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Cardiovascular autonomic neuropathy and the impact on progression of diabetic kidney disease in type 1 diabetes

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ABSTRACT

Introduction We investigated the association between cardiovascular autonomic neuropathy (CAN) and decline in kidney function in type 1 diabetes.

Research design and methods We included 329 persons with type 1 diabetes. CAN was assessed by cardiovascular reflex tests (CARTs): heart rate response to deep breathing (E/I ratio), to standing (30/15 ratio) and to the Valsalva maneuvre. Two or more pathological CARTs defined CAN diagnosis. Outcomes were yearly change in albuminuria or yearly change in estimated glomerular filtration rate (eGFR). An endpoint of eGFR decline >30%, development of end-stage kidney disease (ESKD) or death was examined.

Associations were assessed by linear and Cox regression.

Results Participants were aged 55.2 (9.4) years, 52% were male, with a diabetes duration of 40.1 (8.9) years, HbA_{1c} of 7.9% (62.5 mmol/mol), eGFR 77.9 (27.7) mL/min/1.73 m^2, urinary albumin excretion rate of 14.5 (7–58) mg/24 hours, and 31% were diagnosed with CAN.

CAN was associated with a 7.8% higher albuminuria increase per year (95% CI: 0.50% to 15.63%, p=0.036) versus no CAN. The endpoint of ESKD, all-cause mortality and ≥30% decline in eGFR was associated with CAN (HR=2.497, p=0.0254).

Conclusion CAN and sympathetic dysfunction were associated with increase in albuminuria in individuals with type 1 diabetes suggesting its role as a potential marker of diabetic kidney disease progression.

SIGNIFICANCE OF THIS STUDY

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ Cardiovascular autonomic neuropathy has been associated with the development of diabetic kidney disease and death in patients with and without diabetes. However, cardiovascular autonomic neuropathy as diagnosed with the golden standard measurements of cardiac autonomic reflex tests has never been examined before in relation to diabetic kidney disease.

WHAT ARE THE NEW FINDINGS?

⇒ We investigated associations of cardiovascular autonomic neuropathy and markers of diabetic kidney disease in a case–control cohort of 329 patients from Steno Diabetes Center.

⇒ During a median (IQR) follow-up of 6.1 (5.8–6.5) years, participants suffering from cardiovascular autonomic neuropathy had a 7.8% higher albuminuria increase per year (95% CI: 0.50% to 15.63%, p=0.036) compared with no cardiovascular autonomic neuropathy after adjustment for confounders.

⇒ A composite endpoint of end-stage kidney disease, all-cause mortality and ≥30% decline in estimated glomerular filtration rate was associated with cardiovascular autonomic neuropathy (HR=2.497, p=0.0254).

HOW MIGHT THESE RESULTS CHANGE THE FOCUS OF RESEARCH OR CLINICAL PRACTICE?

⇒ The presence of cardiovascular autonomic neuropathy is associated with risk markers for the progression of diabetic kidney disease. Cardiovascular autonomic neuropathy is easily diagnosed with four simple tests. As such, screening patients for cardiovascular autonomic neuropathy may lead to earlier diagnosis of patients with type 1 diabetes at risk of developing diabetic kidney disease.

INTRODUCTION

Type 1 diabetes is associated with an increased risk of developing microvascular complications such as retinopathy, nephropathy and neuropathy, along with macrovascular complications such as ischemic cardiovascular disease. Individuals with type 1 diabetes are at increased risk of renal complications due to presence of hyperglycemia and hypertension compared with healthy individuals. Diabetes is the leading cause of end-stage kidney disease (ESKD) in the USA.[1] Chronic kidney disease (CKD) in diabetes or diabetic kidney disease (DKD) may be manifested as increased blood pressure, increased urinary albumin excretion rate (UAER) and decreased glomerular filtration rate (GFR).[1] Even mild degrees of

DKD are associated with increased risk of death.[2] Despite substantial improvements in glycemic control and management of other risk factors such as hypertension over the years,[3] DKD prevention remains a challenge.[1 4–7]

