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ARTICLE

HbA_{1c} variability is associated with microalbuminuria development in type 2 diabetes: a 7-year prospective cohort study

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Abstract
Aims/hypothesis HbA_{1c} variability has been shown to be an independent risk factor for nephropathy in patients with type 1 diabetes. In this study, we aimed to explore the association between HbA_{1c} variability and microalbuminuria development in patients with type 2 diabetes. We also intended to test the applicability of serially measured HbA_{1c} over 2 years for this risk assessment.
Methods Between 2003 and 2005, we recruited 821 middle-aged normoalbuminuric individuals with type 2 diabetes and followed them through to the end of 2010. The average follow-up time was 6.2 years. We defined microalbuminuria as a urine albumin to creatinine ratio of 30 mg/g (3.4 mg/mmol) or higher. HbA_{1c} variability was calculated by the SD of serially measured HbA_{1c}. The Cox proportional hazards model was used to evaluate the association between HbA_{1c} SD quartile and development of microalbuminuria.
Results The incidence of microalbuminuria for the overall population was 58.4, 58.6, 60.8 and 91.9 per 1,000 person-years for Q1- to Q4-adjusted HbA_{1c} SD, respectively (p for trend=0.042). Compared with patients in Q1, those in Q4 were about 37% more likely to develop microalbuminuria. The HR derived from a series of 2 year HbA_{1c} measurements was similar to that from data collection for longer than 4 years.
Conclusions/interpretation In addition to mean HbA_{1c} values, HbA_{1c} variability, even measured as early as 2 years, is independently associated with the development of microalbuminuria in patients with type 2 diabetes.

Keywords HbA_{1c} variability · Microalbuminuria · Type 2 diabetes mellitus

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Abbreviations

ACR	Albumin to creatinine ratio
DMIDS	Diabetes Management through an Integrated Delivery System project
FinnDiane	Finnish Diabetic Nephropathy study

Introduction

The DCCT in type 1 diabetes and the UK Prospective Diabetes Study in type 2 diabetes both concluded that a rise in HbA_{1c} can increase the development of microvascular complications [1–3]. Recently, glycaemic variability has also been demonstrated to affect the risk of micro- and macrovascular consequences in diabetes [4–7]; however, its association with diabetic complications has not been consistently confirmed [8–12].

Data from the Finnish Diabetic Nephropathy (FinnDiane) study indicated that HbA_{1c} variability in type 1 diabetes patients is predictive of incident microalbuminuria and progression of renal disease [13]. In type 1 diabetes, HbA_{1c} variability was similarly shown to be an independent risk factor for microalbuminuria development, even among the young, who are highly vulnerable to vascular complications [14]. Until now, however, the relationship between HbA_{1c} variability and the development of nephropathy has not been investigated in type 2 diabetes.

Currently, there is no clear consensus as to how long HbA_{1c} should be measured for to unwaveringly reflect the clinical impacts of HbA_{1c} variability. The DCCT and the FinnDiane study undertook 9 and 5.7 year serial HbA_{1c} measurements, respectively, to examine HbA_{1c} variability [13, 15]. However, the follow-up study of the UK Prospective Diabetes Study demonstrated the important role of early strict glycaemic control in preventing vascular complications [16], implying that an indicator that needs to track HbA_{1c} measurements for more than 5 years to correlate its clinical implications may be late for a prompt intervention.

The primary aim of this study was to explore the relationship between HbA_{1c} variability and microalbuminuria development in patients with type 2 diabetes. Furthermore, in order to emphasise the importance of early stabilisation in glycaemic control, we also intended to determine whether HbA_{1c} variability derived from 2 year measurements is an early indicator independently associated with diabetic nephropathy in type 2 diabetes.

Methods

Participants The study participants were type 2 diabetes patients who were enrolled for the Diabetes Management through an Integrated Delivery System (DMIDS) project (ClinicalTrials.gov NCT00288678) [17]. The detailed inclusion and exclusion criteria for the DMIDS project are described elsewhere [18]. Briefly, 1,209 participants with type 2 diabetes were recruited from 2003 to 2005 and followed through to the end of 2010. Of these enrolees, 143 with fewer than three eligible urine albumin to creatinine ratio (ACR) tests and 245 with microalbuminuria at baseline (ACR ≥3.4 mg/mmol in two consecutive urine tests) were excluded from the analysis. The remaining 821 participants were selected for further investigation. Written informed consent was obtained from all enrolees. The institutional review board at the National Health Research Institutes reviewed and approved this study.

