

Homocysteine and diabetic retinopathy^{1,2,3,4,5}

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Abstract

Background: Homocysteine is an emerging risk factor for cardiovascular and non-diabetic ocular vaso-occlusive diseases. However, studies of the relationship between homocysteine and diabetic retinopathy have reported inconsistent results.

Objective: The purpose of this study was to evaluate the relationship between plasma total homocysteine concentration and diabetic retinopathy.

Design: We assessed the homocysteine-retinopathy relationship in 168 men and women with type 2 diabetes in a community-based, cross-sectional study. We photodocumented diabetic retinopathy status and measured plasma total homocysteine concentration using a commercial FPIA enzymatic kit. Data for selected clinical/demographic variables and established risk factors for diabetic retinopathy were obtained from fasting blood samples and an interviewer-assisted lifestyle questionnaire.

Results: A higher mean (95% CI) plasma total homocysteine concentration was observed in diabetic individuals with retinopathy than in those without retinopathy (11.5 [10.4- 12.5] umol/L vs. 9.6 [9.1-10.2] umol/L, $p = 0.001$). Furthermore, the relationship between homocysteine and diabetic retinopathy was not explained by renal dysfunction and was independent of the other major risk factors for diabetic retinopathy [duration of diabetes, HbA1c, systolic blood pressure] and determinants of higher homocysteine concentrations [age, gender, red cell folate], (OR 1.20 [1.023-1.41], $p = 0.024$).

Conclusion: Plasma total homocysteine concentration may be a useful biomarker and/or a novel risk factor for increased risk of diabetic retinopathy in people with type 2 diabetes.

Introduction

Homocysteine has generated considerable interest in recent years as both a sensitive biomarker of folate deficiency and as an emerging risk factor for cardiovascular disease [even within the normal range of homocysteine concentrations](1), and has been linked to vaso-occlusive diseases in the eye (2).

The known determinants of higher fasting plasma homocysteine levels are older age, male gender, and certain genetic abnormalities, while the major risk factors for hyperhomocysteinaemia (elevated plasma total homocysteine concentration) are impaired renal function and poor vitamin B status (particularly folate status, but also vitamin B6 and B12 status). In the elderly (>75 years), hyperhomocysteinaemia is generally associated with low folate status or renal impairment (3).

In addition, a multitude of physiological, lifestyle, and drug associations with higher homocysteine concentrations have also been reported, including positive associations with smoking, and alcohol and coffee intake (4).

Numerous studies have evaluated the diabetic retinopathy-homocysteine relationship (Table 1), but have yielded inconsistent results (5-28), possibly due to methodological differences and residual confounding. We hypothesized that homocysteine is associated with diabetic retinopathy in type 2 diabetes, independent of the major determinants of both retinopathy and homocysteine levels, and that homocysteine concentrations would be higher in diabetic individuals with than without retinopathy.

Methods

Diabetes status Self-reported diabetes status was confirmed biochemically, according to the WHO diagnostic criteria for the classification of diabetes (29).

Subject selection In order to broaden the range of dietary intakes and lifestyle exposures, we sourced subjects from the

Melbourne Collaborative Cohort Study (MCCS), a community-based prospective cohort of 41,528 male and female volunteers, aged 40–69 years at baseline (1989-1994), recruited from the electoral roll, and ethnic radio, clubs, and churches (30). We invited 248 men and women with type 2 diabetes from the MCCS to participate in this study. Of these, we excluded three (two men with type 1 diabetes and one man with ungradable photographs). Sixty eight percent (n=168) of the eligible subjects (n=245), participated in the study. Ethics approval was obtained from the MCCS scientific committee and Deakin and Monash Universities (Melbourne, Australia), and written informed consent was obtained from every participant.

Diabetic retinopathy We used a mydriatic retinal fundus camera (Kowa FX-500S, Japan) to photodocument retinal status. Diabetic retinopathy grading was based on the Eurodiab protocol (validated against the Airlie House classification) in which the overall grading was that of the worse eye and non-proliferative diabetic retinopathy was defined as more than one microaneurysm and/or haemorrhage(31). A medical retina specialist (CAH), masked to all other participant information, graded the slides on two separate occasions to assess internal validity, and agreement between gradings was excellent (kappa value of 0.986).

