Association of Subclinical Inflammation With Polyneuropathy in the Older Population

KORA F4 study

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OBJECTIVE—Inflammatory processes have been implicated in the pathogenesis of diabetic distal sensorimotor polyneuropathy (DSPN), but their possible relationship has not been assessed at the population level.

RESEARCH DESIGN AND METHODS—We determined serum concentrations of mediators of subclinical inflammation among 1,047 participants 61–82 years of age from the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study (Germany). Logistic and linear regression models were fitted to assess associations between immune mediators (log-transformed) and the presence of clinical DSPN (dichotomous variable) or Michigan Neuropathy Screening Instrument (MNSI) examination score (continuous variable), respectively.

RESULTS—Serum concentrations of the anti-inflammatory interleukin (IL)-1 receptor antagonist (IL-1RA) were positively associated with the presence of DSPN and higher MNSI scores in age-adjusted and sex-adjusted analyses, whereas IL-6, IL-18, and soluble intercellular adhesion molecule-1 were positively associated with only MNSI scores. No associations were observed for adiponectin, C-reactive protein, or tumor necrosis factor-α. Associations for IL-1RA and IL-6 with the MNSI score remained statistically significant after additional adjustment for waist circumference, height, hypertension, cholesterol, smoking, alcohol intake, physical activity, history of myocardial infarction or stroke, presence of neurological conditions, and use of nonsteroidal anti-inflammatory drugs.

CONCLUSIONS—We conclude that DSPN is linked to proinflammatory and anti-inflammatory, possibly compensatory, processes in the older general population. Future studies should clarify the temporal sequence and causality of these associations.

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Distal sensorimotor polyneuropathy (DSPN) contributes to significant morbidity and increased mortality among diabetic subjects (1) but is frequently underdiagnosed by physicians (2) and is unrecognized by patients (3). Several, but not all, studies suggest that DSPN is more prevalent not only in subjects with diabetes but also in individuals with prediabetes when compared with those with normal glucose tolerance (4,5).

The putative mechanisms implicated in the pathogenesis of diabetic neuropathy remain a matter of debate, but a multifactorial course of events is likely (6). There is accumulating evidence indicating that inflammatory processes play an important pathogenetic role in diabetic neuropathy (7). Moreover, findings in other neuropathic conditions suggest the involvement of a number of inflammatory mediators such as interleukins (ILs) and chemokines (8). Recent studies in type 2 diabetic patients suggest that DSPN is associated with increased circulating concentrations of several proinflammatory immune mediators (9,10), whereas anti-inflammatory mediators were rarely ever investigated in this context. Moreover, no data regarding the relationship between subclinical inflammation and DSPN are available for the general population. "Subclinical inflammation" in this context refers to the alterations of systemic levels of immune mediators (i.e., mainly increased concentrations of proinflammatory cytokines) that are typically found in individuals with type 2 diabetes and diabetes complications or in those at increased risk for these conditions. Therefore, the aim of our study was to investigate whether circulating concentrations of seven proinflammatory and anti-inflammatory immune mediators are associated with clinically diagnosed DSPN and neuropathic impairments in elderly subjects participating in the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study.

RESEARCH DESIGN AND METHODS

Study population

The current study was based on the KORA F4 survey (2006–2008), which is the follow-up examination of the population-based KORA S4 Survey (1999–2001) (11). KORA was initiated to study the prevalence and incidence of various chronic diseases in the general

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population, including diabetes, and to identify novel risk factors of these diseases.

The study design and subject enrollment in the KORA S4 survey are described in detail elsewhere (12). Briefly, 2,656 men and women in the range of 55–74 years of age were randomly selected from the region of Augsburg in the south of Germany to participate in the KORA S4 survey. From the 2,564 eligible subjects, 1,653 (64%) completed the survey and a subsequent 1,353 subjects without known diabetes successfully completed an oral glucose tolerance test (OGTT).

The current study uses data from the 7-year follow-up examination (F4 survey) of this cohort that occurred in 2006–2008 and included a second OGTT. Of the aforementioned 1,353 subjects who participated in the KORA S4 survey, a total of 1,209 also participated in the follow-up examinations; 177 participants had physician-diagnosed diabetes and another 923 participants successfully completed the OGTT, resulting in a sample size of 1,100 subjects. Fifty-three of these individuals had to be excluded because of incomplete information regarding glucose tolerance status, clinical DSPN, and immune mediators, which resulted in a final sample size of 1,047 subjects. All participants gave written informed consent and the study was approved by the Ethics Committee of the Bavarian Medical Association.