Laboratory tests Fasting (overnight for ≥8 h) venous blood and morning spot urine specimens were collected every 6 months. HbA_{1c} was measured by high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA, USA). Triacylglycerol and HDL-cholesterol levels were measured by an automatic analyser (Hitachi 7060; Hitachi High Technologies Co., Tokyo, Japan). Urinary albumin was measured by the immunoturbidimetric method (Hitachi 7060). All blood and urine samples were kept at 2–8°C and measured within 8 h at a central laboratory.

Definition of outcome, HbA_{1c} variability and covariates Participants who had an ACR of 3.4 mg/mmol or higher in two consecutive urine tests were defined as having developed microalbuminuria. Urine samples were excluded from analysis if microscopic urinalysis showed erythrocytes, white blood cells or epithelial cells of more than five per high-power field, or the appearance of casts or bacteria.

HbA_{1c} variability was defined as the SD of serial HbA_{1c} measurements, the CV of HbA_{1c} to correct for the mean or the adjusted HbA_{1c} SD—in which SD was divided by the square root of $k/(k-1)$, where k stands for the number of HbA_{1c} measurements—to control for the effects of variation in the number of HbA_{1c} measurements [15]. Because of similar results derived from all three SD definitions, we used the adjusted HbA_{1c} SD to account for HbA_{1c} variability in multivariable survival analysis.

Waist circumference was measured at the level of the midpoint between the lowest rib and the iliac crest. Blood pressure was measured three times separated by 1 min; the mean of these three measurements was recorded. Smoking status was categorised into three groups: current smokers, ex-smokers (having stopped smoking for ≥1 month) and non-smokers (having smoked <100 cigarettes in a lifetime). Those who had ever chewed betel nuts were defined as chewers. Those who had not performed any leisure-time physical activity in the past 2 weeks were defined as the sedentary group.

Statistical analysis Data are expressed as mean ± SD for continuous variables, or as counts and proportions for categorical variables. Student's *t* tests and χ^2 analyses were used for continuous and categorical variables, respectively, to compare characteristics between non-progressors and progressors (with microalbuminuria development). The incidence of microalbuminuria was estimated by the number of observed new microalbuminuria cases per 1,000 person-years. Person-years were calculated as the time elapsed from the date of recruitment until the date of death, loss to follow-up, microalbuminuria development or the end of follow-up, whichever came first. The calculation of a 95% CI for the incidence rate was based on the assumption that the observed incident cases followed a Poisson distribution. We estimated the incidence of microalbuminuria in different quartiles of adjusted HbA_{1c} SD for overall participants and also for different subgroups according to their number of HbA_{1c} measurements and baseline HbA_{1c}. In order to test the predictability of HbA_{1c} variability for microalbuminuria development in different subgroups, we calculated the mean HbA_{1c} and adjusted HbA_{1c} SD for three or four measurements (all HbA_{1c} from recruitment to the end of year 2), and for those with baseline HbA_{1c} ≤8% or >8% (64 mmol/mol), respectively, for subgroup analysis.

Kaplan–Meier analyses and univariate Cox proportional hazard models were used to explore the association between quartiles of adjusted HbA_{1c} SD and microalbuminuria development. The covariates used in Cox proportional hazard models included baseline demographic and metabolic profiles (age at diabetes onset, sex, education, diabetes duration, smoking status, waist circumference, triacylglycerol and HDL-cholesterol levels, mean HbA_{1c} and BP). Multivariable Cox proportional hazards modelling was used to determine the independent effects of HbA_{1c} variability on microalbuminuria development. Study entry was defined as the date of enrolment. Observations were censored at the end of the study or the date that patients died or dropped out of the study, whichever occurred first. Results are expressed as HR compared with the group in the lowest quartile of adjusted HbA_{1c} SD.

The proportional hazard assumption, the constant HR over time, was evaluated by comparing estimated log–log survival curves for all covariates. All assessed log–log survival plots graphically showed two parallel lines, indicating no violation of the assumption. A test for trend was conducted by treating quartiles of adjusted HbA_{1c} SD as a continuous variable.

Analyses were performed with SAS software, version 9.1 (SAS Institute, Cary, NC, USA). A two-sided *p* value of <0.05 was considered statistically significant.

