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Running title

Cardiovascular Autonomic Neuropathy in youth with Type 1 and Type 2 Diabetes

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Cardiovascular Autonomic Neuropathy in Adolescents and Young Adults with Type 1 and Type 2 Diabetes: The SEARCH for Diabetes in Youth Cohort Study.

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ABSTRACT

Aims: To estimate the prevalence of and risk factors for cardiovascular autonomic neuropathy (CAN) in adolescents and young adults with type 1 and type 2 diabetes enrolled in the SEARCH for Diabetes in Youth Study.

Methods: The study included 1646 subjects with type 1 diabetes (age 18 ± 4 years, diabetes duration 8 ± 2 years, HbA1c 9.1 ± 1.9%, 76% Non-Hispanic Whites) and 252 with type 2 diabetes (age 22 ± 4 years, diabetes duration 8 ± 2 years, HbA1c 9.2 ± 3.0%, 45% Non-Hispanic Blacks). Cross-sectional and longitudinal risk factors were assessed at baseline and follow-up visits. Area under the curve (AUC) was used to assess the longitudinal glycemic exposure and cardiovascular risk factors. CAN was assessed by time and frequency domain indices of heart rate variability (HRV). CAN was defined as the presence of e 3 of 5 abnormal HRV indices.

Results: The prevalence of CAN was 12% in adolescents and young adults with type 1 diabetes and 17% in those with type 2 diabetes. Poor long-term glycemic control (AUC HbA1c), high blood pressure, and elevated triglyceride levels were correlates of CAN in subjects with type 1 diabetes. In those with type 2 diabetes, CAN was associated with elevated triglycerides and increased urinary albumin excretion.

Conclusions: The prevalence of CAN in this multiethnic cohort of adolescents and young adults with type 1 and type 2 diabetes are comparable to those reported in adults with diabetes. Suboptimal glycemic control and elevated triglycerides were the modifiable risk factors associated with CAN.

INTRODUCTION

Cardiovascular autonomic neuropathy (CAN) is a serious complication of diabetes affecting the autonomic nerves innervating the heart and blood vessels, with subsequent sympathovagal imbalance and impact on heart rate regulation and cardiac performance (1-3). Although asymptomatic in earlier stages, CAN has been shown to be an independent predictor of cardiovascular disease (CVD) mortality risk (4,5), silent myocardial ischemia (6) and/or major CVD events (7), cardiac remodeling and left ventricular dysfunction (8), and progression of diabetic nephropathy and chronic kidney disease (9,10). Yet, CAN is one of the least recognized complications of diabetes, especially in youth.

Reduction in the heart rate variability (HRV) parameters is the earliest manifestations of CAN. Evaluation of HRV with time and frequency domain indices are non-invasive methods to assess the presence and severity of CAN (2). Several small cross-sectional studies in the various pediatric population, using diverse definitions, have reported CAN prevalence estimates between 18% and 75% (11-15). In one of the largest pediatric epidemiological studies assessing the burden of diabetes-related complications in an Australian cohort of adolescents with diabetes, Eppens and colleagues found strikingly high prevalence of autonomic neuropathy (using pupillometry) in adolescents with type 1 and type 2 diabetes (61% and 57%, respectively) (15).

Apart from the SEARCH study, there have been no systematic efforts to assess the burden of neuropathy, including CAN, in adolescents and young adults with diabetes in the United States. We previously reported that youth with type 1 diabetes have reduced HRV as compared to age-matched healthy controls enrolled in the SEARCH Cardiovascular Disease (CVD) study (16). Although, we have recently reported the age-adjusted prevalence of several diabetic complications (including CAN) in the SEARCH participants as part of the SEARCH Cohort Study (17), the specific objective of this study was

to examine the prevalence of CAN more closely by age group, diabetes duration, gender and race/ethnicity. Moreover, we were specifically interested in examining the cross-sectional and longitudinal risk factors (since diagnosis of diabetes to present time) in adolescents and young adults with and without CAN separately in those with type 1 and type 2 diabetes to better understand the underlying risk factors and the pathological processes that drive CAN in this young cohort.

The overall objectives of the current study were: 1) to estimate the prevalence of CAN in a large, ethnically diverse cohort of adolescents and young adults with type 1 and type 2 diabetes enrolled in the SEARCH for Diabetes in Youth Study by age, gender, diabetes duration and race/ethnicity; and 2) to identify the cross-sectional and longitudinal associations of CAN with anthropometric and metabolic parameters.