Height, weight, waist circumference, systolic blood pressure, and diastolic blood pressure were measured according to standard protocols as described elsewhere (12). Trained medical interviewers collected information regarding medical history, physical activity, smoking behavior, and alcohol consumption.

Cases of self-reported diabetes, as well as the dates of diagnoses, were validated by contacting the general practitioners of the participants. All other participants underwent an OGTT (World Health Organization criteria). After an overnight fasting period of at least 10 h, fasting blood samples were collected and participants were administered an oral dose of 75 g anhydrous glucose (Dextro OGT; Boehringer Mannheim, Mannheim, Germany). Another blood sample was collected 2 h after the glucose load. Blood samples were collected without stasis. After withdrawal, the samples were centrifuged and refrigerated at 4°C until analysis in the central laboratory of the Augsburg Central Hospital (not more than 6 h after withdrawal). Blood glucose levels were assessed using the hexokinase method (Glu-Flex; Dade Behring, Marburg, Germany). Glucose tolerance categories were defined according to the 1999 World Health Organization diagnostic criteria (13). It can be assumed that the majority of cases with newly diagnosed diabetes in this age group had type 2 diabetes.

Assessment of neuropathy

The neurological examination of the F4 survey consisted of two parts. The first part involved a detailed interview addressing the presence of pain in the feet and other parts of the body, the presence of neurological diseases, and history of foot ulcers and amputations. The second part comprised a foot inspection and a series of neurological tests involving sensation to touch, vibration, and temperature, and testing of ankle reflexes and sudomotor function.

We defined the presence of clinical DSPN as bilateral impairment of foot vibration perception and/or bilateral impairment of foot pressure sensation as described (14). Vibration perception threshold was determined at the dorsal side of the left and right first toes using a calibrated 64-Hz Rydel Seiffer tuning fork. Increased thresholds were calculated according to Martina et al. (15). Pressure sensation was measured at the dorsal side of the left and right first toes between the nail fold and the metatarsophalangeal joint using a 10-g monofilament (Twin-Tip). Participants were asked to close their eyes during the test and to respond with "yes" each time the monofilament was sensed. No negative stimuli were tested. At least 8 out of 10 correct responses were considered to indicate normal sensibility (16). Less than eight perceived applications indicated reduced sensibility; in the case in which none of the applications was perceived, sensibility to touch was considered absent. Measurements of vibration perception and pressure sensation were performed by trained investigators under supervision of an experienced diabetologist and according to the practical guidelines for the diabetic foot from the American Diabetes Association and the International Diabetic Foot Working Group (17,18). Our choice for these two specific tests lies in their quantitative nature to detect the insensate foot, and the fact that both tests are predictors of future foot ulceration (19). Also, the two tests previously have been studied and were determined to be the most accurate tools for diagnosing large-fiber polyneuropathy in patients with diabetes. We have validated our clinical DSPN definition against nerve conduction studies as described previously (14).

Ankle reflexes were examined using an appropriate reflex hammer. The ankle reflexes were elicited in the sitting position. The Achilles tendon was percussed directly, and the reflex was graded as present, present after reinforcement, or absent.

We also used the examination portion of the Michigan Neuropathy Screening Instrument (MNSI), which is used to identify individuals at high risk for DSPN (20). Higher MNSI scores were shown to be associated with higher systemic levels of proinflammatory immune mediators in diabetic patients (9). The physical assessment of the MNSI contains items regarding the appearance of feet (normal or deformities, dry skin, callus, infection, fissure, or other irregularities), foot ulceration, ankle reflexes, and vibration perception at the first toes. The minimum score is 0 points (all aspects normal) and the maximum score is 8 points. We applied the MNSI in the analysis as a continuous variable as well as a dichotomous variable using a cutoff of >2 points, as previously suggested (20,21).