Results

Table 1 shows that progressors were more likely to have lower education, longer diabetes duration and poorer metabolic profiles, including higher baseline urine ACR and poorer control of BP and glucose, compared with non-progressors. Those who developed microalbuminuria also had higher HbA_{1c} variability during the follow-up period. With regard to characteristics in different quartiles of adjusted HbA_{1c} SD (Table 2), those who had higher HbA_{1c} variability tended to have earlier diabetes onset, use more glucose-lowering drugs and have poorer glycaemic control at baseline and during follow-up. Patients in the highest quartile (Q4) of HbA_{1c} SD were also more likely to be smokers (32.4% vs 23.6% for Q1–Q3 combined, $p<0.001$), betel nuts chewers (15.1% vs 10.9%, $p=0.031$) and physically inactive (35.7% vs 27.5%, $p=0.011$).

As shown in Table 3, both mean and adjusted SD of HbA_{1c} were significantly related to microalbuminuria development in univariate analysis as well as in separate multivariable regressions (model 1, HR 1.10, $p<0.05$ for mean of HbA_{1c}; model 2, *p* for trend=0.001 for adjusted SD of HbA_{1c}); however, the effect of mean of HbA_{1c} was attenuated (HR 1.04, non-significant) when these two variables were put together in the same model (model 3). Compared with those in the lowest quartile of adjusted HbA_{1c} SD, as shown in Table 3, the patients in the Q4 were 48% more likely to develop microalbuminuria ($p<0.05$ for Q4 and *p* for trend=0.043 in model 3). With regard to other covariates, the impact of lower education was persistent in both univariate and multivariable models; furthermore, diabetes duration, high BP and the subsequent use of ACE inhibitors or angiotensin receptor blockers were also revealed to have marginal effects on the development of microalbuminuria (Table 3) after controlling for other covariates.

As shown in Table 4, the incidences of microalbuminuria for participants overall were 58.4, 58.6, 60.8 and 91.9 per 1,000 person-years for Q1–Q4 adjusted HbA_{1c} SD, respectively (*p* for trend=0.001). The graded association (*p* for trend) between the quartile of adjusted HbA_{1c} SD and risk of microalbuminuria was consistent and little affected by the HbA_{1c} follow-up time (2 vs ≤7 years) or baseline HbA_{1c} (≤8% vs >8% [64 mmol/mol]) (Fig. 1 and Table 4). In contrast, the established effects of mean HbA_{1c} were not significant in those with baseline HbA_{1c} of 8% (64 mmol/mol) or less or for 2 years of follow-up (Table 4). We also used a sex-specific cut-off point [19] to define microalbuminuria and conducted a sensitivity analysis, with similar results (data not shown).

Discussion

Intrapersonal HbA_{1c} variability, as expressed by SD of serially measured HbA_{1c}, is a reliable and stable indicator to predict microalbuminuria development in type 2 diabetes patients. Our findings not only enrich previous knowledge

Table 1 Characteristics of demographics, baseline biomarkers and serially measured HbA_{1c} in type 2 diabetes patients with or without progression to microalbuminuria during a 7-year follow-up

Characteristic	All participants (n=821)	Non-progressors (n=520)	Progressors (n=301)	p value
Demographic characteristics				
Male (%)	46.1	44.0	49.8	0.108
Education ≤6 years (%)	54.9	51.5	60.7	0.012
Age at diabetes onset (years)	51.2±8.3	51.2±8.6	51.1±9.1	0.305
Diabetes duration at recruitment (years)	2.9±2.7	2.7±2.2	3.3±3.3	<0.001
Follow-up (years)	6.2±0.7	6.2±0.6	6.2±0.7	0.542
Smoking status				0.299
Non-smoker (%)	73.1	74.8	70.1	
Ex-smoker (%)	7.1	6.9	7.3	
Current smoker (%)	19.8	18.3	22.6	
Betel nut chewer (%)	8.6	8.1	9.6	0.503
No physical activity in 2 weeks (%)	30.7	30.4	31.1	0.888
Baseline drug treatment				
Glucose-lowering drugs (%)				
Sulfonylurea	88.7	84.2	96.6	0.305
Biguanide	80.6	76.1	88.3	0.265
Other oral glucose-lowering drugs	18.2	15.9	22.2	0.367
Insulin	2.4	1.7	3.6	0.131
Antihypertensive drugs (%)				
ACE inhibitor	32.7	28.4	40.1	0.325
ARB	9.0	7.8	10.9	0.281
CCB	33.8	32.3	36.5	0.737
β-Blocker	32.1	30.0	35.8	0.386
Diuretics	19.0	17.5	21.5	0.619
Baseline biomarkers				
Hypertension[a] (%)	56.1	52.3	63.4	0.002
Systolic BP (mmHg)	129.3±14.3	128.1±11.3	130.1±18.1	<0.001
Diastolic BP (mmHg)	77.2±10.6	77.2±6.3	77.3±13.1	<0.001
Waist circumference (cm)	87.1±10.0	87.0±10.0	87.2±9.9	0.775
HDL-cholesterol (mmol/l)	1.51±3.33	1.37±1.86	1.60±3.99	0.333
Triacylglycerols (mmol/l)	2.76±14.3	2.19±7.05	3.12±17.4	0.362
Urine ACR (mg/mmol)	1.47±2.14	1.15±1.75	2.02±2.60	<0.001
HbA_{1c} characteristics and pattern				
Baseline HbA_{1c}, % (mmol/mol)	8.2±1.8 (66.1)	8.0±1.7 (63.9)	8.4±2.0 (68.3)	0.002
During follow-up				
Number of HbA_{1c} measurements	9.0±2.7	9.1±2.8	8.9±2.7	0.543
Mean of serial HbA_{1c}, % (mmol/mol)	7.9±1.2 (62.8)	7.7±1.1 (60.7)	8.2±1.3 (66.1)	<0.001
SD of serial HbA_{1c}				
Crude SD	1.12±0.53	1.04±0.48	1.23±0.59	<0.001
CV of SD	0.13±0.06	0.13±0.05	0.14±0.06	0.035
Adjusted SD	1.03±0.51	0.97±0.47	1.14±0.54	0.004

