RESEARCH ARTICLE

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Associations between multiple neurological biomarkers and distal sensorimotor polyneuropathy: KORA F4/FF4 study

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Abstract

Aims: The aim of this study was to assess associations between neurological biomarkers and distal sensorimotor polyneuropathy (DSPN).

Materials and Methods: Cross-sectional analyses were based on 1032 participants aged 61–82 years from the population-based KORA F4 survey, 177 of whom had DSPN at baseline. The prevalence of type 2 diabetes was 20%. Prospective analyses used data from 505 participants without DSPN at baseline, of whom 125 had developed DSPN until the KORA FF4 survey. DSPN was defined based on the examination part of the Michigan Neuropathy Screening Instrument. Serum levels of neurological biomarkers were measured using proximity extension assay technology. Associations between 88 biomarkers and prevalent or incident DSPN were estimated using Poisson regression with robust error variance and are expressed as

risk ratios (RR) and 95% CI per 1-SD increase. Results were adjusted for multiple confounders and multiple testing using the Benjamini–Hochberg procedure.

Results: Higher serum levels of CTSC (cathepsin C; RR [95% CI] 1.23 (1.08; 1.39), p_{B-H} = 0.044) and PDGFRα (platelet-derived growth factor receptor A; RR [95% CI] 1.21 (1.08; 1.35), p_{B-H} = 0.044) were associated with prevalent DSPN in the total study sample. CDH3, JAM-B, LAYN, RGMA and SCARA5 were positively associated with DSPN in the diabetes subgroup, whereas GCP5 was positively associated with DSPN in people without diabetes (all p_{B-H} for interaction <0.05). None of the biomarkers showed an association with incident DSPN (all p_{B-H}>0.05).

Conclusions: This study identified multiple novel associations between neurological biomarkers and prevalent DSPN, which may be attributable to functions of these proteins in neuroinflammation, neural development and myelination.

1 | INTRODUCTION

Distal sensorimotor polyneuropathy (DSPN) accounts for considerable morbidity, reduced quality of life and socioeconomic costs, while it is independently associated with high mortality in both people with and without diabetes mellitus.[1] Multiple cohort studies have demonstrated that DSPN cannot only occur early in people with diabetes but also frequently occurs in people without manifest diabetes in the absence of other well-established causes, in whom older age, obesity, prediabetes, dyslipidaemia and other unfavourable metabolic conditions emerged as risk factors.[2,3] Population-based approaches are needed to address this heterogeneity in the development and progression of DSPN.[2]

Previous biomarker studies in DSPN focused on biomarkers of subclinical inflammation and oxidative stress due to their suggested role in the development of the disease and analysed their associations with prevalent and incident DSPN.[4–6] Although a panel of inflammation-related biomarkers improved the prediction of DSPN in a population-based cohort,[7,8] their association with other comorbidities of diabetes might limit their clinical utility due to a lack of specificity for DSPN. More recently, we showed that serum levels of neurofilament light chain (NFL), a biomarker of neuroaxonal damage in several neurodegenerative diseases, were associated with DSPN and peripheral nerve dysfunction in middle-aged individuals with recent-onset diabetes.[9] This association was independent of multiple confounders advocating NFL as a novel biomarker for DSPN in people with diabetes. It can be hypothesised that blood biomarkers that are associated with neurological diseases might be more suitable to detect DSPN and monitor its progression and regression than systemic biomarkers reflecting more general pathomechanisms.[9,10] However, available evidence is still limited, and no previous study has investigated neurological biomarkers in older individuals both with and without diabetes.[11,12]

Therefore, the main aim of this study was to assess associations between multiple neurological biomarkers and prevalent DSPN in a population-based cohort. In exploratory analyses, differences in associations based on diabetes status were assessed. Finally, potential associations between these biomarkers and incident DSPN were investigated.

2 | STUDY POPULATION AND METHODS

2.1 | Study population

This study was based on data from the Cooperative Health Research in the Region of Augsburg (KORA) F4 (2006–2008) and the KORA FF4 surveys (2013–2014), which are the first and second follow-up examinations of the population-based KORA S4 survey (1999–2001). All KORA surveys were conducted in Augsburg and the adjacent counties Augsburg and Aichach–Friedberg in Southern Germany[7,13,14] in line with the Declaration of Helsinki.