METHODS

SEARCH for Diabetes in Youth Study

SEARCH for Diabetes in Youth is a prospective cohort study following children and adolescents of diverse racial and ethnic backgrounds diagnosed with diabetes at less than 20 years of age in the United States of America (USA) (17). SEARCH participants are incident cases of diabetes identified at four geographically defined populations in Ohio, Washington, South Carolina, and Colorado, from health plan enrollees in California, and from Indian Health Service beneficiaries from American Indian populations in Arizona and New Mexico.

Study Population

Adolescents and young adults with diabetes diagnosed at < 20 years of age were identified from a population-derived incident registry network at five USA sites by the SEARCH for Diabetes in Youth Registry Study (17). Cases with newly diagnosed type 1 or type 2 diabetes in 2002-2006 or 2008, who completed a SEARCH baseline examination (on average 9.3 ± 6.4 months from diagnosis) and had at least 5 years of diabetes duration between 2011 and 2015, were recruited into the SEARCH Cohort Study (2011-2015) (on average at 7.9 ± 1.9 years from diagnosis) (**Appendix Figure 1**). Although the parent SEARCH Cohort Study enrolled 2777 individuals, we excluded children < 10 years of age (n=134) at the cohort visit, those with no diabetes antibody measures for the etiological definition of diabetes (n=440), and those with incomplete neuropathy assessment (n=305), which reduced the analytic sample size to 1898 individuals (**Appendix Figure 2**).

Prior to protocol implementation, local institutional review board approval was obtained for each center. Written informed consent was obtained from participants age 18 and older, while assent with parental written informed consent was obtained for participants younger than 18 years.

Baseline and Cohort Visits

The SEARCH baseline and cohort visits included a participant survey; measurement of height, weight, waist circumference, blood pressure; and blood and urine collection. Race and ethnicity were self-reported and categorized as Non-Hispanic White (NHW), Non-Hispanic Black (NHB), Hispanic, Asian or Pacific Islander, and Other. Current cigarette smoking was defined as having smoked cigarettes on e 1 of the 30 days preceding the visit. Individuals who had tried smoking or smoked regularly (at least one cigarette every day for 30 days) but were not current smokers were considered former smokers. Subjects who had never smoked a whole cigarette were considered nonsmokers.

Waist circumference was measured using the natural waist location (17) and divided by height in centimeters to calculate the waist-to-height ratio (WHR). Body mass index (BMI) was defined as weight (kilograms) divided by height (meters)^2. For participants < 20 years of age, the Centers for Disease Control and Prevention (CDC)-derived BMI z20 scores were used; for those e 20 years the observed mean and standard deviation were used to standardize their BMI z20 values.

Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times using an aneroid sphygmomanometer while the participants were seated for at least 5 minutes, and the average of the three measurements was taken.

A blood draw occurred after an 8-hour overnight fast, and medications, including short-acting insulin, were withheld the morning of the visit. Blood samples were obtained under conditions of metabolic stability, as defined by no episodes of diabetic ketoacidosis in the prior month. Specimens were processed locally at the sites and shipped within 24 h to the central laboratory (Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington), where they were analyzed for measurement of high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, and glycated hemoglobin (HbA1c) as previously described (17). Urinary albumin and creatinine levels were assessed on a random spot urine sample to evaluate renal function using the albumin-to-creatinine ratio (ACR). The accuracy of HbA1c data was monitored by participation in the National Glycohemoglobin Standardization Program, and the accuracy and consistency of lipid data were monitored regularly by comparing results obtained by enzymatic methods with those obtained by CDC reference methods (CDC Reference Laboratory) (18).

In addition to a SEARCH baseline and a cohort visit, 57% of participants (n=1082) had one or more intermediate visits (at 1, 2 or 5 years after the baseline visit) at which the same cardio-metabolic risk

factors were measured, including HbA1c, lipids, waist circumference, WHR, and BMI. The assay of biological samples has remained consistent over time.

Diabetes Type

Diabetes type was defined using an etiological classification developed by SEARCH (19) based on diabetes autoantibodies [Glutamate decarboxylase-2 (GAD-65), insulinoma-associated-2 antibodies IA-2A), and Zinc-T8 autoantibody] and estimated insulin sensitivity score (validated equation including waist circumference, HbA1c, and triglyceride levels) at the baseline visit (19). Type 1 diabetes was defined as at least one positive antibody, regardless of insulin sensitivity, or no positive antibodies and insulin sensitivity (score > 8.15). Type 2 diabetes was defined as negative antibodies and insulin resistance (score < 8.15) (19).