Data are expressed as proportion (%) or mean ± SD

Crude SD = $\sqrt{\frac{\Sigma(x_i-\bar{x})^2}{k-1}}$ where k=number of HbA_{1c} measurements and $\bar{x}$ = mean of serially measured HbA_{1c}

CV of SD=crude SD/$\bar{x}$

Adjusted SD=crude SD/$\sqrt{\frac{k}{K-1}}$

[a]Participants who had been diagnosed by a physician as having hypertension, were currently taking antihypertensive drugs or had BP >130/80 mmHg at recruitment

ARB, angiotensin receptor blocker; CCB, calcium-channel blocker

Table 2 Baseline characteristics according to quartiles of intrapersonal adjusted SD of serial HbA_{1c} measurements

Characteristic	Q1	Q2	Q3	Q4	*p* value
Patients (*n*)	204	206	202	209	
Range of adjusted HbA_{1c} SD	0.09–0.66	0.67–0.95	0.96–1.29	1.30–3.48	
Demographic characteristics					
Male (%)	47.5	42.7	45.5	48.8	0.624
Education ≤6 years (%)	54.9	50.0	56.9	57.8	0.377
Annual household income <US$10,000 (%)	26.3	32.6	22.1	28.7	0.572
Age at diabetes onset (years)	53.1±8.7	51.2±8.9	50.7±8.6	49.7±8.7	0.001
Diabetes duration at recruitment (years)	3.0±2.7	3.0±3.4	2.7±2.1	3.0±2.5	0.555
Follow-up duration (years)	6.2±0.6	6.2±0.6	6.3±0.7	6.2±0.7	0.357
Ever-smoker (%)	21.8	25.1	27.6	32.4	0.114
Betel nut chewer (%)	8.2	12.8	13.4	15.1	0.041
No physical activity in 2 weeks (%)	29.4	26.1	27.5	35.7	0.126
Baseline drug treatment					
Glucose-lowering drugs (%)					
Sulfonylurea	84.8	81.5	92.0	96.6	0.017
Biguanide	72.0	77.1	86.6	86.6	0.013
Other oral glucose-lowering drugs	12.2	17.9	19.8	22.9	0.434
Insulin	0.9	2.4	2.4	3.8	0.370
Antihypertensive drugs (%)					
ACE inhibitor	43.6	33.4	33.6	40.6	0.079
ARB	14.2	5.8	8.9	7.1	0.011
CCB	36.7	33.9	29.7	34.9	0.351
β-Blocker	32.8	30.5	33.1	32.0	0.931
Diuretics	19.1	14.5	19.3	22.9	0.333
Baseline biomarkers					
Hypertension[a] (%)	53.9	53.4	57.7	60.3	0.433
Systolic BP (mmHg)	129.5±11.1	129.5±11.5	129.0±14.6	129.1±18.7	0.983
Diastolic BP (mmHg)	77.5±6.2	78.7±6.9	77.6±11.6	77.7±15.0	0.623
Waist circumference (cm)	87.3±10.2	87.6±9.8	87.0±9.5	86.6±10.5	0.801
HDL-cholesterol (mmol/l)	1.26±0.34	1.24±0.31	1.24±0.30	1.25±0.40	0.785
Triacylglycerols (mmol/l)	1.66±0.96	1.73±1.41	2.02±1.82	1.85±2.08	0.116
Urine ACR (mg/mmol)	1.28±1.72	1.44±1.40	1.54±2.08	1.63±2.16	0.014
HbA_{1c} characteristics and pattern					
Baseline HbA_{1c} ,% (mmol/mol)	7.3±1.2 (56.3)	7.6±1.4 (59.6)	8.3±1.5 (67.2)	9.4±2.2 (79.2)	<0.001
≤7% (53 mmol/mol) (%)	38.2	35.0	21.3	17.2	<0.001
7–9% (53–75 mmol/mol) (%)	56.9	45.2	39.6	25.4	
>9% (75 mmol/mol) (%)	4.9	19.8	39.1	57.4	
During follow-up					
Number of HbA_{1c} measurements	8.0±3.1	9.4±2.5	9.3±2.6	9.4±2.5	<0.001
Mean of serially measured HbA_{1c}, % (mmol/mol)	7.3±1.3 (56.3)	7.6±1.4 (59.6)	8.4±1.5 (68.3)	9.4±2.3 (79.2)	<0.001