Clinical measures and retinopathy risk factors Systolic and diastolic blood pressure was recorded using a Dinamap XL portable automated adult vital signs monitor [Model 9300 (Critikon, Florida, USA)]. Blood pressure was recorded as the average of the last two of three consecutive readings, obtained from the right arm of seated subjects at one minute intervals after a 10 minute rest period. Weight was measured to within 0.1kg before breakfast and following a 12-hour fast, with subjects wearing light clothing and no shoes, using digital electronic scales (UC-300; A.N.D, Tokyo,

Japan). Height was measured to within 0.1cm using a wall mounted stadiometer (Harpenden, Holtain Limited, Crymych, UK). Body mass index (BMI) was calculated as weight (kg) / height (m)². A 'current smoker' was defined as a subject who smoked at least 7 cigarettes a week at the time of completing the questionnaire.

Plasma biochemistry Homocysteine concentration in EDTA-treated plasma was assayed using a commercially available Fluorescence Polarisation Immunoassay (Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL) (32). Plasma total homocysteine concentrations were read on an IMx System® Analyser (Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL). Plasma glucose concentrations were analysed using an automatic analyser (Hitachi model 705, Tokyo, Japan) and a commercial enzymatic kit (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) by the glucose oxidase method. Plasma cholesterol and triacylglycerol concentrations were analysed with an automatic analyser (Hitachi model 705, Tokyo, Japan) using a commercial enzymatic kit (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany).

Red blood cell folate concentration Red blood cell folate was measured in haemolysed whole blood (1/22 dilution) using a Bio-Rad Quantaphase Folate radioassay [detection range: 0.2 to 3.0 ng/ml, CVs: 9.8-12.1%] (33). Red cell folate concentration was calculated as follows: Red Cell Folate (adjusted) = (22 x unadjusted Red Cell Folate concentration) / Hematocrit

Urinary biochemistry Urinary albumin concentration was measured using immunonephelometry (Kallestadt QM300 or Beckman 360 Array nephelometers; interassay CV was 3%-5%). Urinary creatinine concentration was measured using an alkaline picrate method (Olympus AU800 autoanalyser; interassay CV was 2%). The urinary albumin to creatinine ratio was then calculated as albumin (mg) / creatinine (mmol).

Statistical analyses We used SPSS version 13 for Windows (SPSS Inc., Chicago IL, USA) software to perform the statistical analyses. The data were cross-sectional observations. Descriptive statistics for the exposure and outcome variables were obtained, and variables with distributions that were not normally distributed were log-transformed prior to analysis. Associations between retinopathy and continuous variables were assessed using ANOVA for variables with homogeneous variances or the corresponding non-parametric test when variances were non-homogeneous. Associations between categorical variables were analyzed using chi-square tests.

Initially, variables assessed in univariate analyses, including known risk factors for diabetic retinopathy, were modelled using binomial logistic regression analysis to determine the best clinical predictors of diabetic retinopathy. Plasma total homocysteine concentration was then added to subsequent models that controlled for the major risk factors for diabetic retinopathy and the established determinants of homocysteine. The fit of each model was tested and the Nagelkerke R² approximation was compared for each of the logistic regression models. P <0.05 was considered statistically significant.

Results

Characteristics of the participants are shown in Table 2. Diabetic retinopathy was identified in 28.6% of people with type 2 diabetes. As expected, the HbA1c level was higher in participants with than without retinopathy, although both groups demonstrated poor metabolic control (HbA1c >7%). Participants with diabetic retinopathy had a significantly longer duration of diabetes, were more likely to use hypoglycaemic medication(s), and had a higher albumin to creatinine ratio (ACR). The majority in both retinopathy and non-retinopathy groups had systolic hypertension (SBP>140mmHg), with a trend evident for a higher systolic and pulse blood pressure in the retinopathy group.

Lipid levels were not associated with diabetic retinopathy, and there were few smokers in the study population. Male gender was not associated with diabetic retinopathy (31% vs. 25%, $p = 0.388$), and we observed a non-significant gender difference in the median homocysteine concentration (9.0 $\mu\text{mol/l}$ women vs. 9.9 $\mu\text{mol/l}$ men, $p = 0.063$, respectively).