Assessment of CAN

CAN was assessed by HRV testing using the SphygmoCor (Atcor, PA). SEARCH staff from each center were centrally trained and certified to perform the HRV test. The HRV tests were performed under standardized conditions that included overnight fasting, avoidance of caffeine and tobacco products for 8 hours before the test, and withholding prescriptions and over-the-counter medicines (except for basal insulin) until testing was completed. Participants underwent a 5-minute continuous electrocardiogram recording while supine after a 10-minute rest. All traces were reviewed and analyzed to ensure R-waves were adequately identified from artifacts and ectopic beats. The term “NN interval” is used instead of RR interval of the ECG to emphasize that the processed beats are normal sinus rhythm (i.e., every QRS complex preceded a P-wave). We analyzed the following time- and frequency-domain HRV parameters from the SphgmoCor device: standard deviation of NN interval (SDNN), root mean square difference of the successive NN interval (RMSSD), high frequency (HF) power, low frequency

(LF) power and LF: HF ratio. SDNN is a measure of overall HRV, while the RMSSD and HF power represent the parasympathetic component of the autonomic system and LF power the sympathetic component. HRV test was considered abnormal if the values were below the 5th percentile observed in 206 age- and sex-matched healthy controls (age 10-28 years, 54% females) from the SEARCH CVD study (16). CAN, as our primary outcome measure, was defined as the presence of e 3 abnormal HRV indices.

Statistical Analysis

Cross-sectional Data: Anthropometric, demographic, and metabolic data collected at the cohort visit as described above were used to compare the characteristics distinguishing adolescents and young adults with and without CAN stratified by diabetes type.

Students t-test and Wilcoxon two-sample tests were used to compare the distribution of normally and non-normally (triglycerides, SDNN, RMSSD) distributed continuous variables, respectively, and the Ç2 test was used for categorical variables separately for type 1 and type 2 diabetes participants. Fisher's exact test was used whenever a cell count for a particular test was less than 5. The data is presented as mean ± standard deviation for normally distributed variables and as median (inter quartile range) for non-normally distributed variables such as triglycerides and log transformation was done for others such as ACR.

The prevalence of CAN was estimated overall and based on the age at diagnosis (e 10 years and < 10 years) and duration of diabetes (5 years, 6-10 years, and > 10 years) separately for persons with type 1 or type 2 diabetes.

Longitudinal Data: In addition to the data collected at baseline and at the cohort visit, the area under the curve (AUC) was computed to summarize the longitudinal trajectory of HbA1c and other

continuous variables, such as lipids, blood pressure, and BMI collected over time (at the baseline, 1, 2, or 5-year follow-up and cohort visits), adjusting for the time interval between the first and last measurement.

To assess the association of long-term glycemic control with CAN, logistic regression models treating the presence of CAN as the outcome were fitted separately for participants with type 1 or type 2 diabetes. These models were adjusted for potential confounders (collected at current SEARCH 3 cohort visit) such as age and sex (model 2), BMI (model 3), blood pressure (model 4), triglycerides (model 5), and ACR (model 6). A fully adjusted model that included all of these variables as covariates was also fitted (model 7). Models were stratified by diabetes type to limit confounding effects of age and adiposity. Diagnostic tests were performed to ensure that modeling assumptions were satisfied. The data were analyzed using SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

Characteristics of adolescents and young adults with type 1 (n=1646) and type 2 diabetes (n = 252) stratified by their CAN status are shown in **Table 1**. The prevalence of CAN was 12% in adolescents and young adults with type 1 diabetes, and 17% among those with type 2 diabetes (**Table 1**). Subjects with type 1 diabetes and CAN were older (mean age 19 ± 4 vs. 18 ± 4 years), more likely to have developed diabetes at age 10 or older, and had a larger waist circumference (81 ± 12 vs. 78 ± 12 cm), higher blood pressure (SBP 110 ± 11 vs. 106 ± 11 and DBP 72 ± 9 vs. 69 ± 9 mm Hg), poorer glycemic control (HbA1c 9.6 ± 2.1 vs. 9.1 ± 1.8 %), and elevated levels of triglycerides [median (IQR) 82(61,120) vs. 74(55,104) mg/dl] than those without CAN (all $P < 0.05$) (**Table 1**). Males with type 1 diabetes had a higher prevalence of CAN as compared to females (15% vs. 10%, $P = 0.001$). Subjects with type 2

diabetes and CAN had higher DBP (80 ± 12 vs. 75 ± 10 mm Hg) and elevated triglyceride levels [median (IQR) 151(102,254) vs. 110(78,183) mg/dl) and ACR (3.2 ± 1.5 vs. 2.7 ± 1.7 mg/g) compared to those without CAN (all $P < 0.05$) (**Table 1**). Hispanic and NHW subjects with type 2 diabetes had a higher prevalence of CAN (29% and 27%, respectively) as compared to NHB (7%) and other minority groups (12%) ($P = 0.001$).