Data are expressed as proportion (%) or mean ± SD

Adjusted SD of HbA1c=crude SD/$\sqrt{\frac{k}{k-1}}$ where k=number of HbA_{1c} measurements

[a]Participants who had been diagnosed by a physician as having hypertension, were currently taking antihypertensive drugs or had BP >130/80 mmHg at recruitment

ARB, angiotensin receptor blocker; CCB, calcium-channel blocker

about the impact of HbA_{1c} variability on type 1 diabetes [13–15], but also provide the first empirical evidence for a possible association of HbA_{1c} variability with the development of microalbuminuria in patients with type 2 diabetes.

Table 3 Risk factors in type 2 diabetes patients contribute to progression to microalbuminuria during a 7-year follow-up

Risk factor	Univariate HR (95% CI)	Multivariable HR (95% CI)		
		Model 1	Model 2	Model 3
Sex (female/male)	1.22 (0.98, 1.54)	1.25 (0.94, 1.67)	1.23 (0.92, 1.64)	1.24 (0.93, 1.65)
Education (≤6/>6 years)	1.33 (1.05, 1.68)*	1.45 (1.10, 1.90)*	1.43 (1.08, 1.88)*	1.41 (1.07, 1.86)*
Diabetes onset age (per 1 year increment)	1.00 (0.99, 1.01)	0.99 (0.97, 1.00)	0.99 (0.97, 1.00)	0.99 (0.98, 1.00)
Diabetes duration (per 1 year increment)	1.07 (1.03, 1.10)*	1.03 (0.99, 1.07)	1.03 (1.00, 1.08)*	1.04 (1.00, 1.07)
Smoking status				
Ex-smoker/non-smoker	1.04 (0.67, 1.62)	0.91 (0.58, 1.45)	0.88 (0.56, 1.40)	0.89 (0.56, 1.41)
Current smoker/non-smoker	1.32 (1.01, 1.74)*	1.26 (0.93, 1.72)	1.21 (0.89, 1.65)	1.21 (0.89, 1.66)
ACE inhibitor and/or ARB use (yes vs no)	1.27 (1.02, 1.59)*	1.26 (1.00, 1.58)*	1.26 (1.00, 1.58)	1.26 (1.00, 1.58)
Baseline biomarkers				
Blood pressure (≥130/80 vs <130/80 mmHg)	1.45 (1.14, 1.83)*	1.30 (1.03, 1.65)*	1.25 (0.98, 1.59)	1.25 (0.98, 1.59)
Waist circumference (high vs low)	0.92 (0.70, 1.20)	1.02 (0.77, 1.39)	1.01 (0.75, 1.35)	1.01 (0.75, 1.36)
HDL-cholesterol (low vs high)	0.81 (0.65, 1.02)	0.89 (0.69, 1.15)	0.87 (0.68, 1.12)	0.88 (0.68, 1.13)
Triacylglycerols (≥1.69 vs <1.69 mmol/l)	0.93 (0.74, 1.17)	0.92 (0.73, 1.18)	0.95 (0.75, 1.21)	0.95 (0.75, 1.21)
HbA_{1c} characteristics during follow-up				
Mean of serial HbA_{1c} (per 1% increment)	1.13 (1.04, 1.23)*	1.10 (1.00, 1.20)*		1.04 (0.94, 1.14)
Adjusted standard deviation of HbA_{1c}				
Quartile 2/Quartile 1	1.13 (0.83, 1.56)		1.06 (0.74, 1.50)	1.03 (0.72, 1.48)
Quartile 3/Quartile 1	1.35 (1.04, 1.77)*		1.13 (0.80, 1.60)	1.09 (0.75, 1.57)
Quartile 4/Quartile 1	1.73 (1.26, 2.38)**		1.57 (1.13, 2.17)**	1.48 (1.03, 2.12)*
p for trend	<0.001		0.001	0.043