Presence of diabetic autonomic neuropathy, as measured using markers for cardiovascular autonomic neuropathy (CAN) is cross-sectionally and temporally associated with DKD.[8 9] The prevalence of CAN in populations with diabetes ranges from around 20%[10] and up to 65% in persons with longstanding diabetes.[11] It has been shown that associations exist between CAN, especially increased sympathetic tone of the autonomic nervous system, and the development of DKD. It has also been suggested that CAN may be a part of the pathophysiology leading to DKD, or alternatively that the conditions occur together because of shared risk factors.[8 12] Sympathetic dysfunction is seen early in the onset of CAN.[13 14]

The objective of this study was to investigate the possible association between CAN, assessed by robust and internationally recognized indices, and future progression of DKD including increase in albuminuria, decline in renal function and a composite outcome comprising ESKD, all-cause mortality and ≥30% decline in estimated GFR (eGFR), in a cohort of persons with type 1 diabetes followed prospectively to allow identification of baseline risk markers of future progression of DKD.

RESEARCH DESIGN AND METHODS

This study is based on data from an original cohort of 900 participants with type 1 diabetes included in a case–control study at Steno Diabetes Center Copenhagen (conducted from 1993 to 2001). The cohort setup is described in depth previously.[15] These subjects were recruited based on either longstanding normoalbuminuria (control) or DKD with persistent albumin excretion above 300 mg/24 hours in their history (cases). Of the 900 subjects in the original cohort, 571 were alive at the time of a cross-sectional follow-up study in 2009–2010. The follow-up study constitutes the baseline for the present analyses. In total, 375 subjects responded to a study invitation of whom 20 were excluded for severe comorbidities such as cancer or non-DKD. A register-based follow-up was made in 2016 using the Danish national healthcare registries and local medical records.

Analysis of CAN

CAN assessments were performed at the subjects' baseline visit. Subjects rested lying down for 5 min in a quiet room at room temperature (18°C–23°C) before assessment of their CAN status. CAN assessment included 2-minute heart rate variability and a cardiac autonomic reflex measure using SD of normal to normal intervals (SDNNs). Standardized cardiovascular reflex test (CART) measurements were done with the following three measurements: lying to standing test (30/15 ratio), deep breathing test (E/I ratio) and the Valsalva maneuver. CART and SDNN measures were analyzed as continuous variables and as binary variables with age-stratified cut-off values defined by Cardone.[16] The diagnosis of CAN was defined as two or more pathological CART measurements as per the recommendation of the American Diabetes Association.[17] Subjects with two or more abnormal CARTs were considered to have CAN. Subjects with two or more normal CARTs were considered not to have CAN. Subjects with one or no CART measurements were classified as 'no CAN estimation'. Resting heart rate, SDNN and CARTs were recorded by trained technicians using a Vagus device (Medicus Engineering, Aarhus, Denmark).

Anthropometric, blood pressure and lifestyle measures

Height and weight were measured with light indoor clothing, without shoes, using a fixed rigid stadiometer (Seca, Chino, California, USA) and an electronic scale (Mettler Toledo, Glostrup, Denmark), respectively.[18]

Oscillometric (UA787; A&D Medical, Abingdon, UK) office blood pressure was measured in a supine position after a 15-minute rest using an appropriate cuff size. Three measurements were obtained and averaged.[18]

Lifestyle measures were obtained by questionnaires. Participants were classified as current smokers if they smoked ≥1 cigarette, cigar or pipe per day, and all others were classified as non-smokers. Physical activity was defined as being regularly physically active or not.[18]

Biochemical measures

HbA_{1c} was measured using high-performance liquid chromatography (Variant; Bio-Rad Laboratories, Munich, Germany) and serum creatinine concentration using an enzymatic method (Hitachi 912; Roche Diagnostics, Mannheim, Germany). UAER was measured in three consecutive 24-hour urine collections by an enzyme immunoassay. The CKD Epidemiology Collaboration Equation was used to calculate eGFR from plasma creatinine.[18]

Endpoint assessment

Details on the assessment of endpoints have previously been published.[19] Briefly, all participants were traced with no lost to follow-up in the Danish National Death and Health Registries on December 31, 2016. Information on cause of death was available until December 31, 2015. Participants were also traced in the electronic laboratory records for data on eGFR and urine albumin to creatinine ratio (UACR) obtained at regular outpatient visits.