The association of CAN with the AUC of cardio-metabolic risk factors is depicted in **Table 2**. Long-term poor glycemic control, summarized as AUC for HbA1c, triglyceride levels (AUC triglycerides), and blood pressure (AUC SBP and AUC DBP), were significantly higher among type 1 diabetes subjects with CAN as compared to those without CAN. Only higher triglycerides over time were significantly associated with CAN among subjects with type 2 diabetes (**Table 2**).

Table 3 summarizes the results from the multiple logistic regression analyses for the association between longitudinal glycemic control (AUC HbA1c as the independent variable) and CAN (dependent variable) adjusted sequentially for covariates. Long-term poor glycemic control (AUC HbA1c) was significantly associated with CAN independent of age, sex, blood pressure, BMI, triglyceride levels, and ACR in subjects with type 1 diabetes, but not in those with type 2 diabetes (**Table 3**).

Since nearly 32% (n= 879) of the 2777 SEARCH participants were excluded from the analysis data set due to various reasons (age < 10 years, missing etiological definition of diabetes type, incomplete CAN assessment), we examined whether there were any significant differences in the anthropometric, demographic, and metabolic characteristics of the participants who were excluded versus included in this data set that could potentially affect the prevalence estimates. Individuals excluded (n=879) from the analysis were more likely to have a longer duration of diabetes (9 vs. 8 years, $P < 0.001$) and were

younger at the time of diabetes diagnosis (9 vs. 11 years, $P < 0.001$), as compared to those included in the analytic sample (n=1898) (**Appendix Table 1**).

DISCUSSION

This study evaluating a large, multiethnic cohort of adolescents and young adults in the USA found high prevalence of CAN in subjects with type 1 and type 2 diabetes. Poor glycemic control and higher triglyceride levels over time were consistently associated with CAN. This is the first population-derived study in the USA that carefully characterized differences in the cross-sectional and longitudinal risk factors for CAN in a large, racially/ethnically diverse cohort of adolescents and young adults with type 1 diabetes and type 2 diabetes.

Placing our findings in perspective , in a meta-analysis including 3943 participants from 19 studies, the prevalence of subclinical CAN defined by either cardiovascular reflex tests or baroreflex sensitivity in young people (age < 24 years) with type 1 diabetes varied between 16 and 75% depending on the outcomes reported (20). For instance, in this meta-analysis pooled prevalence of CAN defined by measures of HRV was 21%, and ranged from 4-11% if CAN was defined by a single cardiovascular reflex test such as deep breathing or Valsalva (20). In contrast, a relatively small study that assessed HRV (LF and HF power) and included only 20 pediatric patients with type 1 diabetes (mean diabetes

duration 7 years, mean HbA1c 8.2%) reported prevalence rates for CAN as high as 75% (11). The prevalence estimates for CAN we found in the SEARCH cohort are lower compared with the report by Eppens and colleagues in an Australian cohort that included 1433 youth with type 1 and 68 type 2 diabetes who found that 61% of the youth with type 1 diabetes and 57% of those with type 2 diabetes had evidence of autonomic neuropathy (15). However, in that cohort autonomic neuropathy was defined as an abnormal pupillometry test (assessed by measuring the pupil size before and 3 seconds after a light stimulus was delivered using an infrared pupillometer) (15). The difference in the method of assessment (pupillometry vs. HRV testing) likely explains the higher prevalence of autonomic neuropathy in the Australian cohort, in spite of that cohort being younger, with shorter diabetes duration and better glycemic control compared with the SEARCH cohort (15). Although pupillometry was considered in the past a simple non-invasive test to assess parasympathetic autonomic function, it has not been widely used in the research setting due to the lack of standardization in the techniques employed, and the lack of validation studies. Thus, differences in outcome definitions, type of autonomic dysfunction evaluated, and the methods of assessments are likely the reasons accounting for the high variability in the reported prevalence reported in the few pediatric populations studied.

Poor glycemic control, longer duration of diabetes, increasing age, microalbuminuria, DBP, and dyslipidemia (lower HDL, increased triglycerides) are some of the established risk factors for CAN in adults with diabetes (1, 21-25). This study found that poor glycemic control and hypertriglyceridemia over time were the strongest risk factors associated with CAN in adolescents and young adults with type 1 diabetes. There is ample biologic plausibility and evidence for the causal role of hyperglycemia in the development and progression of chronic complications, including CAN (1, 26-29). Hyperglycemia induces abnormal signaling of the autonomic neurons via accumulation of advanced glycation end

products and microangiopathy, causing ischemic atrophy of autonomic nerve fibers innervating cardiac and vascular tissues (30). In this study, unfortunately, glucose control was quite poor in adolescents and young adults with type 1 or type 2 diabetes, with a mean HbA1c of ~ 9%, far exceeding the target HbA1c (<7.5%) recommended by the American Diabetes Association (31). These data further confirm that there is an urgent need for efforts focused at improving glycemic control among adolescents and young adults with diabetes to mitigate the elevated risk of the adverse outcomes associated with CAN and its downstream consequences, including increased CVD risk, in addition to numerous other adverse effects.