Figure 1 provides an overview of the study sample, which is almost identical to those from previous studies on biomarkers of inflammation and oxidative stress in this cohort.[7,8,15] Out of 1161 participants of the KORA F4 survey aged 61–82 years, 46 had no neurological biomarker measurements. Additionally, 83 individuals were excluded because of missing Michigan Neuropathy Screening Instrument (MNSI) score (examination part) at F4, diabetes forms other than type 2 diabetes (type 1 diabetes, drug-induced diabetes or unclear glucose tolerance status), heavy alcohol consumption or missing covariables for statistical analysis, which left 1032 participants for the cross-sectional analysis. For the prospective analysis, we further excluded individuals with prevalent DSPN at F4 (n = 177) and those with missing data for the MNSI score at FF4, death, those
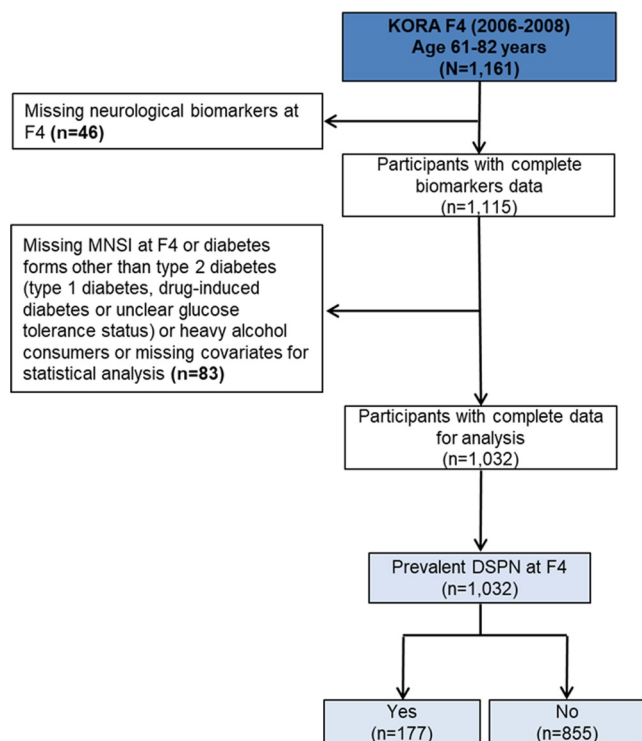
FIGURE 1 Flow chart of the study population.

who moved out of the study area, who refused or were too ill, not interested or too busy to participate, or who could not be contacted (n = 349), which left a sample of 505 individuals. The mean follow-up time was 6.5 years.

2.2 | Measurement of protein biomarkers

Circulating concentrations of protein biomarkers were measured in fasting serum samples using the Olink Target 96 Neurology assay from Olink Proteomics. The 92 biomarkers in this multiplex assay comprise a selection of proteins either associated with neurobiological processes and neurological diseases or with more general functions in cellular regulation, immunology, development and metabolism (see Table S1 for a complete list of biomarkers, UniProt IDs and Olink IDs). Therefore, the term 'neurological biomarkers' in this manuscript refers to the analytes from this panel although some of them may also be considered biomarkers reflecting more general pathways beyond neurology.

The multiplex assay uses proximity extension assay technology[16] and provides a relative quantification of analytes which are given as normalised protein expression (NPX) units and are comparable in their distribution to log2-transformed protein concentrations. Table S1 also lists the limits of detection, intra-assay coefficients of variation (CV) and inter-assay CVs. Intra- and inter-assay CVs were calculated based on three control sera measured in duplicates on each plate (n = 14). Four analytes were excluded because ≥25% of the samples yielded NPX values below the limit of detection. The biomarker SCARB2 had 127 missing values mainly for technical reasons. As described before[17]

we had defined threshold levels of 20% for intra- and inter-assay CVs as a priori criteria for exclusion of analytes but further exclusions were not necessary because intra- and inter-assay CVs ranged between 0.6%–11.1% and 0.7%–11.7%, respectively.

2.3 | Assessment of DSPN and covariates

In both KORA F4 and FF4 surveys, the clinical examination comprised all items of the MNSI, that is, appearance of feet, foot ulceration, ankle reflexes and vibration perception threshold at the great toes. Vibration perception was examined using the Rydel–Seiffer graduated C 64 Hz tuning fork.[18] Normal vibration perception threshold accounted for age-dependent threshold values.[19] The MNSI score included the bilateral assessment of touch/pressure sensation using a 10-g monofilament (Neuropen).[20] Thus, the range of the total MNSI score was from 0 to 10. DSPN was defined based on a cut-off value of >3 points.[7,8] This definition of DSPN is in line with the diagnostic criteria for possible DSPN as described by the Toronto Diabetic Neuropathy Expert Group.[21]

Assessment of anthropometric, demographic, clinical and metabolic variables, lifestyle factors and glucose tolerance status using standard 75-g oral glucose tolerance tests has been described in detail before.[7,14]

2.4 | Statistical analysis

Baseline characteristics of study participants are given as mean ± SD for continuous variables and frequency (%) for categorical variables. Differences between groups were compared using the t-test and χ^2 test, respectively.

Correlations between neurological biomarkers were assessed using Pearson correlation coefficients (r). A Gaussian graphical model was calculated to illustrate the conditional dependence structure between all neurological biomarkers.[22] Each edge in the Gaussian graphical model represents the partial correlation between two biomarkers corrected for all remaining biomarkers in the model.