Incident ESKD was defined as CKD stage 5 (International Classification of Diseases (ICD-10) N18.5), chronic dialysis (procedural code BJFD2), kidney transplantation (procedural code KKAS 00, 10, and

20), or eGFR <15 mL/min/1.73 m^2. The composite endpoint consisted of ESKD, >30% decline in eGFR from baseline, and all-cause mortality. The combined endpoint was included based on recent trends in larger studies to include this specific composite endpoint. The yearly change in UACR was calculated based on all available measurements from outpatient visits during follow-up, in participants with at least two measurements and a minimum follow-up time of 3 years. Decline in eGFR was assessed as time to the first occurrence of ≥30% decrease from baseline without requiring confirmation and as yearly change

Table 1 Baseline characteristics

	No CAN (n=181)	Definite CAN (n=101)	P for difference between no CAN and definite CAN	No CAN estimation (n=47)
Men, n (%)	86 (47.5)	57 (56.4)	0.086	27 (57.4)
Age, years	55.4 (9.7)	53.9 (7.7)	0.608	60.1 (10.0)
HbA_{1c}, mmol/mol	60.9 (9.8)	66.3 (12.1)	0.008	60.9 (11.1)
HbA_{1c}, %	7.7 (0.9)	8.2 (1.1)	0.008	7.7 (1.0)
Body mass index, kg/m^2	24.8 (3.7)	25.0 (4.4)	0.888	24.3 (3.1)
Systolic blood pressure, mm Hg	131.0 (17.5)	136.7 (18.5)	0.002	131.3 (18.4)
Diastolic blood pressure, mm Hg	74.1 (8.8)	74.8 (9.3)	0.795	72.6 (8.7)
Diabetes duration, years	38.6 (8.6)	40.9 (8.3)	0.003	44.0 (10.2)
Regular exercise, n (%)	136 (75.1)	64 (63.4)	0.107	31 (66)
eGFR, mL/min/1.73 m^2	84.4 (21.2)	63.9 (28.3)	<0.001	75.6 (24.8)
Urinary albumin excretion rate, mg/24 hours	9.5 (6–24)	44.3 (13–314.0)	<0.001	11.0 (6.66)
Microalbuminuria 30–300 mg/24 hours (n)	21 (11)	27 (27)	0.064	15 (32)
Macroalbuminuria >300 mg/24 hours (n)	11 (6)	22 (22)	0.493	3 (6)
LDL cholesterol, mmol/L	2.4 (0.7)	2.4 (0.7)	0.542	2.5 (0.7)
Chronic kidney disease (CKD) classification category, n (%)				
Normal: eGFR ≥90 mL/min/1.73 m^2	103 (56.9)	26 (25.7)	<0.001	17 (36.2)
Mild CKD: 60≥eGFR<90 mL/min/1.73 m^2	55 (30.4)	29 (28.7)	0.991	15 (31.9)
Moderate CKD: 30≥eGFR<60 mL/min/1.73 m^2	19 (10.5)	32 (31.7)	0.028	10 (21.3)
Severe CKD: 15≥eGFR<30 mL/min/1.73 m^2	4 (2.2)	11 (10.9)	<0.001	4 (8.5)
Kidney failure: eGFR <15 mL/min/1.73 m^2	0 (0)	3 (3.0)	0.117	1 (2.1)
Medication, n (%)				
Beta-blockers	13 (7.4)	20 (20.4)	<0.001	9 (19.1)
RAAS blockers	176 (97.2)	97 (96)	<0.001	9 (19.1)
Statins	93 (54.1)	72 (80.9)	0.002	22 (50)
Autonomic function measures				
E/I ratio	1.2 (1.1–1.3)	1.0 (1.0–1.1)	<0.001	1.1 (1.1–1.2)
30/15 ratio	1.1 (1.1–1.2)	1.0 (1.0–1.0)	<0.001	1.1 (1.0–1.1)
Valsalva maneuver	1.5 (1.4–1.8)	1.2 (1.1–1.3)	<0.001	1.5 (1.2–1.8)
SDNN, ms	30.5 (20.5–42.5)	10.0 (7–15)	<0.001	17.0 (13–33)
Heart rate, beats/min	66.0 (10.6)	75.6 (12.2)	<0.001	68.7 (12.1)