Dyslipidemia has also been implicated in the pathogenesis of diabetic neuropathy in a non-glucocentric paradigm involving linked metabolic and inflammatory insults that trigger neurodegeneration (32- 36). However, there is a close link between glucose and lipid metabolism, as hypertriglyceridemia and reduced HDL commonly occur in poorly controlled type 1 and type 2 diabetes (33). Hyperglycemia and dyslipidemia are also known as pro-inflammatory triggers to the neurodegenerative processes (23). In a prior analysis of youth participating in the SEARCH CVD study, we found an atherogenic lipid profile in youth with type 1 diabetes and reduced HRV as compared to age-matched healthy controls (16).

The findings from this study have important clinical implications. We observed that youth and young adults with type 1 and type 2 diabetes have evidence of CAN, as documented by changes in HRV at a mean diabetes duration of 8 years. Emerging epidemiological evidence also suggests that CAN is associated with increased arterial stiffness in adolescents (37) and adults (38) with type 1 diabetes, and thus could have an additive effect on the risk of future cardiovascular events, which occur earlier and with poorer prognosis in individuals with diabetes compared with the general population (39,40,4).

Considering that SEARCH participants are a representative cohort of USA youth with diabetes and have suboptimal glycemic control (mean HbA1c well above the American Diabetes Association recommended target of < 7.5%) (19), these data provide evidence that similar screening for CAN, as is recommended for adults, may be beneficial in adolescents and young adults as in adults with diabetes (1), especially in those individuals who have additional risk factors associated with CAN. Recent recommendations from the American Heart Association call for a multifactorial approach to mitigate the increased CVD risk in youth with diabetes (41). Thus, targeting poor glycemic control and dyslipidemia in adolescents and young adults with diabetes as early as possible, which is also in line with current standard of care in diabetes (31), could help mitigate the increased CVD risk in this young population (28,36).

The large sample size, multiethnic composition of the cohort, use of a non-invasive, simple, validated instrument to assess CAN, and evaluation of the longitudinal and cross sectional risk factors are among the strengths of our study. The limited power to examine the association between long-term glycemic control and CAN among persons with type 2 diabetes (despite similar levels of HbA1c to those with type 1 diabetes) may have been due to a comparatively small sample size (although the association was in the same direction as that of the type 1 diabetes group) and is one of the limitations of our study. The lack of longitudinal measures of CAN is also a limitation of our study, although a subset of this cohort (2002-2012 incidence cases e 10 years of age with least 5 years of duration of diabetes) will be re-evaluated for CAN as part of the next phase of SEARCH (2016-2020). Finally, although the SEARCH Cohort Study is drawn from population-based registries of youth with diabetes, those excluded from the analytic sample were more likely to be older at time of diagnosis and had a longer duration of diabetes.

Each of these variables is associated with increased prevalence of CAN and may influence our estimates of CAN prevalence in youth and young adults with diabetes.

Overall, the current data support the contention that good glycemic control and better approaches to manage dyslipidemia, which have been the accepted standard of care for diabetes, need to be the mainstay, as they may also prevent the development and worsening of CAN in this young population. Given the independent risk of CAN for cardiovascular events and death, health care providers should motivate pediatric patients to reach and maintain optimal glucose control and better management of other risk factors including triglycerides to ameliorate the risk of premature cardiovascular events

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention and the National Institute of Diabetes and Digestive and Kidney Diseases.

DUALITY OF INTEREST

All authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

MJ ,JD, RPB and DD designed the study and analysis plan . MJ wrote the manuscript. JD performed the analysis and provided critical input to the manuscript. RPB reviewed data, provided input

in study design, and critically reviewed the manuscript for intellectual content. EMU, DD, RAB, DJP, GI, CP, LMD, ADL, SM, BL, and ELF critically reviewed the manuscript and provided input. JD 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 the data analysis.

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TABLES

Table 1. Clinical characteristics of adolescents and young adults with type 1 and type 2 diabetes stratified by cardiovascular autonomic neuropathy status at the current SEARCH Cohort research visit(2011-2015).