Immune mediator measurements

Concentrations of all immune mediators were assessed in serum samples using commercially available ELISA kits, apart from hsCRP and IL-18 that were measured in EDTA plasma samples. IL-1 receptor antagonist (IL-1RA), total adiponectin, and soluble intercellular adhesion molecule (sICAM)-1 were measured with Quantikine ELISA kits from R&D Systems (Wiesbaden, Germany). IL-6 and tumor necrosis factor (TNF)-α levels were determined with the Quantikine HS ELISA kits from R&D Systems. IL-18 was measured using the ELISA kits from MBL (Nagoya, Japan). Intra-assay coefficients of variation for IL-1RA, adiponectin, sICAM-1, IL-6, TNF-α, and IL-18 were 2.8, 3.8, 3.5, 7.2, 6.3, and 7.6%, respectively. Interassay coefficients of variation for IL-1RA, adiponectin, sICAM-1, IL-6, TNF-α, and IL-18 were 7.0, 8.0, 6.4, 11.8, 14.4, and 9.4%, respectively. High-sensitivity C-reactive protein was determined using a high-sensitivity latex-enhanced nephelometric assay on a BN II

analyzer (Dade Behring), with intra-assay and interassay coefficients of variation of 2.7 and 6.3%, respectively.

Statistical analysis

Data analyses were based on cohort members with complete information regarding glucose tolerance status as assessed by the OGTT, clinical DSPN, and immune mediators (n = 1,047). Participants' characteristics and immune marker concentrations were presented as means ± SD for normally distributed variables and as median (25th/75th percentiles) for variables without a normal distribution. Using ANOVA, age-adjusted and sex-adjusted differences in metric variables were evaluated for subjects with and without clinical DSPN, and for those with MNSI scores ≤2 and >2. For log-normal variables, ANOVA was performed on a log-scale. Differences in dichotomous variables were examined using logistic regression.

Logistic regression models were fitted to study associations between the proinflammatory and anti-inflammatory immune markers (log-transformed continuous variables) and the presence of clinical DSPN (dichotomous variable). Both univariable and multivariable models were calculated to estimate odds ratios (ORs) and 95% CIs. The multivariable model included age (years), sex, height (cm), waist circumference (cm), hypertension (yes/no), total cholesterol (mg/dL), smoking (never/former/current), alcohol consumption (abstain/moderate/high), and physical activity (low/high). The selection of covariables was based on those variables that altered the estimate for the exposure coefficient between statistical models with and without the potential confounder by >10% or those that have been described previously in the literature as potential risk factors for clinical DSPN. In a third model, we additionally adjusted for history of acute myocardial infarction or stroke (yes/no), the presence of neurological conditions that might cause nerve damage, such as cancer, stroke, dementia, and hernias (yes/no), and use of nonsteroidal anti-inflammatory drugs (yes/no).

Using linear regression, we applied these three models for the additional end points MNSI score (continuous variable), mean ankle reflex score (average score of the left and right tests; continuous variable), mean monofilament score (average score of the left and right tests; continuous variable), and mean tuning fork test score (average score of the left and right tests; continuous variables).

$P < 0.05$ indicated statistical significance. All analyses were performed with the Stata statistical software package (version 11; StataCorp LP, College Station, TX).

RESULTS—The prevalence of clinical DSPN was 13.9% (95% CI, 11.9–16.2%) in our study population. When compared with individuals without clinical DSPN, subjects with clinical DSPN were older, more frequently male, taller, had a larger waist circumference, had lower diastolic blood pressure, had lower total and LDL cholesterol levels, had higher HbA_{1c} levels, and were less likely to have normal glucose tolerance but more likely to have combined impaired fasting glucose/impaired glucose tolerance or known diabetes (Table 1).

When the study population was stratified by MNSI score (MNSI score >2 vs. MNSI score ≤2), individuals with MNSI score >2 were older, taller, had a higher BMI, had larger waist circumference, had higher HbA_{1c} levels, and were less likely to have normal glucose tolerance but more likely to have known diabetes than individuals with MNSI score ≤2 (Tables 1 and 2). The prevalence of clinical DSPN was 28.1% (95% CI, 23.7–32.9%) among subjects with MNSI score >2.

Among the immune mediators, serum IL-1RA levels were higher in individuals with DSPN than in those without DSPN (median [25th/75th percentiles] 335 [248–472] vs. 304 [233–400] pg/mL; age-adjusted and sex-adjusted P = 0.011; Table 1). Individuals with MNSI score >2 had higher levels of IL-1RA (329 pg/mL [239–442] vs. 300 pg/mL [231–385] ; age-adjusted and sex-adjusted P = 0.001) and IL-6 (1.7 pg/mL [1.2–2.8] vs. 1.5 pg/mL [1.0–2.3]; age-adjusted and sex-adjusted P = 0.018) than individuals with MNSI ≤2 (Table 2).