A higher mean (95% CI) plasma total homocysteine concentration was observed in diabetic individuals with than without retinopathy (11.5 [10.4- 12.5] vs. 9.6 [9.1- 10.2], $p = 0.001$). Hyperhomocysteinaemia (plasma total homocysteine $\geq 12 \mu\text{mol/L}$ for a folate-fortified population) was present in 22% ($n = 37$) of people with type 2 diabetes, and 7.7% ($n = 14$) had a plasma total homocysteine concentration greater than 15 $\mu\text{mol/L}$. Amongst those with diabetic retinopathy, 33% ($n = 17$) had hyperhomocysteinaemia, and 14.6% ($n = 8$) had a plasma total homocysteine concentration greater than 15 $\mu\text{mol/L}$.

In both participants with and without retinopathy, the mean (95% CI) red cell folate concentration was in the normal range and was not (statistically) significantly different between retinopathy and non-retinopathy cases (312 [252-373] vs. 371 [322-421] ng/ml , $p = 0.174$). However, folate depletion (red cell folate concentration $< 160 \text{ng/ml}$) or deficiency (red cell folate concentration $< 120 \text{ng/ml}$) was observed in 8.1% ($n = 12$) of participants. As expected, a higher red cell folate concentration (adjusted for the hematocrit) was associated with lower homocysteine levels ($r = -0.359$, $p = < 0.0001$).

Table 3 demonstrates that, of the established risk factors and clinical characteristics in Table 2, duration of diabetes and the ACR ratio were the only independent predictors of diabetic retinopathy in our study group. In logistic regression models of homocysteine as a predictor of increased risk of diabetic retinopathy (Table 4), the significant association between homocysteine and diabetic retinopathy identified in univariate testing remained

significant in multivariate testing (model 1). The increased risk of diabetic retinopathy predicted by higher homocysteine concentrations was not explained by renal dysfunction after controlling for both the other major risk factors for diabetic retinopathy [duration of diabetes, HbA1c, systolic blood pressure] and the other determinants of homocysteine concentrations [age, gender, red cell folate], (OR 1.20 [1.023-1.41], $p = 0.024$), as shown in model 2. Model 3 demonstrates that biguanide (metformin) use did not explain the significant relationship between plasma homocysteine concentration and diabetic retinopathy. Age was inversely associated with diabetic retinopathy in the models that adjusted for systolic blood pressure.

Discussion

Several interesting observations were made in this study: Firstly, a higher plasma homocysteine concentration was associated with prevalent retinopathy in people with type 2 diabetes. Secondly, the difference in the mean plasma homocysteine concentration between retinopathy and non-retinopathy cases was relatively small (less than 2 $\mu\text{mol/L}$). Thirdly, the mean plasma homocysteine concentration for both retinopathy and non-retinopathy cases was below the upper limit for normal for this age group i.e. 12 $\mu\text{mol/L}$ for mean age less than 65 yr (34). Finally, a 1 $\mu\text{mol/L}$ increase in plasma homocysteine concentration was an independent predictor of increased risk of diabetic retinopathy of between 15% - 20%, after adjusting for the established risk factors for diabetic retinopathy (duration of diabetes, HbA1c, systolic blood pressure) and the major determinants of the plasma total homocysteine concentration (age, gender, red cell folate concentration, and renal function).

Prospective studies are now needed to confirm our findings, which may have implications for the management of diabetes: Dietary modulation of homocysteine levels is possible (35). Interestingly, poor folate status did not

account for the observed homocysteine-retinopathy relationship in our study, possibly due to the adequate folate status of the majority of participants. Future studies that evaluate the association between poor folate status and diabetic retinopathy may help to clarify the basis of the observed homocysteine-retinopathy relationship.

Homocysteine may be a good biomarker for increased risk of diabetic complications, since retinopathy, nephropathy, and CVD have all been linked to higher homocysteine levels. Our finding that a difference in homocysteine concentration of less than 2umol/L separated retinopathy and non-retinopathy cases suggests that a relatively small increase in the plasma homocysteine concentration in the order of 1umol/L may be a useful trigger for intensification of treatment of the major risk factors for diabetic complications (blood pressure, blood glucose, and blood lipids). Moreover, for the timely identification of individuals at greater risk of the vascular complications of diabetes, the upper limit of the normal range for plasma homocysteine (12umol/L for folate-fortified and/or adult populations and 20umol/L for folate-unfortified and/or older populations [>65 years]) may need to be lowered, as has been the case for blood pressure and lipid target levels.