	Type 1 diabetes = 1646			Type 2 diabetes = 252		
Variable	**No CAN**	**CAN**	**P-value**	**No CAN**	**CAN**	**P-value**
N (%)	**1443(88%)**	**203(12%)**		**209(83%)**	**43(17%)**	
Age, years	18 ± 4	19 ± 4	<0.0001	22 ± 4	22 ± 3	0.99
Age at diagnosis, years	10 ± 4	11 ± 3	<0.0001	14.1 ± 3	14 ± 3	0.82
Age at diagnosis e 10	723(84%)	137(16%)	<0.0001	200(83%)	40(17%)	0.45
Age at diagnosis < 10	720(92%)	66(8%)		9(75%)	3(25%)	
Diabetes duration, years	7.8 ± 1.8	7.9 ± 1.9	0.44	7.9 ± 1.9	7.8 ± 2.0	0.82
Diabetes duration, years						
5-10 years	1233(88%)	167(12%)	0.24	174(82%)	38(18%)	0.49
e10 years	210(85%)	36(15%)		35(87%)	5(13%)	
Sex						
Female	746(90%)	79(10%)	0.001	141(83%)	28(17%)	0.77
Male	697(85%)	124(15%)		68(82%)	15(18%)	
Race/ethnicity						
Non-Hispanic White	1089(87%)	158(13%)	0.63	48(73%)	18(27%)	0.001
Asian/Pacific Islander	24(92%)	2(8%)		3(100%)	0(0%)	
Non-Hispanic Black	143(91%)	14(9%)		105(93%)	8(7%)	
Hispanic	175(86%)	28(14%)		37(71%)	15(29%)	
Others	12(87%)	1(13%)		16(88%)	2(12%)	
Smoking						
Never	967(89%)	122(11%)	0.15	80(84%)	15(16%)	0.76
Former	259(85%)	46(15%)		61(80%)	15(20%)	
Current	188(86%)	30(14%)		63(84%)	12(16%)	
Oral diabetes medication						
Yes	68(87.2%)	10(12.8%)	0.40	109(82.6%)	23(17.4%)	0.90
No	1372(87.7%)	192(12.3%)		99(83.2%)	20(16.8%)	
Insulin dose	56.84 ± 29.09	64.22 ± 30.97	0.0007	66.19 ± 38.48	76.35 ± 36.26	0.20
Insulin dose, per kg	0.85 ± 0.40	0.90 ± 0.40	0.06	0.73 ± 0.39	0.86 ± 0.36	0.19
Waist circumference, cms	78 ± 12	81 ± 12	0.00011	104 ± 19	111 ± 20	0.06
Waist-to-height ratio	0.5 ± 0.1	0.5 ± 0.1	0.48	0.7 ± 0.1	0.7 ± 0.1	0.33
BMI, kg/m^2	24 ± 5	25 ± 5	0.12	35 ± 9	37 ± 9	0.38
SBP, mm Hg	106 ± 11	110 ± 11	<0.0001	118 ± 13	121 ± 17	0.36
DBP, mm Hg	69 ± 9	72 ± 9	<0.0001	75 ± 10	80 ± 12	0.01

HbA1c, %	9.1 ± 1.8	9.6 ± 2.1	0.001	9.2 ± 3	9.6 ± 2.8	0.42
HbA1c, mmol/mol	75 ± 7	80 ± 8	0.001	77 ± 9	80 ± 9	0.42
HDL-cholesterol mg/dl	55 ± 14	54 ± 13	0.15	42 ± 12	39 ± 10	0.16
LDL-cholesterol mg/dl	96 ± 28	99 ± 32	0.50	106 ± 38	104 ± 37	0.88
Triglycerides mg/dL	74 (55,104)	82 (61,120)	0.005	110 (78,183)	151 (102,254)	0.002
CRP mg/dL	0.2 ± 0.5	0.4 ± 1.5	0.74	0.6 ± 0.8	0.7 ± 0.7	0.16
Log ACR, µg/mg	2.0 ± 0.93	2.1 ± 0.95	0.33	2.7 ± 1.7	3.2 ± 1.5	0.014

All data are presented as mean ± SD or n (%) or median (IQR). CAN: cardiovascular autonomic neuropathy, BMI: body mass index, SPB and DBP: systolic and diastolic blood pressure, HDL: high density lipoprotein, LDL: low density lipoprotein, CRP: C-reactive protein, ACR: albumin:creatinine ratio

Table 2. Cardio-metabolic risk factor burden over time by cardiovascular autonomic neuropathy status in adolescents and young adults with type 1 and type 2 diabetes.