Associations between neurological biomarkers and prevalent or incident DSPN were estimated per 1-standard deviation increase using Poisson regression with robust error variance in models of increasing complexity (separate models for each biomarker) in line with previous analyses of inflammation-related biomarkers and DSPN in the same cohort.[8] Model 1 was adjusted for age (years) and sex (male/female). Model 2 was additionally adjusted for waist circumference (cm), height (cm), hypertension (yes/no), total cholesterol (mmol/L), HbA1c (mmol/mol or %), alcohol consumption (none/moderate/high), smoking (never/ex/current), physical activity (active/inactive), use of lipid-lowering drugs (yes/no), use of non-steroidal anti-inflammatory drugs (NSAIDs) (yes/no), estimated glomerular filtration rate (eGFR; mL/min per 1.73 m^2), prevalent myocardial infarction (yes/no) and prevalent stroke (yes/no).

Results were expressed as risk ratios (RRs) and 95% confidence intervals (95% CIs). A vulcano plot was used to visualise the results. Effect modification by type 2 diabetes status (yes/no) was assessed using interaction terms.

All statistical analyses were conducted with SAS version 9.4 (SAS Institute). p values < 0.05 were considered to indicate nominal statistical significance. The Benjamini–Hochberg (B–H) procedure was used to adjust for multiple testing. The visualisation was carried out with RStudio version 4.0.5 (https://posit.co/download/rstudio-desktop).

3 | RESULTS

3.1 | Study population for the cross-sectional analysis

As described before,[7,8,15] people with DSPN in the KORA F4 survey were older, more likely to be male and had higher body mass index (BMI), waist circumference and height. In addition, they were characterised by a higher HbA1c level, higher prevalence of type 2 diabetes, lower kidney function, lower cholesterol levels, higher alcohol consumption, lower level of physical activity, more frequent history of stroke and more frequent use of non-steroidal anti-inflammatory drugs than people without DSPN (Table 1).

As presented in Table S2, serum levels of 46 neurological biomarkers were higher in people with DSPN than in people without DSPN, whereas only one neurological biomarker showed higher serum levels in people without DSPN than in those with DSPN (all $p < 0.05$). Most of the biomarkers showed positive correlations with each other (see correlation matrix in Figure S1), which is also reflected in a dense network of partial correlations between pairs of biomarkers corrected for all remaining biomarkers in the Gaussian graphical model (Figure S2).

3.2 | Associations between neurological biomarkers and prevalent DSPN in the total study sample

In model 1 (adjusted for age and sex), 18 biomarkers were positively and one biomarker was inversely associated with prevalent DSPN. After adjustment for multiple testing, positive associations remained significant ($p_{B-H}<0.05$) for CPM (carboxypeptidase M), CTSC (cathepsin C), EDA2R (tumour necrosis factor receptor superfamily member 27, also known as ectodysplasin A2 receptor) and Siglec-9 (sialic acid-binding Ig-like lectin 9) (Table S3).

In model 2 (fully adjusted), effect sizes were attenuated for some of the biomarkers resulting in 11 positive and one inverse associations at $p < 0.05$. After adjustment for multiple testing, CTSC (RR [95% CI] 1.23 [1.08, 1.39], $p_{B-H} = 0.044$) and PDGFRα

TABLE 1 Baseline characteristics of the study population according to prevalent DSPN status.

Characteristic	Prevalent DSPN ($n = 177$, 17%)	No prevalent DSPN ($n = 855$, 83%)	p
Age, years	72.7 ± 5.2	69.7 ± 5.2	**<0.0001**
Sex (females), %	39.6	51.1	**0.005**
BMI, kg/m^2	30.2 ± 5.3	28.4 ± 4.2	**<0.0001**
Waist circumference, cm	103.9 ± 13.1	97.0 ± 11.7	**<0.0001**
Height, cm	168 ± 9	165 ± 9	**0.0002**
HbA1c, mmol/mol	42 ± 9	39 ± 7	**0.0008**
HbA1c, %	6.0 ± 0.8	5.7 ± 0.6	**0.0007**
Glucose tolerance status, %			**0.0005**
NGT	45.2	54.8	
Prediabetes	23.7	27.1	
T2D	31.1	18.1	
eGFR, mL/min per 1.73 m^2	72.1 ± 15.8	76.9 ± 14.2	**0.0003**
Total cholesterol, mmol/L	5.46 ± 0.98	5.76 ± 1.04	**0.0005**
LDL cholesterol, mmol/L	3.42 ± 0.86	3.64 ± 0.92	**0.003**
HDL cholesterol, mmol/L	1.38 ± 0.31	1.45 ± 0.37	**0.009**
Triacylglycerols, mmol/L	1.45 ± 0.78	1.52 ± 0.93	0.284
Smoking, %			0.297
Never	44.6	49.8	
Former	49.2	42.8	
Current	6.2	7.4	
Alcohol consumption, %			**0.003**
None	34.5	32.2	
Moderate	48.6	58.7	
High	16.9	9.1	
Physically active, %	42.9	52.4	**0.022**
Hypertension, %	63.8	61.0	0.487
Myocardial infarction, %	9.0	5.4	0.062
Stroke, %	7.3	3.2	**0.009**
Use of NSAIDs, %	7.3	3.5	**0.020**
Use of lipid-lowering drugs, %	28.8	24.2	0.198

Note: Data are given as mean ± SD or percentages.