No CAN estimation: subject with one or no CAN measurements.
CAN, cardiovascular autonomic neuropathy; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; E/I ratio, expiration/inspiration ratio; LDL, low-density lipoprotein; RAAS, Renin–Angiotensin–Aldosterone System; SDNN, SD of normal to normal interval.

Table 2 Presence of CAN versus yearly change in eGFR (mL/min/1.73 m^2) and UACR (%)

	N	Outcomes	Estimate (95% CI)	P value
Model 1	220	UACR	9.905 (3.04 to 17.23)	0.004
	224	eGFR	−0.263 (−0.98 to 0.45)	0.469
Model 2	220	UACR	11.820 (4.95 to 19.14)	0.001
	224	eGFR	−0.138 (−0.850 to 0.57)	0.703
Model 3	219	UACR	11.241 (4.06 to 18.92)	0.002
	223	eGFR	0.328 (−0.38 to 1.04)	0.366
Model 4	217	UACR	7.484 (0.45 to 15.01)	0.037
	222	eGFR	0.294 (−0.461 to 1.05)	0.445
Model 5	194	UACR	7.800 (0.50 to 15.63)	0.036
	193	eGFR	0.324 (−0.486 to 1.13)	0.433
Model 6	167	UACR	7.664 (*−0.093 to 16.02*)	0.052
	165	eGFR	0.272 (*−0.558 to 1.103*)	0.520

Model 1: unadjusted. Model 2: adjusted for age and sex. Model 3: as model 2 and additionally adjusted for duration of diabetes, Hba_{1c}, BMI, smoking, exercise, beta-blocker use, LDL cholesterol and systolic blood pressure. Model 4: as model 3, and additionally adjusted for baseline eGFR. Model 5: as model 4 and additionally adjusted for urinary albumin excretion rate.
BMI, body mass index; CAN, cardiovascular autonomic neuropathy; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; UACR, urine albumin to creatinine ratio.

in eGFR from available measurements from outpatient visits.

Statistical analysis

Characteristics are presented as means with SD, medians with IQRs, or as percentages depending on measurement format. Linear regression was performed to assess associations between CAN and DKD parameters (UAER and eGFR). Indices of CAN were evaluated as continuous measurements. The correlation between CAN at baseline and renal outcomes was examined using five distinct models for confounder adjustment. Model 1 was unadjusted. Model 2 included adjustment for age and sex. Model 3 as model 2 with additional adjustment for diabetes duration, HbA_{1c}, body mass index, smoking, exercise, beta-blocker use, low-density lipoprotein cholesterol level and systolic blood pressure. Model 4 as model 3 with additional adjustment for eGFR. Model 5 as model 4 with additional adjustment for baseline UAER. Additionally, a sensitivity analysis, model 6, was performed by adjusting for Renin–Angiotensin–Aldosterone System (RAAS) in addition to model 5 adjustments.

Group (CAN vs no CAN) differences between continuous baseline variables were assessed by Student's t-test. The X^2 test was used for categorical variables. Linear regression was performed for both standardized CART values and non-standardized estimates of CAN status and CART values to assess yearly changes in UACR and eGFR, respectively. For standardized estimates, determinants were standardized by dividing the determinant by the SD of the given determinant. Cox regression analysis was applied to assess the association between CAN status and CART values and risk of ESKD and the composite renal endpoint.

A complete case analysis approach was used, and the two-sided level of significance was set at 5%. Analyses were performed using SAS Enterprise Guide V.7.15 HF7 (SAS Institute). eGFR and UACR slopes were estimated using general linear modeling in R (www.r-project.org) as reported previously (PMID: 31705008).