	Type 1 diabetes = 1646			Type 2 diabetes = 252		
Variable	**No CAN**	**CAN**	**P-value**	**No CAN**	**CAN**	**P-value**
AUC BMI-z	20.6 ± 0.9	20.6 ± 0.9	0.75	21.9 ± 0.7	22 ± 0.6	0.50
AUC DBP	65.9 ± 7.2	68 ± 7.6	<0.001	73.1 ± 6.7	75.6 ± 9.7	0.18
AUC SBP	103.5 ± 9	107.5 ± 9	<0.001	116.6 ± 9.3	119.7 ± 11.8	0.22
AUC HbA1c	8.5 ± 1.3	8.8 ± 1.5	0.005	8.4 ± 2.4	8.6 ± 2.3	0.65
AUC LDL-cholesterol	93.5 ± 22.3	95.1 ± 24.2	0.53	104.1 ± 30.7	99.6 ± 26.6	0.54
AUC Triglycerides	79.2 ± 43.8	92 ± 59.6	0.001	149.6 ± 121.7	225.3 ± 221.8	0.005
AUC HDL-cholesterol	56.1 ± 11.8	54.7 ± 10.6	0.10	42.4 ± 10.9	40.1 ± 10.4	0.13

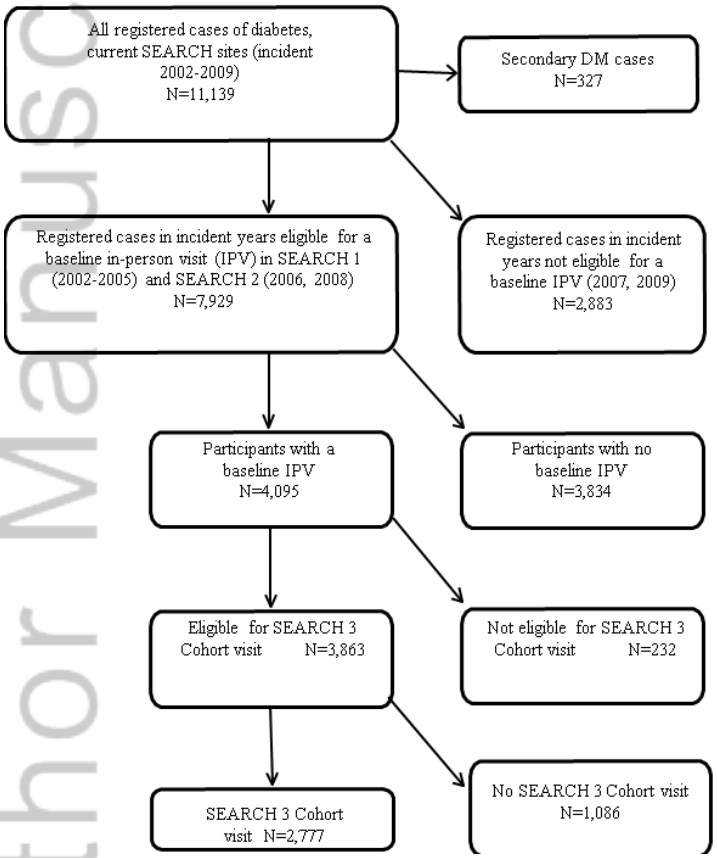
All data are presented as mean ± SD. CAN: cardiovascular autonomic neuropathy, BMI: body mass index, SPB and DBP: systolic and diastolic blood pressure, HDL: high density lipoprotein, LDL: low density lipoprotein, AUC: area under the curve derived from data collected at baseline, 1,2, 5 year follow-up and the current cohort visits

Table 3. Multivariable regression models for association between long term glycemic control and cardiovascular autonomic neuropathy collected at the current SEARCH Cohort visit(2011-2015)