Abbreviations: BMI, body mass index; DSPN, distal sensorimotor polyneuropathy; eGFR, estimated glomerular filtration rate; NGT, normal glucose tolerance; NSAIDs, non-steroidal anti-inflammatory drugs; T2D, type 2 diabetes.

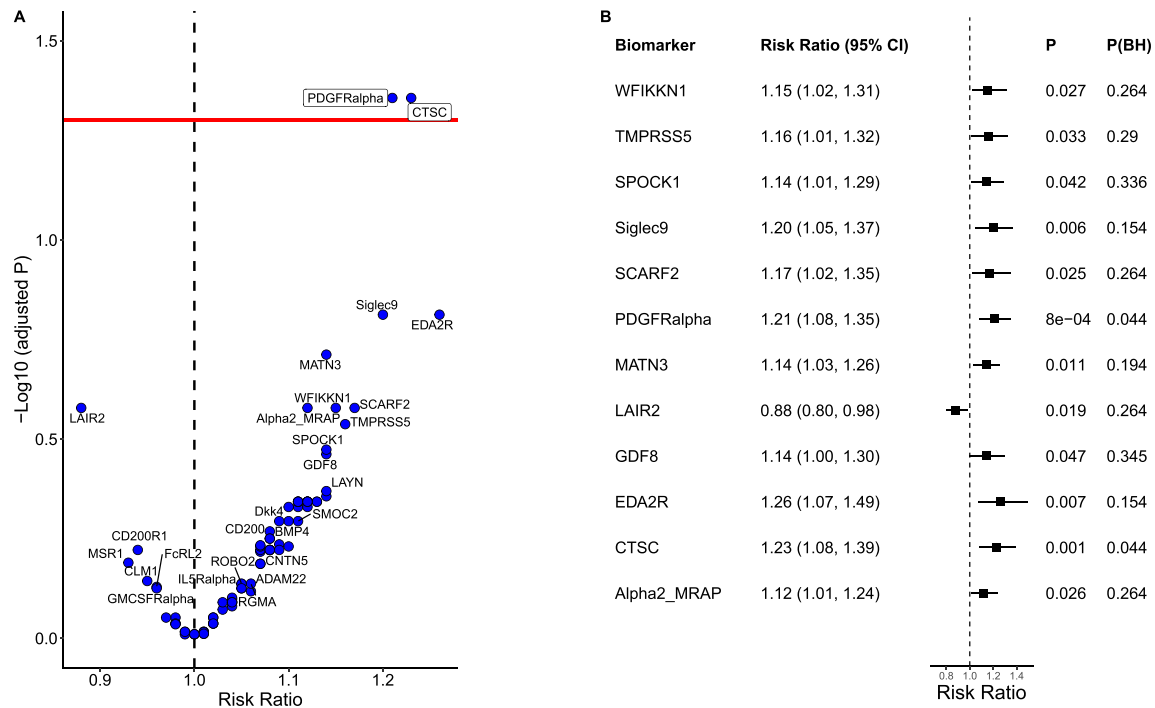Bold print indicates statistical significance ($p < 0.05$).

FIGURE 2 Associations between neurological biomarkers and prevalent DSPN in the total study sample (model 2). (A) Volcano plot showing risk ratios (x-axis) and –log10 transformed Benjamini–Hochberg adjusted p values (p_{B-H}; y-axis). The red line indicates $p_{B-H} = 0.05$. (B) Forest plot showing all biomarkers with associations at $p < 0.05$. Full biomarker names are given in Table S1.

(platelet-derived growth factor receptor-α; RR [95% CI] 1.21 [1.08, 1.35], $p_{B-H} = 0.044$) were associated with prevalent DSPN (Table S3). Results from model 2 are also visualised in a volcano plot (Figure 2A) and a forest plot (Figure 2B). As shown in Figure S3, pairwise correlations between these 12 biomarkers were mainly positive.

3.3 | Effect modification by diabetes status on the associations between neurological biomarkers and prevalent DSPN

When the analysis for model 2 was repeated stratified by diabetes status, effect estimates were higher in the subgroup with type 2 diabetes for 23 biomarkers and lower for one biomarker compared to the subgroup without type 2 diabetes (all $p_{interaction} < 0.05$; Table 2; full results in Table S4).