RESULTS

A total of 355 participants, of which 26 were outside the age range of validated CAN measurements (20–80 years of age), were eligible for the study (table 1). No subjects were excluded based on missing confounder variables. This left us with 329 subjects included in the analysis. At baseline, participants' mean (SD) age was 55.2 (9.4) years, 52% were male, with diabetes duration of 40.1 (8.9) years, HbA_{1c} of 62.5 (11.0) mmol/mol, 7.9%, eGFR 77.9 (27.7) mL/min/1.73 m^2, median (IQR) UAER of 14.5 (7.0–59.5) mg/24 hours and 31% were diagnosed with CAN. For subjects without CAN, 11 had macroalbuminuria, and 21 had microalbuminuria. For subjects with CAN, these numbers were 22 and 27, respectively. Median (IQR) follow-up time was 6.1 (5.8–6.5) years. During follow-up, 18 subjects died and 10 subjects developed ESKD. A total of 44 subjects reached the composite endpoint of ESKD, all-cause mortality or ≥30% decline in eGFR.

Yearly changes in eGFR were −0.73 mL/min/1.73 m^2 and −0.99 mL/min/1.73 m^2, respectively, for no CAN versus CAN group (p=0.54). Yearly changes in UACR were a 2% decrease and a 7% increase for no CAN versus CAN group (p=0.01), respectively.

In the adjusted model 5, participants with CAN had an increase in albuminuria of 7.80 percentage points per

Table 3 Adjusted* continuous CAN indices versus slope of GFR and UACR

	N	Parameter	Outcomes	Estimate (95% CI)	P value
Model 1	141	Valsalva	UACR	−0.04 (0.02 to −0.07)	0.031
	144	Valsalva	GFR	0.108 (−0.307 to 0.522)	0.611
	208	30/15 ratio	UACR	−0.04 (−0.07 to 0.0003)	0.053
	212	30/15 ratio	GFR	0.058 (−0.271 to 0.387)	0.730
	211	E/I ratio	UACR	−0.04 (−0.07 to −0.01)	0.010
	215	E/I ratio	GFR	0.215 (−0.149 to 0.588)	0.247
	213	SDNN	UACR	−0.04 (−0.07 to −0.003)	0.047
	217	SDNN	GFR	0.171 (−0.228 to 0.57)	0.401
Model 2	141	Valsalva	UACR	−0.031 (−0.07 to 0.01)	0.097
	144	Valsalva	GFR	0.057 (−0.364 to 0.478)	0.790
	208	30/15 ratio	UACR	−0.042 (−0.07 to −0.02)	0.003
	212	30/15 ratio	GFR	0.050 (−0.276 to 0.376)	0.763
	211	E/I ratio	UACR	−0.039 (−0.07 to −0.01)	0.014
	215	E/I ratio	GFR	0.222 (−0.145 to 0.589)	0.234
	213	SDNN	UACR	−0.034 (−0.068 to −0.001)	0.054
	217	SDNN	GFR	0.156 (−0.243 to 0.554)	0.444
Model 3	141	Valsalva	UACR	−0.036 (−0.07 to 0.002)	0.061
	144	Valsalva	GFR	−0.118 (−0.538 to 0.303)	0.582
	207	30/15 ratio	UACR	−0.041 (−0.07 to −0.013)	0.004
	211	30/15 ratio	GFR	−0.046 (−0.366 to 0.274)	0.779
	210	E/I ratio	UACR	−0.031 (−0.064 to 0.002)	0.069
	214	E/I ratio	GFR	0.003 (−0.376 to 0.382)	0.988
	212	SDNN	UACR	−0.03 (−0.07 to 0.05)	0.090
	216	SDNN	GFR	−0.084 (−0.484 to 0.315)	0.679
Model 4	141	Valsalva	UACR	−0.034 (−0.07 to −0.004)	0.077
	144	Valsalva	GFR	−0.034 (−0.447 to 0.378)	0.870
	205	30/15 ratio	UACR	−0.034 (−0.06 to −0.006)	0.017
	210	30/15 ratio	GFR	−0.025 (−0.350 to 0.301)	0.882
	208	E/I ratio	UACR	−0.016 (−0.05 to 0.017)	0.344
	213	E/I ratio	GFR	0.037 (−0.361 to 0.435)	0.855
	210	SDNN	UACR	−0.018 (−0.05 to 0.02)	0.322
	215	SDNN	GFR	−0.045 (−0.460 to 0.369)	0.830
Model 5	129	Valsalva	UACR	−0.039 (−0.07 to −0.001)	0.044
	126	Valsalva	GFR	0.120 (−0.318 to 0.558)	0.591
	185	30/15 ratio	UACR	−0.033 (−0.06 to −0.005)	0.023
	184	30/15 ratio	GFR	−0.057 (−0.395 to 0.282)	0.742
	185	E/I ratio	UACR	−0.018 (−0.053 to 0.017)	0.317
	184	E/I ratio	GFR	0.008 (−0.424 to 0.439)	0.972
	187	SDNN	UACR	−0.021 (−0.06 to 0.017)	0.262
	186	SDNN	GFR	−0.186 (−0.625 to 0.253)	0.406