As shown in Table 3, IL-1RA levels were positively associated with clinical DSPN and higher MNSI scores in age-adjusted and sex-adjusted analyses, whereas higher levels of IL-6, IL-18, and sICAM-1 were associated with higher MNSI scores only. After further adjustment for multiple confounders (waist circumference, height, hypertension, cholesterol, smoking, alcohol intake, physical activity, history of myocardial infarction or stroke, presence of neurological conditions, and use of nonsteroidal anti-inflammatory drugs), associations for IL-1RA and IL-6 with MNSI scores remained statistically significant. No associations were found for adiponectin, high-sensitivity C-reactive protein, or TNF-α.

Furthermore, we observed several associations between high levels of IL-1RA, IL-6, and sICAM-1 with impaired ankle reflexes, reduced pressure sensation, or reduced vibration perception, but only the association between high IL-1RA and impaired vibration perception persisted after multivariable adjustment (Table 3).

In a sensitivity analysis, we investigated to what extent our findings are independent of the presence of diabetes and the metabolic syndrome. In models 2 and 3, we substituted the three components contributing to the metabolic syndrome (waist circumference, hypertension, total cholesterol) with two new variables, presence of the metabolic syndrome (ATP III definition) and presence of diabetes. All significant results in Table 3 remained statistically significant with increased β-coefficients in models 2 and 3 after the adjustment for diabetes and the metabolic syndrome. Notably, the associations between IL-1RA and clinical DSPN (P = between 0.011 and 0.33) as well as ankle reflexes (P = between 0.003 and 0.020) were statistically significant in all three models. In addition, the associations between IL-6 and ankle reflexes (P = between 0.007 and 0.028) and between sICAM-1 and MNSI as well as ankle reflexes reached statistical significance (all $P < 0.05$), with β-coefficients in models 2 and 3 almost identical to those for the respective first models.

In another sensitivity analysis, we excluded all individuals with neurological diseases (n = 175), which could have had an impact on our results, instead of adjusting for this variable. This analysis (data not shown) led to results that were almost identical to those given in Table 3.

CONCLUSIONS—This is the first study to describe an association between subclinical inflammation and DSPN in a large population-based sample.

Our major novel finding is that individuals with specific manifestations of DSPN have higher serum IL-1RA levels that cannot be explained by anthropometric or metabolic confounders. Differences between median IL-1RA levels of groups stratified by DSPN status or MNSI score were only ~10% with overlapping distributions, but these differences were statistically significant when using the

Table 1—*Characteristics of the study population stratified by presence of clinical DSPN*

Variable	No clinical DSPN	Clinical DSPN*	*P*†
N	901	146	
Age, years	69.9 ± 5.3	71.8 ± 5.7	<0.001
Sex, % male	49	61	0.009
Height, cm	165 ± 9.0	168 ± 9.0	<0.001
BMI, kg/m^2	28.6 ± 4.4	29.2 ± 4.5	0.127
Waist circumference, cm	97.7 ± 12.0	101.6 ± 12.5	0.011
Systolic blood pressure, mmHg	128.5 ± 19.9	128.1 ± 19.1	0.380
Diastolic blood pressure, mmHg	74.5 ± 9.9	71.7 ± 11.1	0.008
Hypertension, %‡	62	64	0.691
Smoking, %, never/former/current	51/41/8	49/45/6	0.602
High alcohol consumption, %§	18	14	0.069
Low physical activity, %¶	48	60	0.058
Previous myocardial infarction, %	6	8	0.670
Previous stroke, %	4	7	0.249
Presence of neurological diseases, %	14	31	<0.001
Absent ankle reflexes, %	8	25	<0.001
Foot ulcer present, %	0	2	0.001
MNSI score	2 (1–2.5)	3 (2.5–4)	<0.001
Use of NSAIDs, %	4	7	0.184
NGT, %	56	42	0.020
i-IFG, %	5	2	0.044
i-IGT, %	17	17	0.906
IFG/IGT, %	4	8	0.042
Newly diagnosed diabetes, %	5	7	0.755
Known diabetes, %	13	25	0.001
Diabetes duration, years‖	8 (3–12)	10 (7–17)	0.142
Metabolic parameters			
Fasting glucose, mg/dL**	97 (91–104)	97 (91–105)	0.905
2-h glucose, mg/dL**	118 (99–147)	131 (102–160)	0.104
HbA_{1c}, % (mmol/mol)	5.7 ± 0.5 (39.3 ± 6.8)	5.9 ± 0.8 (41.4 ± 7.9)	<0.001
Total cholesterol, mg/dL	223 ± 40	208 ± 38	<0.001
LDL cholesterol, mg/dL	141 ± 36	132 ± 34	0.030
HDL cholesterol, mg/dL	54 (46–65)	52 (45–60)	0.070
Creatinine, mg/dL	0.91 (0.79–1.05)	0.94 (0.82–1.11)	0.180
Uric acid, mg/dL	5.5 ± 1.4	5.8 ± 1.5	0.543
Immune mediators			
IL-1RA, pg/mL	304 (233–400)	335 (248–472)	0.011
Adiponectin, μg/mL	10.2 (6.8–15.4)	10.0 (6.2–15.0)	0.342
hsCRP, mg/L	1.5 (0.8–3.1)	1.5 (0.9–3.2)	0.705
IL-6, pg/mL	1.6 (1.1–2.4)	1.7 (1.2–2.6)	0.777
IL-18, pg/mL	314 (250–414)	327 (260–414)	0.961
TNF-α, pg/mL	2.0 (1.5–2.9)	2.1 (1.6–3.1)	0.304
sICAM-1, ng/mL	230 (199–262)	228 (197–261)	0.860