Waist circumference high vs low: male (>90 vs ≤90 cm), female (>80 vs ≤80 cm)

HDL-cholesterol low vs high: male (<1.03 vs ≥1.03 mmol/l), female (<1.28 vs ≥1.28 mmol/l)

*$p<0.05$, **$p<0.01$

ARB, angiotensin receptor blocker

Furthermore, the current study also demonstrates that a 2 year estimate of HbA_{1c} variability can be used as a short-term monitoring indicator for the progression of diabetic nephropathy. This prospective cohort study may provide useful guidance for clinical applications.

In addition to the mean value of serially measured HbA_{1c}, HbA_{1c} variability has been frequently shown to be associated with diabetic complications in patients with type 1 diabetes. Adult patients with higher HbA_{1c} variability are more likely to develop cardiovascular events and albuminuria, as shown in the FinnDiane Study [13]. A similar association has also been observed in young type 1 diabetes patients. The Oxford Regional Prospective Study [14] and the Pittsburgh Epidemiology of Diabetes Complications Study [20] demonstrated that higher SD of HbA_{1c} could predict microalbuminuria and coronary artery disease in type 1 diabetes patients younger than 17 years of age. Although the DCCT data [21, 22] could not associate microvascular complications with acute glucose variability derived from the intra-day 7-point blood glucose profile, they revealed a significant link between long-term glycaemic stability and the development of retinopathy and nephropathy by using the 9 year adjusted SD of HbA_{1c} as an indicator [15].

To the best of our knowledge, HbA_{1c} variability has never been used to predict clinical outcomes in patients with type 2 diabetes. Instability of the fasting glucose level has been reported as a risk factor for the development of complications in type 2 diabetes, but the results have been inconsistent. Intra-day glucose variability was shown to be associated with coronary artery disease in type 2 diabetes in a cross-sectional study [7]; however, it could not predict recurrent cardiovascular outcomes in the prospective HEART2D study [23]. The predictability of all-cause and cardiovascular mortality from 3-year fasting glucose variability in type 2 diabetes patients was shown in the Verona Diabetes Study [24, 25], but is still controversial with regard to an association between glucose variability and microvascular outcomes. A small-scale study ($n=130$) conducted in Spain found that fasting glucose variability was an independent risk factor for retinopathy in patients with type 2 diabetes in a 5.2 year follow-up [26]; however, another Italian study ($n=746$) could not confirm this association [4]. The inconsistency in the results of the aforementioned

Table 4 Incidence and risk of microalbuminuria in individuals in different quartiles of adjusted HbA_{1c} SD and in different levels of mean HbA_{1c}, stratified by HbA_{1c} follow-up time and baseline HbA_{1c}