Continued

Table 3 Continued

	N	Parameter	Outcomes	Estimate (95% CI)	P value
Model 6	129	Valsalva	UACR	−0.021 (−0.058 to 0.016)	0.275
	126	Valsalva	GFR	0.078 (−0.373 to 0.529)	0.734
	185	30/15 ratio	UACR	−0.032 (−0.060 to −0.004)	0.027
	184	30/15 ratio	GFR	−0.059 (−0.398 to 0.281)	0.735
	185	E/I ratio	UACR	−0.016 (−0.053 to 0.021)	0.401
	184	E/I ratio	GFR	−0.013 (−0.459 to 0.433)	0.956
	187	SDNN	UACR	−0.021 (−0.058 to 0.016)	0.275
	186	SDNN	GFR	−0.193 (−0.633 to 0.248)	0.392

Model 1: unadjusted. Model 2: adjusted for age and sex. Model 3: as model 2 and additionally adjusted for duration of diabetes, Hba_{1c}, BMI, smoking, exercise, beta-blocker use, LDL cholesterol and systolic blood pressure. Model 4: as model 3, and additionally adjusted for baseline estimated GFR. Model 5: as model 4 and additionally adjusted for urinary albumin excretion rate.
*Estimates are for change in standardized units (95% CI).
BMI, body mass index; CAN, cardiovascular autonomic neuropathy; E/I ratio, expiration/inspiration ratio; GFR, glomerular filtration rate; LDL, low-density lipoprotein; SDNN, SD of normal to normal interval; UACR, urine albumin to creatinine ratio.

year (95% CI: 0.50 to 15.63, p=0.036) compared with subjects without CAN. There was no correlation between CAN status and change in eGFR in any model of adjustment (table 2).

CAN indices, CARTs and SDNN were analyzed with respect to yearly change in UACR and eGFR. In the unadjusted model, all indices of CAN were negatively associated to changes in UACR (online supplemental table 1). Lower values of the CARTs mainly representing sympathetic autonomic function (Valsalva, 30/15 ratio) remained significantly associated with increase of UACR over time at all levels of statistical adjustments. A one-unit lower (more detrimental) Valsalva and 30/15 ratio was associated with an annual UACR decline of 11.8% (95% CI: 0.76% to 24%, p=0.037) and 30.2% (95% CI: 2.53% to 65.45%, p=0.037), respectively (online supplemental table 1). Standardized regression coefficients for CAN measures did not differ in significance from non-standardized measurements in models 4 and 5 (table 3). Presence of the CAN diagnosis or indices of CAN were not associated with decline in eGFR (table 2).

CAN status and binary CARTs were not associated with the development of ESKD (online supplemental table 2). Adjustment as in model 5 was not possible due to too few events. An analysis of CAN diagnosis and binary CARTs versus the composite endpoint of ESKD, all-cause mortality and a ≥30% decline in eGFR from baseline revealed a significant association in the adjusted models; for CAN the HR was 2.497 (95% CI: 1.119 to 5.571, p=0.0254), whereas it was not significant for 30/15 ratio, E/I ratio and Valsalva (table 4).