After adjustment for multiple testing, there was no significant interaction observed for CTSC and PDGFRα that showed positive associations with prevalent DSPN in the total study population (Table S4). Serum levels of CDH3 (cadherin-3), JAM-B (junctional adhesion molecule B), LAYN (layilin), RGMA (repulsive guidance molecule A) and SCARA5 (scavenger receptor class A member 5) were positively associated with DSPN in the diabetes subgroup but not in the subgroup without type 2 diabetes (all p_{B-H} for interaction <0.05), whereas GCP5 (glypican-5) was positively associated with DSPN in people without diabetes but not in those with type 2 diabetes (p_{B-H} for interaction <0.05) (Table 2).

3.4 | Associations between neurological biomarkers and incident DSPN

As described previously for almost identical study samples,[7,8,15] people who developed DSPN between the KORA F4 and FF4 surveys were characterised by higher age, BMI, waist circumference, height and HbA1c and by a higher frequency of hypertension than those who remained DSPN-free. In addition, cases with incident DSPN had lower levels of eGFR, total cholesterol and physical activity than those without (Table S5).

Both groups differed in serum concentrations of 14 biomarkers, of which 11 were higher in people with incident DSPN (Table S6). After adjustment for age and sex (model 1), five biomarkers were associated with incident DSPN at $p < 0.05$ (one positive, four inverse associations), but no associations were observed after adjustment for multiple testing (all $p_{B-H} \geq 0.176$). In the fully adjusted model 2, three biomarkers were inversely associated with the risk of DSPN at $p < 0.05$, but not after adjustment for multiple testing (all $p_{B-H} = 0.970$) (Table S7).

4 | DISCUSSION

Main findings of this study were the positive associations between serum levels of CTSC and PDGFRα with prevalent DSPN in the older general population. While no effect modification by diabetes status was observed for these two proteins, further analyses indicated in general larger effect sizes in people with type 2 diabetes compared to

TABLE 2 Effect modification of diabetes status on the association between neurological biomarkers and prevalent DSPN.

Biomarker	No T2D[a] RR (95% CI)	T2D[b] RR (95% CI)	$p_{interaction}$	$p_{B-H\ interaction}$
ADAM 22	0.96 (0.79, 1.17)	1.26 (1.02, 1.56)	**0.004**	0.050
CD38	1.04 (0.85, 1.27)	1.06 (0.79, 1.42)	**0.029**	0.151
CDH3	0.91 (0.76, 1.08)	1.28 (1.05, 1.57)	**0.0001**	**0.009**
CDH6	1.02 (0.88, 1.19)	1.29 (0.98, 1.68)	**0.045**	0.165
CLM-1	0.88 (0.76, 1.02)	1.03 (0.81, 1.31)	**0.036**	0.151
EDA2R	1.13 (0.91, 1.41)	1.38 (1.10, 1.73)	**0.040**	0.160
EPHB6	0.94 (0.79, 1.13)	1.17 (0.96, 1.42)	**0.007**	0.059
GCP5	1.23 (1.03, 1.47)	0.82 (0.66, 1.01)	**0.001**	**0.022**
GDNFR-alpha-3	1.03 (0.86, 1.23)	1.28 (1.04, 1.58)	**0.025**	0.151
GFR-alpha-1	0.99 (0.82, 1.20)	1.27 (0.99, 1.62)	**0.033**	0.151
JAM-B	1.01 (0.83, 1.22)	1.29 (1.02, 1.63)	**0.003**	**0.044**
LAYN	1.01 (0.83, 1.22)	1.28 (1.04, 1.58)	**0.003**	**0.044**
PDGF-R-alpha	1.10 (0.94, 1.27)	1.37 (1.15, 1.64)	**0.008**	0.059
RGMA	0.93 (0.78, 1.10)	1.34 (1.06, 1.69)	**0.001**	**0.022**
RGMB	1.03 (0.85, 1.24)	1.20 (0.94, 1.54)	**0.007**	0.059
SCARA5	1.02 (0.87, 1.19)	1.52 (1.16, 2.00)	**0.001**	**0.022**
SCARB2	1.05 (0.85, 1.30)	1.17 (0.93, 1.48)	**0.035**	0.151
SCARF2	1.09 (0.90, 1.32)	1.24 (1.00, 1.55)	**0.030**	0.151
SKR3	1.07 (0.88, 1.29)	1.12 (0.91, 1.38)	**0.045**	0.165
THY 1	1.02 (0.85, 1.21)	1.22 (0.91, 1.64)	**0.032**	0.151
TNFRSF12 A	1.03 (0.86, 1.23)	1.26 (1.00, 1.58)	**0.016**	0.108
TNFRSF21	0.95 (0.80, 1.13)	1.18 (0.90, 1.54)	**0.027**	0.151
UNC5C	0.92 (0.78, 1.08)	1.19 (0.92, 1.54)	**0.007**	0.059
VWC2	0.92 (0.76, 1.11)	1.29 (1.02, 1.64)	**0.008**	0.059

Note: This table lists all biomarkers with $p_{interaction} < 0.05$. Estimates were adjusted for age, sex, waist circumference, height, hypertension, total cholesterol, alcohol consumption, smoking, physical activity, use of lipid-lowering drugs, use of NSAIDs, eGFR, prevalent myocardial infarction and prevalent stroke (model 2). Full results are given in Table S4. Biomarker abbreviations are specified in Table S1. Bold print indicates statistical significance ($p_{interaction} < 0.05$ or $p_{B-H\ interaction} < 0.05$).

