoms might be explained by a higher specificity of DPN simply by the requirement of more than one symptom indicative of DPN (39). Moreover, participants who showed any course of symptoms (i.e., stable with symptoms, progressing symptoms, improving symptoms) during 13 years had a higher risk of DPN than those who stayed free of symptoms (stable without symptoms), yet the finding for the course stable with symptoms did not reach statistical significance. This is in line with previous, mainly cross-sectional studies (39) that showed an association between neuropathic symptoms and clinically confirmed DPN, although symptoms alone have been shown to be of poor diagnostic accuracy in a cross-sectional setting. In conclusion, the results of this study might indicate a potential predictive value of symptoms for later development of DPN. Future larger prospective studies are needed to address such potential predictive value of neuropathic symptoms, which could assist in the early detection of DPN in a clinical setting.

The main strengths of our study are its relatively large size and prospective design with a relatively long follow-up of 13 years from the time of a screening-based diagnosis of type 2 diabetes. We used a robust definition of DPN (18) and studied a cohort of people at an age range around the peak for the prevalence of type 2 diabetes. Additionally, participants were followed in a real-world setting of general practice, which is

well applicable to the largest groups of people with type 2 diabetes (2).

Our study also has limitations. First, the hypothesis of symptom development may be simplistic considering previous reports of disparity between painful symptoms and clinical signs of DPN (5,7,8). The hypothesis is based on the idea that persistence of positive symptoms requires some degree of function of the involved nerve fibers (15). However, we acknowledge that the explanation for development and maintenance of painful symptoms is likely more complex and not necessarily explained by damage of specific peripheral nerve fibers (5,7,25), yet this hypothesis has been proposed in a number of recent studies, including large reviews (1,11,16). Second, as discussed above, the selected symptoms as assessed by the MNSIq have not been validated against the proposed nerve fiber damages. In addition, the assessed symptoms are not specific for DPN and could thus reflect other diseases (4,40). The reporting of symptoms also might be affected by different characteristics of the participants. We addressed this issue by further adjusting our analyses for the presence of comorbidity, educational level, and cohabiting status without finding material changes in the results. Third, we do not know the exact time of onset of DPN because no longitudinal measures of nerve fiber function are available in this study and participants were only assessed for DPN at the 13-year follow-up examination. Therefore, we cannot state to have studied symptoms preceding DPN. However, because DPN is a slowly progressing disease and because we studied people from an early stage of type 2 diabetes on the basis of detection by screening, a relatively small proportion of this cohort is expected to have had DPN at an earlier time point. Finally, our definition of DPN requires abnormal function of large nerve fibers. This might result in overlooking cases of small-fiber neuropathy (i.e., early-stage DPN according to the proposed hypothesis for the course of nerve fiber damage in DPN) or cases where large-fiber damage is present distally from the site of sural nerve assessment. This might cause underestimation of the true prevalence of DPN and the strength of associations between symptoms and clinically confirmed DPN.

The generalizability of our results to the total ADDITION-Denmark cohort is likely to be influenced by selection because of nonattendance at the clinical 13-year follow-up examination. The participants lost to follow-up were older and had more comorbidities than attenders (Supplementary Table 2). Overall, no difference in attendance was seen by the status of neuropathy symptoms at baseline, but selection bias might cause an underestimation of both the true prevalence of DPN and the estimates of association between neuropathic symptoms and DPN.

In conclusion, this study can neither confirm nor refute the hypothesis for the course of nerve fiber damage in DPN. Our results show an overall stable, yet heterogeneous phenotype for the course of neuropathic symptoms from the time of a screening-based diagnosis of type 2 diabetes to clinically confirmed DPN after 13 years. With the prospective design of this study, we provide stronger evidence for an association between neuropathic symptoms and clinically confirmed DPN. Prospective studies investigating concurrent dysfunction of small and large nerve fibers and neuropathic symptoms are needed to clarify the course of DPN.

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