CONCLUSION

In this cohort of 329 persons with type 1 diabetes, we found that presence of CAN was associated with progression in DKD when assessed by increase in albuminuria, but not with decline in renal function (eGFR). The analysis of the composite endpoint 30% decline in eGFR, kidney failure or death revealed an association with CAN status, whereby subjects with CAN had a higher risk of reaching the composite endpoint. These associations were independent of traditional risk factors for DKD including baseline HbA_{1c} UACR, eGFR and blood pressure.

Other studies have shown a similar association between CAN and decline in UACR for both persons with type 1 and 2 diabetes independent of other confounding factors such as glycemic control, blood pressure regulation and diabetes duration.[8 9 11 13] In the current study, we demonstrated an association between CARTs primarily associated with sympathetic nervous function (Valsalva and 30/15 ratio) and future increases in albuminuria. Forsen *et al* previously demonstrated a correlation between E/I ratio and UACR, which was not apparent in our data although we could demonstrate an association with other CARTs.[9] There was no significant association between any CAN measures and changes in eGFR. CAN status (as examined using heart rate variability) has been reported to be associated with decline in eGFR, but not UACR in type 1 diabetes.[20] In accordance with our findings, Lu *et al* found associations between CARTs and UACR, but not between CARTs and eGFR.[21] Orlov *et al* found an association between CAN status and advanced progressive kidney failure defined as CKD stage ≥3 in a large cohort study of persons living with type 1 diabetes.[20] The lacking associations between CAN and development of ESKD in our study may be due to a low number of cases.

DKD can be seen as consisting of two dimensions, a decline in eGFR, and an increase in albuminuria. Albuminuria is thought to be caused due to endothelial damage in the kidneys with glomerular leakage of albumin. A decline in eGFR is seen as a result of interstitial fibrosis in the kidneys. There is no direct correlation

Table 4 CAN and CARTs versus ESKD, all-cause mortality and ≥30% decline in eGFR from baseline

	Events	Outcomes	HR (95% CI)	P value
Model 1	44	CAN	6.352 (3.268 to 12.346)	<0.0001
	44	E/I ratio	5.035 (2.487 to 10.193)	<0.0001
	26	Valsalva	7.103 (3.163 to 15.951)	<0.0001
	43	30/15 ratio	3.832 (2.045 to 7.179)	<0.0001
Model 2	44	CAN	6.843 (3.493 to 13.404)	<0.0001
	44	E/I ratio	5.255 (2.586 to 10.677)	<0.0001
	26	Valsalva	7.453 (3.278 to 16.945)	<0.0001
	43	30/15 ratio	4.228 (2.218 to 8.060)	<0.0001
Model 3	44	CAN	4.957 (2.410 to 10.194)	<0.0001
	44	E/I ratio	3.983 (1.856 to 8.545)	0.0004
	26	Valsalva	4.194 (1.582 to 11.117)	0.0039
	43	30/15 ratio	3.887 (1.905 to 7.933)	0.0002
Model 4	44	CAN	3.138 (1.472 to 6.690)	0.0031
	44	E/I ratio	2.411 (1.077 to 5.4)	0.0324
	26	Valsalva	2.641 (0.975 to 7.159)	0.0562
	43	30/15 ratio	2.459 (1.165 to 5.194)	0.0183
Model 5	39	CAN	2.497 (1.119 to 5.571)	0.0254
	39	E/I ratio	1.811 (0.776 to 4.226)	0.1693
	24	Valsalva	1.903 (0.668 to 5.421)	0.2284
	43	30/15 ratio	2.223 (0.990 to 4.990)	0.0529
Model 6	23	CAN	2.807 (0.874 to 9.014)	0.0829
	23	E/I Ratio	2.520 (0.600 to 10.585)	0.2070
	23	Valsalva	1.903 (0.769 to 6.763)	0.1373
	23	30/15 ratio	2.223 (0.493 to 3.775)	0.5496