A limitation of many homocysteine studies, particularly homocysteine-lowering CVD trials (36), has been their failure to control for renal disease. In the many studies that have evaluated the diabetic retinopathy-homocysteine relationship (Table 1), several did not control for impaired renal function. A number did not control for established retinopathy risk factors, and none controlled for metformin use. All of these factors were accounted for in the present study.

Many of the earlier studies evaluated homocysteine only as a categorical variable (proportion of study sample with hyperhomocysteinaemia i.e. homocysteine concentration above a designated cut-off). However, the cut-off for hyperhomocysteinaemia is arbitrary and differed substantially between studies,

ranging from 11.7 umol/l to 16 umol/l. In our study, retinopathy and non-retinopathy cases had mean homocysteine levels below 11umol/l, indicating the cut-off used in earlier studies may have been too high. Therefore, both the variability in and the level of the selected cut-offs may have contributed to the discordant findings between studies. Consequently, we evaluated the impact of homocysteine as a continuous variable.

Other methodological issues may have also contributed to the disparity between studies: Homocysteine can be determined either directly or after derivatization, making comparisons of findings between studies difficult. We measured plasma homocysteine levels directly, consistent with current expert opinion and recommendations. In addition, there are many different methods and classifications for the assessment of diabetic retinopathy: We photodocumented retinopathy status according to the Eurodiab protocol, validated against the Airlie house classification, unlike many of the earlier homocysteine studies in which retinopathy was assessed using ophthalmoscopy (14). Nevertheless, it is possible that more accurate assessment of diabetic maculopathy would facilitate a better understanding of the nature of the retinopathy-homocysteine relationship.

The main limitation of this study was the use of a cross-sectional design, which precludes determination of temporal direction and therefore of causal inference. Although we controlled for retinopathy risk factors and the most important confounders of homocysteine in this population; specifically age, gender, smoking status, red cell folate levels, metformin use, and renal status, many factors linked to homocysteine in other studies were not assessed in our study, such as genetic and lifestyle factors, for example vitamin B12 and B6 intakes, and conditions associated with diabetes and aging, such as depression and dementia (32). However, in contrast to earlier studies, recruiting from a community-based cohort

enabled evaluation of the homocysteine-retinopathy relationship in a more health-conscious group of individuals at lower risk of co-morbidities and potential confounders than hospital-based populations.

In conclusion, our observations support a role for homocysteine in diabetic retinopathy, at least as a biomarker and potentially as a risk factor if prospective studies confirm our observations. While we acknowledge there is currently insufficient evidence to recommend routine screening of homocysteine for the purpose of treating elevated homocysteine concentrations in the wider adult population, almost one in ten of our participants was folate-depleted and so was at risk of folate deficiency and related functional deficits, suggesting that monitoring homocysteine and folate status

in people with type 2 diabetes may have net health benefits. Finally, this study provides further support for recommending a folate-rich diet based on high intakes of fresh fruit and vegetables for people with type 2 diabetes.

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Table 1 Studies of plasma homocysteine concentrations in populations with diabetes

Authors	Year	Design	Country	Number of subjects			Homocysteine levels umol/L				Association between DR and homocysteine	Other significant associations with homocysteine	
				ND	T1D	T2D	ND	T1D	T2D	DR			
Hultberg et al	1991	CH	Sweden	46	79		11	10.4				Absent if normal renal	Nephropathy
Agardh et al	1994	CH	Sweden		76			**8/9/13				Absent	B folate, S-Creatinine, S-Urea, U-Albumin, SBP, duration of diabetes, nephropathy(not microalbuminuria)
Neugebauer et al	1997	CH	Japan			112						Present (MTHFR)	
Chico et al	1998	CH	Spain	56	75	90	7.4	7	9.2			Not investigated	AER, T2DM, presence and severity of nephropathy
Stabler	1999	Cc	USA			452						Absent	Neuropathy; macroalbuminuria
Smulders	1999	CH	Netherlands			85						Absent	Microalbuminuria
Vaccaro et al ²	2000	CH	Italy	44	66		7.4					Present (proliferative)	MTHFR/C677T mutation, microalbuminuria (not T1D)
Chiarelli	2000	CH	Italy		61							Present (proliferative)	Microalbuminuria
Hoogveen et al ³	2000	Cc	Netherlands	454		171						Present	Not investigated
Agardh et al	2000	CH	Sweden		49			10.4				Absent	Serum creatinine
Buysschaert et al	2001	CH	Belgium		71							Present (univariate)	Age, creatinine, folic acid were independently associated SBP, cholesterol, duration, complications
Agullo-Ortuno	2002	CH	Spain	54	57	32	10.1	11.7	11.7			Present (T1D)	Macroangiopathy and nephropathy but only in T1DM