Data are presented as mean ± SD or as median (25th–75th percentiles). hsCRP, high-sensitivity C-reactive protein; IFG/IGT, combined impaired fasting glucose/impaired glucose tolerance; i-IFG, isolated impaired fasting glucose; i-IGT, isolated impaired glucose tolerance; NGT, normal glucose tolerance; NSAIDs, nonsteroidal anti-inflammatory drugs. *Defined as the presence of impaired bilateral foot vibration perception or impaired bilateral foot pressure sensation (or both). †Adjusted for age and sex. ‡Defined as a blood pressure of ≥140/90 mmHg or the use of antihypertensive medication in subjects who reported previous diagnoses of hypertension (or both). §≥20 g/day for women and ≥40 g/day for men. ¶Defined as <1 h of physical activity per week during leisure time in either winter or summer. ‖Based on 97 participants without and 32 participants with clinical DSPN. **Participants with known diabetes were excluded because they did not undergo an OGTT; based on 787 participants without and 110 participants with clinical DSPN.

appropriate statistical method (linear regression with log-transformed IL-1RA levels to approximate normal distribution). Notably, the association was more pronounced for the continuous MNSI score than for DSPN as a dichotomous diagnosis. IL-1RA is an anti-inflammatory protein that is induced by the proinflammatory cytokine IL-1β and inhibits IL-1β action by blocking its receptor (22). IL-1β release is tightly regulated; in the absence of major acute inflammatory disease, its systemic levels are approximately the detection limit of currently available assays. However, increased IL-1RA concentrations can be considered an indirect indicator of proinflammatory IL-1β–mediated processes (22). We previously reported that systemic IL-1RA levels are upregulated preceding the clinical manifestation of type 2 diabetes (23). Several lines of evidence indicate that IL-1β contributes to the development of type 2 diabetes by impairing both β-cell function and insulin sensitivity, and that factors such as hyperglycemia may be involved in the upregulation of this cytokine (24–28). The mechanistic link between IL-1β, IL-1RA, and DSPN is much less clear. IL-1β has been investigated mainly in rodent models of neuropathic pain. Intraneural injections of IL-1β induced signs of neuropathic pain in rats (29). In mouse models of neuropathy, genetic deletion of IL-1β or the transgenic overexpression of IL-1RA attenuated neuropathic pain after nerve injury (30). Thus, findings from animal models point toward a causal role of IL-1β in the development of neuropathic pain and indicate that the role of this cytokine in the pathogenesis of DSPN also should be investigated in mechanistic studies in humans. It can be hypothesized that the contributions of IL-1β to the development of type 2 diabetes and the development of DSPN are largely independent at the cellular level because they involve local processes in different tissues, but they may be linked by metabolic stimuli that regulate the expression and release of IL-1β. Based on the aforementioned studies, we hypothesize that in both incident type 2 diabetes and prevalent DSPN, increased IL-1RA levels represent a compensatory upregulation in response to immunological disturbances such as an induction of IL-1β, which might occur downstream of metabolic derangements like hyperglycemia and hyperlipidemia.