Abbreviations: CI, confidence interval; DSPN, distal sensorimotor polyneuropathy; eGFR, estimated glomerular filtration rate; NSAIDs, non-steroidal anti-inflammatory drugs; RR, risk ratio for 1-SD increase of biomarker levels; T2D, type 2 diabetes.

[a]$n = 822$ (of whom 122 had DSPN).

[b]$n = 210$ (of whom 55 had DSPN).

Benjamini–Hochberg corrected p values (p_{B-H}) <0.05 are considered statistically significant.

those without diabetes and identified additional five proteins associated with prevalent DSPN in people with type 2 diabetes and one DSPN-associated protein in people without type 2 diabetes. Of note, none of the neurological proteins investigated here was associated with incident DSPN during a follow-up period of 6.5 years after correction for multiple testing.

4.1 | CTSC and PDGFRα: Novel biomarkers of prevalent DSPN

Our results suggest that CTSC and PDGFRα may represent novel biomarkers of prevalent DSPN. These data extend previous studies in the population-based KORA F4/FF4 and other cohorts that mainly focused on biomarkers of inflammation and oxidative stress.[5]

CTSC, also known as dipeptidyl peptidase-I (DPP-I), belongs to the protease family of cathepsins. It is a ubiquitously expressed lysosomal cysteine dipeptidyl aminopeptidase that can also be found in the extracellular space and blood. In particular, neutrophils, mast cells and lymphocytes secrete high levels of CTSC.[23,24] CTSC has not been investigated in the context of DSPN before but may be linked to its pathogenesis through its role in regulating proinflammatory processes. CTSC is part of a proteolytic network in which it has essential functions in activating cytotoxic serine proteases from neutrophils, mast cells, cytotoxic T cells and natural

killer cells which include neutrophil elastase, cathepsin G, and granzymes A and B.[23,25] Upon activation these proteases contribute to tissue damage in various inflammatory diseases.[25] This protease activation may also explain the role of CTSC in the caspase 1-independent activation of the NLRP3 inflammasome and subsequent processing of IL-1β.[26] Several studies have linked CTSC with microglia and macrophage M1 polarisation and activation of the NF-κB pathway contributing to neuroinflammation[27–29] and cardiovascular diseases.[30,31] Of note, overexpression of CTSC in a mouse model increased interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α levels in serum samples and brain tissue, whereas CTSC knockout had the opposite effect.[27] These findings are particularly relevant given our previous reports about positive associations of serum IL-6 and TNFα with incident DSPN[7] and the identification of IL-1β and TNFα as potential upstream regulators of a biomarker pattern associated with the risk of DSPN.[8] In addition, pathway analysis pointed towards an involvement of granulocytes in DSPN.[8]

Hence, it seems likely that higher CTSC levels might exert multiple proinflammatory effects downstream and thereby contribute to the pathogenesis of DSPN. However, it is also possible that CTSC may be a consequence rather than cause of the increased cytokine and chemokine levels that have previously been shown to be linked with DSPN.[7,8] The reason why a positive association between serum levels of CTSC and DSPN was only found in the cross-sectional but not in the prospective analysis remains unclear. However, the findings point towards an increasing complexity of proinflammatory mechanisms in DSPN.

PDGFRα is a plasma membrane bound receptor tyrosine kinase that binds different isoforms of PDGF (PDGF-AA, AB, BB, CC, DD).[32] PDGF/PDGFR signalling has pleiotropic functions for cell growth and proliferation. Given their roles in angiogenesis and neurogenesis[33,34] it is obvious that dysregulated PDGF/PDGFR signalling has been implicated in vascular complications of diabetes[35,36] and in the development of neurodegenerative diseases.[32,37] The role of PDGFs is context-dependent as they can be neuroprotective but also trigger proinflammatory signalling pathways.[35,36] The link between PDGF/PDGFR signalling and neuroinflammation involving cytokines such as TNFα or chemokines[32] suggests that the association between serum PDGFRα and DSPN could be biologically plausible, but validation of this hypothesis would require a comprehensive measurement of PDGF ligands in the circulation, which was not feasible in this study. Of note, shedding from the plasma membrane has been reported for PDGFRα through exosomes[38] and for PDGFRβ through a metalloproteinase.[39] Increased levels of soluble PDGFRβ have been suggested as biomarkers of pericyte injury under stress conditions.[40] Currently, it is not known to what extent higher serum levels of PDGFRα which is expressed on oligodendrocyte precursor cells in the central nervous system may also reflect peripheral cell damage in DSPN, which needs to be investigated in future studies.