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Abdella et al	2002	CH	Kuwait			358			10.2	Absent	Male gender, CHD, HbA1c, creatinine, apoB (not microalbuminuria), neuropathy, smoking
Matteucci et al	2002	CH	Italy	133	79		12.2	9.2		Absent	Gender, age smoking in controls; gender, age, smoking, Creatinine, lipopn(a), nephropathy inT1D
Guo et al	2002	CH	China	28	32		8.9	11.6	13.9	Present	T2D, MTHFR genotype, metabolites of NO
Yang G et al ¹	2002	CH	China	19	55		*22.3	*25	*37.3	Present	No association with T2D
Sun J et al	2003	CH	China	57	208					Present	T1D, T2D, T1 complications
Looker et al	2003	Pc	USA (Pima)			396				Present (proliferative)	Nephropathy
Goldstein et al ⁴	2004	CH	Israel	156	179		11.8	13.5	N14.6 P15.9	Present	Not investigated
Saeed et al	2004	CH	UK		48			7.7	8.8	Present	Microalbuminuria
Yucel et al	2004	CH	Turkey	30	40					Present (NP and P)	Not investigated
Soedamah-Muthu	2005	CH	13 European countries		533					Present (NP and P)	Hypertension, macroalbuminuria, CVD, GFR
De Luis et al	2005	CH	Spain		155					Absent*** (Hcy≥15umol/l)	Peripheral arteriopathy, nephropathy, fibrinogen, lipoprotein (a), systolic and diastolic blood pressure
Huang et al	2006	CH	Taiwan	204	257		10.0	12.9		Present	Duration of diabetes >10years

Excluded abnormal renal and ACR ; 2. Excluded diabetes duration <10 yr; 3. Adjusted for diabetes, age, sex, HbA1c, hypertension; 4. N non-proliferative; P proliferative diabetic retinopathy ; T1D type 1 diabetes;

T2D type 2 diabetes * post methionine loading ** normal/ micro/ macroalbuminuria (clinical nephropathy) *** Hyperhomocysteinaemia defined as homocysteine ≥15umol/L ND non-diabetic DR diabetic retinopathy; NO nitric oxide

GFR glomerular filtration rate; C cross-sectional, Prospective, H hospital-based, c community-based; MTHFR Retinopathy associated with homocysteine-related MTHFR genotype

Table 2 Characteristics of people with type 2 diabetes, by retinopathy status (n = 168)

	Retinopathy absent		Retinopathy present		Pvalue
	(n = 120)		(n = 48)		
	Median	Percentiles	Median	Percentiles	
	50	25 75	50	25 75	
Age, yrs	65.0	59.0 69.0	66.5	60.3 69.0	0.455
Fasting glucose, mM	10.0	7.9 11.7	10.4	8.5 12.3	0.266
HbA1c, %	7.6	6.6 8.7	8.6	7.1 10.2	0.003
Duration of diabetes, yr	7.0	4.8 12.0	12.0	7.3 21.5	<0.0001
Hypoglycaemic medx, %	63		90		0.001
Body mass index, kg/m ²	30	27 33	28	25 32	0.122
Systolic blood pressure, mmHg	142	131 156	148	134 167	0.186
Diastolic blood pressure, mmHg	73	69 81	74	69 79	0.946
Pulse blood pressure, mmHg	66	58 79	73	62 89	0.123
Current smoker, %	9.1		4.3		0.292
Urinary ACR, mg/mmol	1.1	0.7 2.8	1.9	0.9 12.1	0.017
Plasma total cholesterol, mmol/l	5.5	4.7 6.1	5.1	4.6 5.7	0.079