In addition, our findings involving the older general population are in line with our previous finding of the association between IL-6 and the MNSI examination score in patients with type 2 diabetes (9). It is important to note that the association between IL-6 and MNSI reported here is not a mere replication of the previous study, because we now show that this association also is present in the older population even after adjustment for diabetes and the metabolic

Table 2—*Characteristics of the study population stratified by MNSI score*

Variable	MNSI score ≤2	MNSI score >2	*P**
N	656	391	
Age, years	69.2 ± 5.3	71.9 ± 5.2	<0.001
Sex, % male	49	55	0.053
Height, cm	165 ± 8.8	167 ± 9.2	<0.001
BMI, kg/m^2	28.2 ± 4.1	29.6 ± 4.9	<0.001
Waist circumference, cm	96.2 ± 11.3	101.5 ± 12.9	<0.001
Systolic blood pressure, mmHg	128.3 ± 19.6	128.8 ± 20.0	0.866
Diastolic blood pressure, mmHg	74.8 ± 9.9	72.9 ± 10.4	0.141
Hypertension, %†	60	66	0.976
Smoking (%), never/former/current	49/42/9	53/42/5	0.050
High alcohol consumption, %‡	17	18	0.506
Low physical activity, %§	46	56	0.038
Previous myocardial infarction, %	5	7	0.831
Previous stroke, %	3	6	0.137
Presence of neurological diseases, %	14	21	0.001
Absent ankle reflexes, %	1	25	<0.001
Foot ulcer present, %	0	1	0.016
Clinical DSPN¶	5	28	<0.001
Use of NSAIDs, %	3	6	0.086
NGT, %	59	46	0.004
i-IFG, %	5	6	0.428
i-IGT, %	17	17	0.690
IFG/IGT, %	4	5	0.169
Newly diagnosed diabetes, %	5	6	0.936
Known diabetes, %	11	20	0.002
Diabetes duration, years‖	7 (2–9)	10 (7–18)	<0.001
Metabolic parameters			
Fasting glucose, mg/dL**	96 (91–104)	98 (92–106)	0.081
2-h glucose, mg/dL**	118 (99–146)	122 (99–153)	0.751
HbA_{1c}, % (mmol/mol)	5.7 ± 0.5 (38.8 ± 5.7)	5.9 ± 0.8 (40.9 ± 8.5)	<0.001
Total cholesterol, mg/dL	224 ± 41	215 ± 40	0.064
LDL cholesterol, mg/dL	142 ± 36	137 ± 36	0.155
HDL cholesterol, mg/dL	55 (46–65)	53 (45–62)	0.252
Creatinine, mg/dL	0.91 (0.79–1.05)	0.94 (0.2–1.11)	0.032
Uric acid, mg/dL	5.5 ± 1.3	5.7 ± 1.5	0.246
Immune mediators			
IL-1RA, pg/mL	300 (231–385)	329 (239–442)	0.001
Adiponectin, μg/mL	10.1 (6.4–15.2)	10.3 (6.9–15.5)	0.449
hsCRP, mg/L	1.5 (0.8–3.1)	1.5 (0.8–3.2)	0.916
IL-6, pg/mL	1.5 (1.0–2.3)	1.7 (1.2–2.8)	0.018
IL-18, pg/mL	312 (247–409)	326 (255–429)	0.385
TNF-α, pg/mL	2.0 (1.5–2.9)	2.0 (1.5–2.9)	0.969
sICAM-1, ng/mL	227 (199–258)	235 (200–269)	0.057

Data are presented as mean ± SD or as median (25th–75th percentiles). hsCRP, high-sensitivity C-reactive protein; IFG/IGT, combined impaired fasting glucose/impaired glucose tolerance; i-IFG, isolated impaired fasting glucose; i-IGT, isolated impaired glucose tolerance; NGT, normal glucose tolerance; NSAIDs, nonsteroidal anti-inflammatory drugs. *Adjusted for age and sex. †Defined as a blood pressure of ≥140/90 mmHg or the use of antihypertensive medication in subjects who reported previous diagnoses of hypertension. ‡≥20 g/day for women and ≥40 g/day for men. §Defined as <1 h of physical activity per week during leisure time in either winter or summer. ¶Defined as the presence of impaired bilateral foot vibration perception or impaired bilateral foot-pressure sensation (or both). ‖Based on 61 participants with MNSI score ≤2 and 68 participants with MNSI score >2. **Participants with known diabetes were excluded because they did not undergo an OGTT; based on 583 participants with MNSI score ≤2 and 314 participants with MNSI score >2.

syndrome as potential confounders. Serum levels of sICAM-1 were associated with MNSI score and ankle reflexes in our first sensitivity analysis, which is, again, in line with results of a previous study involving patients with diabetes (10), but it represents a novel finding because of the different study population. Associations between C-reactive protein and TNF-α and DSPN or MNSI in patients with diabetes (9,10) could not be corroborated in the general population.