Variable	Adjusted SD				*p* for trend (Q1 to Q4)[a]	HR per 1% HbA_{1c} increment[b]	*p* for interaction (adjusted SD × mean of HbA_{1c})[c]
	Q1	Q2	Q3	Q4			
HbA_{1c} follow-up time							
Overall (up to 7 years)							
Cases/person-years	66/1,125	67/1,143	70/1,150	102/1,109			
Incidence	58.4	58.6	60.8	91.9			
HR (95% CI)[a]	1	1.06 (0.74, 1.50)	1.13 (0.80, 1.60)	1.57 (1.13, 2.17)	0.001	1.10 (1.00, 1.20)[b]	0.951
2 years							
Cases/person-years	62/1,112	65/1,137	69/1,145	101/1,103			
Incidence	55.7	57.2	60.2	91.5			
HR (95% CI)[a]	1	1.19 (0.77, 1.84)	1.32 (0.86, 2.03)	1.42 (0.93, 2.17)	0.019	1.03 (0.92, 1.15)[b]	0.920
Baseline HbA_{1c}							
≤8%							
Cases/person-years	33/650	33/653	37/656	54/660			
Incidence	50.7 (35.5, 70.4)	50.5 (35.3, 70.1)	56.4 (40.3, 76.9)	81.8 (62.0, 106.0)			
HR (95% CI)[a]	1	1.00 (0.60, 1.65)	1.05 (0.65, 1.71)	1.42 (0.91, 2.22)	0.026	1.13 (0.91, 1.39)[b]	0.371
>8%							
Cases/person-years	33/475	34/490	33/494	48/449			
Incidence	69.4 (48.6, 96.4)	69.3 (44.8, 95.8)	66.8 (46.7, 92.7)	106.9 (79.7, 140.6)			
HR (95% CI)[a]	1	1.01 (0.61, 1.67)	1.29 (0.82, 2.03)	1.64 (1.04, 2.59)	0.047	1.18 (1.04, 1.34)[b]	0.365

Incidence is expressed as events per 1,000 person-years observed

[a]Models were controlled for sex, age of diabetes onset, education, diabetes duration, smoking status, BP, waist circumference, HDL-cholesterol, triacylglycerols, ACE inhibitor/ARB use and adjusted SD of HbA_{1c}

[b]Models were controlled for sex, age of diabetes onset, education, diabetes duration, smoking status, BP, waist circumference, HDL-cholesterol, triacylglycerols, ACE inhibitor/ARB use and mean of HbA_{1c}

[c]Models included the following variables: sex, age of diabetes onset, education, diabetes duration, smoking status, BP, waist circumference, HDL-cholesterol, triacylglycerols, ACE inhibitor/ARB use, mean of HbA_{1c}, adjusted SD of HbA_{1c} and (mean × adjusted SD) of HbA_{1c}

ARB, angiotensin receptor blocker

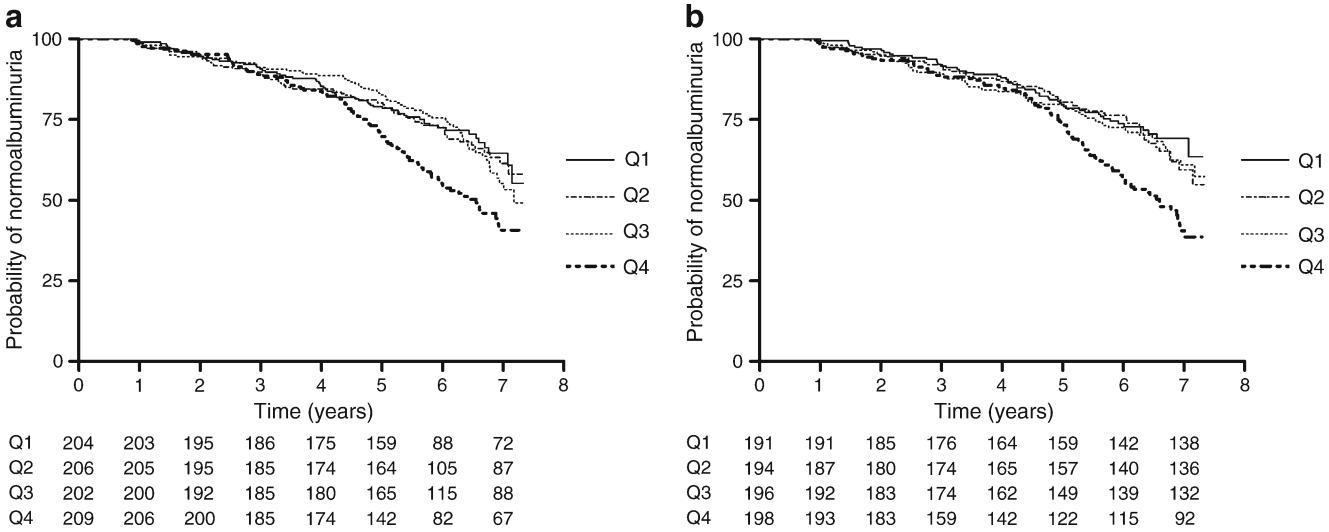

Fig. 1 Probability of remaining in normoalbuminuria status, by quartile of adjusted HbA_{1c} SD. (**a**) Adjusted HbA_{1c} SD was calculated using all HbA_{1c} measurements during the follow-up period. Logrank tests Q2 vs Q1 p=0.415, Q3 vs Q1 p=0.107; Q4 vs Q1 p<0.001 (**b**) Adjusted HbA_{1c} SD was calculated using the first three or four HbA_{1c} measurements (from recruitment to the end of year 2). Logrank tests Q2 vs Q1 p=0.335; Q3 vs Q1 p=0.397; Q4 vs Q1 p<0.001