4.2 | Interaction by diabetes status

Further analyses pointed towards an interaction between diabetes status and neurological biomarkers regarding associations with prevalent DSPN for a subset of the biomarkers investigated here. Overall, effect sizes tended to be larger in the subgroup of people with type 2 diabetes. After full adjustment, we identified positive associations between CDH3, JAM-B, LAYN, RGMA and SACAR5 with DSPN in people with type 2 diabetes. Of note, LAYN and RGMA have previously been linked to diabetic kidney disease.[41,42] In addition, higher GPC5 levels were significantly associated with DSPN in people without type 2 diabetes. Table 3 provides an overview of these biomarkers and how they could be linked with DSPN.[43–55] JAM-B and RGMA represent the best candidates for validation given their effects on myelination in other studies.[46,50,53] LAYN and SCARA5 could be relevant given their roles in hyaluronan and iron metabolism, respectively.[47–49,51,55] CDH and GCP5 have not been linked to DSPN yet, but members of their protein families have been implicated in neural development as well as in the development of neurodegenerative diseases.[43,44]

All these six biomarkers are cell surface proteins that also have soluble forms in the circulation. Mechanistic follow-up studies will need to investigate both expression patterns in different cell types and circulating levels to establish any causal links with the pathophysiology of DSPN. Nevertheless, there seem to be plausible biological links between several neurological biomarkers and DSPN that render them promising candidates for future mechanistic as well as clinical studies.

4.3 | Clinical implications

Major challenges in the clinical management of DSPN are the late diagnosis of the disease and a lack of circulating biomarkers that could be used for screening purposes to predict its onset and monitor its progression.[5] Additionally, due to the paucity of disease-modifying therapies, there remains an unmet need to identify novel drug targets.[5]

The current study extends the list of biomarkers that represent promising candidates for future studies that should also include people with DSPN confirmed by other established methods beyond the MNSI. Given their potential links with DSPN, it is biologically plausible that dysregulations of some of the proteins at the cellular level might contribute to the pathogenesis of DSPN, for example, through their role in inflammation, neurogenesis, or myelination. In this case, they could be targets for novel disease-modifying therapeutic approaches. An increase in the circulating levels of the membrane-bound proteins most likely represents an indicator of tissue damage rather than a direct contributor to DSPN and could be relevant for disease monitoring if these circulating levels are also associated with disease severity. In addition, further studies are needed to determine whether dynamic changes in these biomarkers

TABLE 3 Biomarkers associated with DSPN in people with or without type 2 diabetes: Potential links to DSPN.

Protein biomarker	Physiological function	Potential links to DSPN
CDH3 (cadherin-3)	Calcium-dependent cell-cell adhesion protein	While some cadherins have roles in neural development, there is as yet no evidence linking CDH3 to DSPN or other neurological diseases.[43]
GPC5 (glypican-5)	Cell surface heparan sulphate proteoglycan	Several members of the glypican family are important for neuronal network formation and have also been implicated in neurodevelopmental disorders, but evidence for GPC5 in this context is lacking.[44]
JAM-B (junctional adhesion molecule-2, JAM2)	Junctional adhesion protein that mediates heterotypic cell-cell interactions	JAM-B contributes to leucocyte extravasation from the circulation to sites of tissue damage and infection, thus linking the protein to inflammatory processes.[45] JAM-B also inhibits somatodendritic myelination of neurons without affecting differentiation, proliferation or migration of oligodendrocyte precursor cells, whereas JAM-B deficiency leads to aberrant somatodendritic myelin wraps.[46] Based on this effect in the central nervous system, effects on myelination of peripheral nerves appear possible.
LAYN (layilin)	Cell surface hyaluronan receptor	Hyaluronan is a component of the extracellular matrix important in neural differentiation, survival, proliferation, migration and cell signalling. Changes in hyaluronan regulation have been implicated in various pathological conditions in the central nervous system and in peripheral neuropathies of different aetiologies.[47–49] This makes a link between LAYN as hyaluronan receptor and DSPN possible.
RGMA (repulsive guidance molecule A)	Axon guidance protein	RGMA appears to have complex functions in the central nervous system. RGMA is an axon guidance protein in the developing and adult central nervous system, but also has repulsive function on axonal growth and can inhibit axon regeneration.[50] In addition, interaction with granulocytes and T cells resulting in context-dependent pro- and anti-inflammatory activities have been described.[50–52] RGMA is upregulated in multiple neurological conditions and has been suggested as therapeutic target of diabetes-related and other neuropathies.[50,53]
SCARA5 (scavenger receptor class a member 5)	Ferritin receptor	SCARA5 has as yet not been linked with any neurological processes or disorders. However, its role in iron trafficking and delivery may be relevant in this context, because dysregulations of iron homoeostasis have been implicated in impaired Schwann cell maturation and myelination of peripheral neurons and in the development of peripheral diabetic neuropathy in animal models.[54,55]