Overall, our data indicate that inflammatory processes contribute to the pathogenesis of DSPN (31), and that anti-inflammatory therapy may be beneficial for its treatment (7). Recombinant IL-1RA was shown in a randomized clinical trial to improve glycemia and to attenuate subclinical inflammation in patients with long-standing type 2 diabetes (32). Moreover, IL-1β inhibition has been tested to attenuate inflammatory processes in atherosclerosis (33), and future research may be helpful to investigate the potential for comparable approaches in individuals at high risk for DSPN.

Inflammation also has been linked to the autonomic nervous system. In a small group of subjects with newly diagnosed type 2 diabetes, IL-6 was higher and the high-molecular-weight adiponectin-to-leptin ratio was lower than in control subjects. IL-6 was inversely related to parasympathetic activity (34). In middle-aged men free of cardiovascular disease participating in the Twins Heart Study, autonomic nerve dysfunction detected by reduced heart rate variability was associated with higher levels of IL-6 and C-reactive protein even after controlling for cardiovascular risk factors (35). Autonomic dysregulation leading to subclinical inflammation may represent one pathway through which traditional risk factors promote the development of coronary artery disease. However, neuropathic pain is mediated through neuroinflammatory mechanisms affecting nervous system tissue that is controlled by inflammatory responses to the initial insult (36). Higher systemic levels of C-reactive protein and sICAM-1 have been associated with painful compared with painless neuropathy in patients with diabetes (10), but a detailed investigation of this association was not the focus of the current study. We previously reported a strong association between prevalent polyneuropathy and peripheral arterial disease (37). Thus, it is tempting to speculate that subclinical inflammation not only contributes to atherosclerosis but also may constitute a "common ground" between the latter and alterations in the nervous system.

Our study provides a prevalence estimate of 13.9% for clinical DSPN in the KORA F4 study participants 61 to 82 years of age. Thus, the prevalence that we

Table 3—*Associations between immune mediators and clinical DSPN, MNSI examination score, and individual measures of neuropathic impairment*

		Outcome variables									
		Clinical DSPN		MNSI (continuous)		Ankle reflexes		Pressure sensation		Vibration sensation	
Immune mediators	Model	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
IL-1RA	1	**0.53**	**0.011**	**0.42**	**<0.001**	**0.14**	**0.003**	**−0.26**	**0.021**	**−0.30**	**0.007**
	2	0.36	0.119	**0.22**	**0.031**	0.02	0.639	−0.12	0.347	**−0.26**	**0.034**
	3	0.40	0.099	**0.21**	**0.034**	0.02	0.669	−0.13	0.276	**−0.26**	**0.038**
Adiponectin	1	−0.17	0.323	−0.05	0.490	0.01	0.726	0.11	0.252	0.05	0.611
	2	−0.01	0.968	0.09	0.251	0.07	0.066	0.04	0.690	−0.01	0.921
	3	−0.02	0.921	0.09	0.269	0.07	0.063	0.05	0.592	−0.01	0.932
hsCRP	1	0.03	0.756	0.06	0.128	0.01	0.522	−0.07	0.147	−0.01	0.815
	2	0.03	0.800	0.01	0.762	−0.02	0.379	−0.03	0.500	−0.01	0.825
	3	0.03	0.756	0.01	0.724	−0.02	0.389	−0.05	0.349	−0.01	0.788
IL-6	1	0.03	0.807	**0.22**	**<0.001**	**0.08**	**0.007**	**−0.17**	**0.022**	−0.11	0.160
	2	−0.10	0.517	**0.13**	**0.042**	0.03	0.275	−0.10	0.207	−0.07	0.346
	3	−0.11	0.475	**0.13**	**0.035**	0.04	0.267	−0.10	0.207	−0.08	0.316
IL-18	1	0.01	0.949	**0.21**	**0.033**	0.06	0.188	0.03	0.835	−0.22	0.077
	2	−0.12	0.601	0.09	0.362	0.01	0.765	0.11	0.345	−0.14	0.230
	3	−0.16	0.515	0.09	0.375	0.01	0.773	0.10	0.385	−0.14	0.237
TNF-α	1	0.15	0.303	0.07	0.280	0.01	0.686	−0.02	0.814	−0.10	0.209
	2	0.12	0.411	0.03	0.633	−0.01	0.842	0.01	0.925	−0.10	0.191
	3	0.16	0.305	0.04	0.539	−0.01	0.932	−0.01	0.864	−0.12	0.132
sICAM-1	1	−0.08	0.857	**0.35**	**0.045**	**0.18**	**0.036**	−0.21	0.317	−0.27	0.208
	2	−0.22	0.588	0.25	0.151	0.12	0.161	−0.06	0.783	−0.31	0.152
	3	−0.21	0.617	0.26	0.135	0.13	0.155	−0.12	0.575	−0.32	0.137