studies may be attributable to the influence of food intake on serial glucose measurements. A sporadically measured acute glucose profile may also not be able to reflect a long-term dynamic pattern of glycaemic variability. Moreover, the standard measurement of acute glucose fluctuation using continuous glucose monitoring or intra-day 7-point glucose profile to calculate SD or the mean amplitude of glycaemic excursion [27] is not clinically applicable for most non-insulin-using type 2 diabetes patients.

In this study, we used HbA_{1c}, an indicator reflecting glycaemic control over 2–3 months [28], to detect microalbuminuria development. The mean and SD derived from three or four HbA_{1c} measurements in 2 years were adequate to predict microalbuminuria. This differs from most previous studies, which used HbA_{1c} variability from long-term observations, varying from 5 to 16 years [13, 15, 20], to delineate its impacts on diabetic complications in type 1 diabetes. A series of HbA_{1c} measurements is able to reveal a general pattern of glycaemic control during a certain period for risk assessment. However, it is difficult to apply in clinical practice if the required data collection period for a reliable indicator is too long; clinicians usually need to be aware of their patients' risks at the earliest possible time to make prompt clinical decisions. Apart from the fact that the long-term follow-up of serial HbA_{1c} levels is essential for better diabetes care, our findings indicate the use of 2-year variability and mean of HbA_{1c} to correlate microalbuminuria development is a clinically responsive indicator, which emphasises the importance of optimising an unfluctuating HbA_{1c} early to prevent diabetic nephropathy.

High variability of HbA_{1c} implies that poor glycaemic control does exist, at least temporarily, although the average HbA_{1c} may be desirable in our patients. According to 'metabolic memory' theory [29], poor glycaemic control, even if it lasts only a short time, can be 'memorised' and still cause detrimental effects later on. Glucose fluctuations have been demonstrated to cause oxidant overproduction and endothelial dysfunction, and this effect is even stronger in stable higher glucose status in type 2 diabetic patients [30, 31]. The overproduction of reactive oxygen species is the common mediator of several hyperglycaemia-activated pathways in the pathogenesis of diabetic nephropathy. Patients with high HbA_{1c} variability often live unhealthier lifestyles and, as shown in this study, may also intensify their vulnerability to the development of diabetic nephropathy. Furthermore, persistent epigenetic changes could be induced by transient hyperglycaemia [32], although other mechanisms of nephropathy caused by higher HbA_{1c} variability are still unknown.

We have to be cautious when interpreting the results of this study because we may not be able to fully control all confounding factors in an observational study. To clarify the possibility of reverse causation, which is often suspected in observational cohort design, a randomised clinical trial is needed to further validate the effects of the proposed 2-year HbA_{1c} variability on diabetic nephropathy. Other limitations of this study are the measurement issues. We checked HbA_{1c} every 6 months, from which the glycaemic variability was derived; however, HbA_{1c} is an indicator reflecting glycaemic control over 2–3 months. Therefore, the HbA_{1c} variability in the current study may be an underestimate, owing to the inadequate monitoring period. Furthermore, for practical reasons, instead of measuring 24 h albumin excretion or early-morning first voiding urine, ACR was measured using morning spot urine in this study. This is acceptable according to the

Kidney Disease Outcome Quality Initiative Clinical Practice Guideline [33], but may overestimate the incidence of microalbuminuria.

In conclusion, the current study is the first prospective study showing that higher HbA_{1c} variability is associated with the development of microalbuminuria in type 2 diabetes patients. The predictability of the 2 year HbA_{1c} SD for development of microalbuminuria conveys a clinical message that sustaining glycaemic control at the early stage is crucial for the management of type 2 diabetes.

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Contribution statement CCH and SJS designed the study and conceived the idea. CCH, HYC SJH, TYT, and YSL analysed data and interpreted results. MCH and YCY collected and maintained the research data. CCH and SJS drafted the article. HYC, MCH, SJH, YCY YSL, and TYT critically revised the article for important intellectual content. All authors reviewed the manuscript and had final responsibility for the decision to submit for publication.

Duality of interest All authors declare that there is no duality of interest associated with this manuscript.

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