Abbreviation: DSPN, distal sensorimotor polyneuropathy.

correlate with changes in the severity of DSPN and whether they can be used as surrogate outcome measures in clinical trials to better assess response to treatment. Moreover, the lack of significant prospective associations is a limitation of our study, which may be related to lower statistical power than in the cross-sectional analysis and/or a different kinetics, that is, altered biomarker levels may indicate higher risk of DSPN within a shorter or also a longer timeframe than the 6.5-year follow-up of our study.

Overall, one could conclude from our results that only few neurological biomarkers may be associated with DSPN. However, it is important to keep in mind that the multimarker panel that we used was designed for the investigation of multiple neurological diseases and processes. Therefore, it is conceivable that more neuropathy-focused panels or untargeted proteomics approaches could lead to a more comprehensive identification of DSPN-related biomarkers in the future.

Currently, proximity extension assay technology is available in a small number of research facilities, which limits its clinical applicability. Progress in the identification of DSPN-related biomarkers with valid diagnostic or prognostic utility should lead to the development of assays that could be established in clinical laboratories.

In a recent study, we demonstrated that higher serum levels of NFL are associated with peripheral nerve dysfunction and prevalent DSPN.[9] NFL is a biomarker of neuroaxonal damage, and further

studies are needed to validate to what extent NFL and the biomarker candidates identified in this study could be used to monitor the progression of DSPN and other neuropathies in clinical practice and intervention studies.[10,12,56]

4.4 | Strengths and limitations

A major strength of this study is its population-based design so that associations between biomarkers and DSPN could be assessed in people with and without type 2 diabetes from the older general population. Additionally, the study was based on a large biomarker panel with multiple proteins that have not been investigated before in the context of DSPN. Associations with DSPN were adjusted for multiple confounders. The comprehensive approach with a large biomarker panel required correction for multiple testing, which was implemented throughout the study.

The study also has limitations. First, possible DSPN was defined clinically using the examination part of the MNSI since nerve conduction studies to confirm DSPN were not feasible in this population-based setting. Second, we used a targeted proteomics assay rather than an unbiased approach such as mass spectrometry (LC-MS/MS) so that additional neurological biomarkers which would have been measurable with LC-MS/MS could not be assessed. Third, neurological biomarker levels were not available in KORA FF4, which precluded analysing the associations of changes in their levels over time with changes in the MNSI. Fourth, biomarker measurements in serum cannot distinguish between upregulated proteins due to central or peripheral nerve degeneration. Fifth, study participants were older and mainly of European descent so that the data cannot be generalised to other age groups and ethnicities. Finally, the number of participants developing DSPN over the observed 6.5-year time period might have been too small to detect associations between neurological biomarkers and incident DSPN.

4.5 | Conclusions

The present study identified CTSC and PDGFRα as potential novel biomarkers of prevalent DSPN in older individuals from the general population. CDH3, JAM-B, LAYN, RGMA and SCARA5 were positively associated with DSPN in people with type 2 diabetes, whereas GCP5 was positively associated with DSPN in people without diabetes. Given the role of these proteins in pathways such as neuroinflammation, neural development and myelination, a link with DSPN appears plausible. However, follow-up studies need to address the question to what extent upregulation of these proteins reflects the severity of tissue damage and whether it is linked to an active involvement in the pathophysiology of DSPN.

AUTHOR CONTRIBUTIONS

Christian Herder acquired funding. Christian Herder and Haifa Maalmi designed the study. Christian Herder, Alexander Strom, Wolfgang Rathmann, Margit Heier, Wolfgang Koenig, Dan Ziegler, Annette Peters, Gidon J. Bönhof and Barbara Thorand contributed data. Christian Herder and Haifa Maalmi drafted the analysis plan. Haifa Maalmi performed the statistical analyses. Christian Herder, Barbara Thorand, Helen Morrison and Haifa Maalmi contributed to data interpretation. Christian Herder wrote the manuscript. Haifa Maalmi contributed to the draft of the manuscript. All authors reviewed and edited the manuscript and approved its submission. Christian Herder and Haifa Maalmi are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

CONFLICT OF INTEREST STATEMENT

All authors declare that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

The data are subject to national data protection laws. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with KORA. To obtain permission to use KORA data under the terms of a project agreement, please use the digital tool KORA.PASST (https://epi.helmholtz-muenchen.de/).

ETHICS STATEMENT

The KORA surveys were approved by the ethics board of the Bavarian Chamber of Physicians (Munich, Germany). All participants provided written informed consent.

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PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/dmrr.3807.

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