N = 1,049. For clinical DSPN, regression coefficients (β) and *P* values are calculated using logistic regression modeling for all other end points using linear regression modeling. Bold print indicates statistically significant associations. Model 1: adjusted for age and sex. Model 2: model 1 + waist circumference (cm), height (cm), hypertension (yes/no), total cholesterol (mg/dL), smoking (never/former/current), alcohol intake (abstain/moderate/high), and physical activity (active/inactive). Model 3: model 2 + history of myocardial infarction or stroke (or both), presence of neurological conditions that might cause nerve damage, and use of nonsteroidal anti-inflammatory drugs. hsCRP, high-sensitivity C-reactive protein.

found is higher than the prevalence of distal symmetric neuropathies (7.0%) observed in the Italian Longitudinal Study on Aging (ILSA) (38). The ILSA study design is comparable with ours because the ILSA also was population-based and examined a similar range (65–84 years of age). However, the ILSA diagnosis of neuropathy was based on a two-step procedure consisting of an interview and a brief neurological examination (without measurement of the vibration perception threshold, which was included in KORA F4) as the first step and a more detailed clinical examination of all individuals who had positive screening results after the first step, but without the option of ascertaining individuals with negative screening results. These methodological differences and the distinct risk factor profile (lower prevalence of diabetes and lower BMI in ILSA) (39) may have contributed to the almost two-fold difference in the prevalence estimates between both studies.

With respect to determinants of DSPN, our study design was cross-sectional; therefore, causal risk factors cannot be identified. Age, height, waist circumferences, known diabetes, and HbA_{1c} were positively associated with presence of clinical DSPN and MNSI score >2, as was expected. The finding of lower total and LDL cholesterol in subjects with clinical DSPN was surprising. These differences were statistically significant after adjustment for age and sex, as shown in Tables 1 and 2, and persisted after further adjustment for lipid-lowering drugs. However, inclusion of further risk factors of DSPN in the regression models indicated that both cholesterol variables were not independently associated with DSPN (data not shown).

Strengths of our study include the large population-based sample that was well-phenotyped with respect to DSPN and subclinical inflammation, and the statistical analysis that adjusted for a range of potential confounders. Limitations include the cross-sectional design and the fact that data were not generalizable to other ethnic groups or younger age ranges. Moreover, our definition of DSPN only satisfies the minimal diagnostic criteria for possible DSPN, not for probable or confirmed DSPN, according to the Toronto Diabetic Neuropathy Expert Group (1), and also corresponds to the lowest likelihood of DSPN according to the report of the American Academy of Neurology (40). We did not adjust for multiple testing because our outcomes and the immune mediators investigated were not independent of each other, so Bonferroni correction would have been too conservative. However, we acknowledge that our main findings need replication in other samples. Our results are independent of myocardial infarction and stroke as important potential confounders, but we cannot entirely exclude that other age-related conditions may have affected our findings.

In conclusion, we observed associations between serum IL-1RA, IL-6, and specific manifestations of DSPN, indicating the presence of proinflammatory and anti-inflammatory processes in DSPN in the older population. Prospective and intervention studies are needed to understand

the temporal sequence and causality of the relationship between subclinical inflammation and DSPN.

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C.H. planned the study, contributed to data analysis and interpretation, and wrote the manuscript. B.W.C.B. performed data analysis and wrote the manuscript. W.R. planned the study, contributed to data analysis and interpretation, and reviewed and edited the manuscript. M.H. contributed data and reviewed and edited the manuscript. B.K. reviewed and edited the manuscript. W.K. contributed data and reviewed and edited the manuscript. B.T. contributed data and reviewed and edited the manuscript. M.R. reviewed and edited the manuscript. C.M. contributed data and reviewed and edited the manuscript. D.Z. planned the study, contributed to data analysis and interpretation, and reviewed and edited the manuscript. W.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis.

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