Model 1: unadjusted. Model 2: adjusted for age and sex. Model 3: as model 2 and additionally adjusted for duration of diabetes, Hba_{1c}, BMI, smoking, exercise, beta-blocker use, LDL cholesterol and systolic blood pressure. Model 4: as model 3, and additionally adjusted for baseline eGFR. Model 5: as model 4 and additionally adjusted for urinary albumin excretion rate. CARTs were evaluated as binary variables based on age-specific cut-off values.
BMI, body mass index; CAN, cardiovascular autonomic neuropathy; CARTs, cardiac autonomic reflex tests; eGFR, estimated glomerular filtration rate; E/I ratio, expiration/inspiration ratio; ESKD, end-stage kidney disease; LDL, low-density lipoprotein.

between albuminuria and eGFR, and as such, patients are capable of having one, without the other.[22] An association between neuropathy and progression of DKD can be due to shared risk factors such as hyperglycemia. It has also been suggested that neuropathy is involved in the pathogenesis of DKD. CAN is associated with a lack of nocturnal dipping in blood pressure, which is related to a decline in kidney function.[23] During the early stages of CAN, a decrease in cardiac autonomic parasympathetic tone and an increase in sympathetic tone are seen.[14] The increased activation of the sympathetic nervous system results in higher levels of circulating catecholamines, which results in higher blood pressure. This increase in blood pressure can lead to increased glomerular pressure, and in turn increased renal damage. The autonomic nervous system has a direct influence on the kidneys through input on the renal vasculature and juxtaglomerular apparatus. Longitudinal studies have shown that higher levels of circulating inflammatory factors may contribute to decline in kidney function.[24] Exploring these possible mechanisms will lead to future studies. One might imagine a setup where the function of the sympathetic nervous system could be improved and the effect on the kidneys reduced, using vagal stimulation methods.[25]

The national healthcare registries provide us with a rare opportunity for following persons throughout their life and treatment regime. This enables us to collect highly valid follow-up information. In this study, we have used the gold standard measurements to evaluate CAN by applying three internationally recommended CARTs.

CAN status diagnosis, as defined by the three recommended CARTs and SDNN measurement, has not previously been used in studies examining subjects for changes in kidney function.

Subjects were not recruited randomly, but rather as part of a follow-up study to an earlier cohort. The cohort studied was originally selected as either longstanding

normoalbuminuria (with low risk of later progression to kidney disease) or established DKD. Thus, short-term normoalbuminuria or microalbuminuria was not included in the cohort and thus there is a selection bias of non-progressive subjects. As such, biases introduced in the recruitment of the cohort could have been carried over.

The applied measurements of CAN in this study are not specific for either the sympathetic or parasympathetic part of the autonomous nervous system, and as such, neither can be ruled out as an influencing factor.

Subjects with normoalbuminuria would not be expected to progress to ESKD, and persons with albuminuria might have already died from complications at the time of re-examination.

There was no correlation found between ESKD, eGFR >30% and CAN, hence the combined endpoint might be driven by all-cause mortality.

A sensitivity analysis of RAAS blockade was performed by additional adjustment for the use of these drugs. Some significant associations were lost. However, it is unclear whether RAAS can be considered a true confounder, as it is unclear whether it impacts both CAN measures and outcomes. RAAS blockade reduces the progression of DKD. However, it is not known if RAAS blockade confounds the possible effect CAN has on kidney disease. The results of RAAS adjustment may indicate that RAAS treatment could ameliorate the effect of CAN on kidney disease. However, such conclusions cannot be drawn due to confounding by indication, Hence, the focus of our analyses has been but on model 5.

Presence of CAN was a risk marker for progression of DKD, when assessed by longitudinal UACR measures and when a composite renal endpoint comprising ESKD, all-cause mortality and decline in eGFR ≥30% was applied, but not annual decline in eGFR, in persons with type 1 diabetes. These correlations were primarily driven by sympathetic nervous function at baseline. The current study identifies the association between CAN and progression of DKD in type 1 diabetes.

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