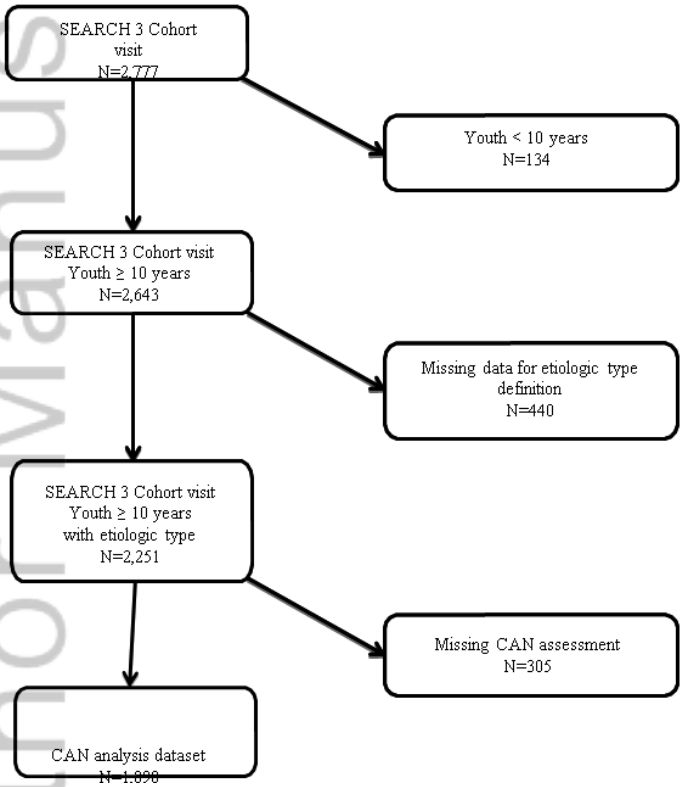
Dependent Variable: CAN **Independent Variable:** AUC A1c adjusted for time between measures	**Type 1 diabetes**		**Type 2 diabetes**	
	OR (95%CI)	**P-value**	**OR (95%CI)**	**P-value**
Model 1 = AUC A1c	1.28 (1.11;1.48)	0.0006	1.07 (0.77;1.48)	0.69
Model 2 = Model 1+ age, sex	1.3 (1.13;1.5)	0.0002	1.07 (0.77;1.49)	0.68
Model 3 = Model 1+ BMI	1.29 (1.11;1.49)	0.0006	1.11 (0.79;1.56)	0.54
Model 4 = Model 1 + SBP, DBP	1.26 (1.09;1.47)	0.002	1 (0.71;1.41)	1.00
Model 5 = Model 1 + Trig	1.22 (1.05;1.43)	0.010	0.93 (0.65;1.33)	0.69
Model 6 = Model 1 + ACR	1.26 (1.07;1.47)	0.005	1.27 (0.86;1.88)	0.22
Model 7 = Model 1 + age, sex, BMI, SBP, DBP, Trig, ACR	1.24 (1.04;1.46)	0.01	1.08 (0.69;1.7)	0.72

CAN: cardiovascular autonomic neuropathy, OR: odds ratio, CI: confidence interval, BMI: body mass index, SPB and DBP: systolic and diastolic blood pressure, trig: triglycerides, ACR: albumin: creatinine ratio, AUC: area under the curve

Appendix Figure 1: SEARCH 3 consort diagram.



Appendix Figure 2: Exclusion and inclusion criteria



APPENDIX

Appendix Table 1. Characteristics of the participants excluded and included in the cardiovascular autonomic neuropathy analysis dataset at the current SEARCH Cohort visit(2011-2015).

Variable	Excluded (N=879)	Included (N=1898)	P-value
Age at cohort visit (years)	18 ± 5	19 ± 4	<0.001
Age at diagnosis (years)	9 ± 5	11 ± 4	<0.001
Sex (Female)	53%	52%	0.74
Diabetes duration (months)	104 ± 24	95 ± 23	<0.001
Race/ethnicity			0.080
Non-Hispanic White	63.8%	69.3%	
Non-Hispanic Black	18.7%	14.1%	
Hispanic	13.7%	13.4%	
Asian/Pacific Islander	1.9%	1.5%	
Native Americans	1.5%	1.3%	
Other	0.4%	0.3%	
BMI z-score	0.8 ± 1.2	0.7 ± 1.1	0.29
SBP (mm Hg)	102 ± 14	102 ± 13	0.62
DBP (mm Hg)	64 ± 11	64 ± 10	0.86
HbA1c (%)	9.2 ± 2.1	9.2 ± 2.1	0.90
Triglycerides (mg/dL)	102 ± 100	105 ± 107	0.43
LDL-cholesterol (mg/dL)	97 ± 30	98 ± 30	0.49
HDL-cholesterol (mg/dL)	55 ± 15	53 ± 14	0.06

All data are presented as mean ± SD or n (%). BMI: body mass index, SPB and DBP: systolic and diastolic blood pressure, HDL: high density lipoprotein, LDL: low density lipoprotein

TABLES

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Age at diagnosis, years	10 ± 4	11 ± 3	<0.0001	14.1 ± 3	14 ± 3	0.82
Age at diagnosis e 10	723(84%)	137(16%)	<0.0001	200(83%)	40(17%)	0.45
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Male	697(85%)	124(15%)		68(82%)	15(18%)	
Race/ethnicity						
Non-Hispanic White	1089(87%)	158(13%)	0.63	48(73%)	18(27%)	0.001
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Non-Hispanic Black	143(91%)	14(9%)		105(93%)	8(7%)	
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Model 7 = Model 1 + age, sex, BMI, SBP, DBP, Trig, ACR	1.24 (1.04;1.46)	0.01	1.08 (0.69;1.7)	0.72

CAN: cardiovascular autonomic neuropathy, OR: odds ratio, CI: confidence interval, BMI: body mass index, SPB and DBP: systolic and diastolic blood pressure, trig: triglycerides, ACR: albumin: creatinine ratio, AUC: area under the curve