					<i>Homocysteine and diabetic retinopathy</i>		
Plasma HDL-cholesterol, mmol/l	1.1	0.9	1.4	1.1	0.9	1.3	0.659
Plasma triglycerides, mmol/l	1.6	1.2	2.5	1.6	1.1	2.5	0.289
Plasma homocysteine, umol/l	9.2	7.5	11.5	10.5	9.0	13.8	0.003
Red cell folate, ng/ml [^]	282	199	438	239	181	402	0.200

HDL high-density lipoprotein, ACR albumin to creatinine ratio; [^]adjusted for hematocrit; current smoker and gender data are prevalence (%)

P values reported are Chi-square for categorical variables (prevalence reported), Anova for parametric data, and Mann-Whitney for non-parametric data

Table 3 Multivariate model of predictors of retinopathy in people with type 2 diabetes

Independent variables	B	S.E.	Exp(B)	95.0% C.I. for EXP(B)		P value
				Lower	Upper	
Age, yrs	-0.06	0.04	0.94	0.88	1.01	0.082
Fasting glucose, mM	-0.01	0.09	0.99	0.83	1.18	0.922
Duration of diabetes, yr	0.72	0.33	2.06	1.08	3.96	0.029
HbA1c, %	0.20	0.15	1.22	0.92	1.63	0.172
Hypoglycaemic medx, %	0.58	0.60	1.79	0.55	5.83	0.332
Body mass index, kg/m ²	-0.06	0.05	0.94	0.85	1.04	0.245
Systolic blood pressure, mmHg	0.00	0.01	1.00	0.98	1.03	0.846
Diastolic blood pressure, mmHg	0.00	0.03	1.00	0.95	1.06	0.916
Current smoker, %	-0.82	0.89	0.44	0.08	2.54	0.360
Urinary ACR, mg/mmol	0.40	0.17	1.50	1.07	2.09	0.018
Plasma total cholesterol, mmol/l	-0.19	0.24	0.83	0.52	1.32	0.426
Plasma HDL-cholesterol, mmol/l	0.31	0.75	1.36	0.31	5.95	0.685
Plasma triglycerides, mmol/l	-0.12	0.23	0.89	0.57	1.40	0.610

Log-transformed data were modelled for duration of diabetes and ACR
 Model R² = 0.30

Table 4 Regression models of homocysteine as a predictor of retinopathy in type 2 diabetes

Mode							
1							
		95.0% C.I.for					
1		B	S.E.	Exp(B)	EXP(B)		Pvalue
R ²					Lower	Upper	
0.25	Duration, yr	0.85	0.33	2.34	1.23	4.46	0.009
	HbA1c, %	0.16	0.13	1.17	0.91	1.52	0.229
	Age, yr	-0.05	0.03	0.95	0.89	1.01	0.113
	Male gender	-0.57	0.64	0.57	0.16	2.01	0.380
	Red cell folate, ng/mL	-0.01	0.01	0.99	0.97	1.02	0.479
	Homocysteine, umol/L	0.16	0.08	1.17	1.01	1.37	0.041
	ACR, mg/mmol	0.02	0.01	1.02	1.00	1.05	0.093
		95.0% C.I.for					
2		B	S.E.	Exp(B)	EXP(B)		Pvalue
R ²					Lower	Upper	
0.24	Duration, yr	0.90	0.33	2.46	1.30	4.66	0.006
	HbA1c, %	0.17	0.13	1.18	0.91	1.53	0.208
	Age, yr	-0.07	0.04	0.93	0.87	1.00	0.049
	Male gender	-0.31	0.65	0.74	0.20	2.65	0.638
	Red cell folate, ng/mL	-0.01	0.01	0.99	0.97	1.02	0.529
	Homocysteine, umol/L	0.18	0.08	1.20	1.02	1.41	0.024

		0.19	0.17	1.20	0.87	1.67	0.265
		0.02	0.01	1.02	0.99	1.04	0.153
		95.0% C.I. for					
3		B	S.E.	Exp(B)	EXP(B)		Pvalue
R ²					Lower	Upper	
0.30	Duration, yr	0.10	0.03	1.10	1.03	1.17	0.003
	HbA1c, %	0.22	0.14	1.25	0.95	1.64	0.111
	Age, yr	-0.08	0.04	0.93	0.86	1.00	0.043
	Male gender	-0.49	0.69	0.61	0.16	2.38	0.478
	Red cell folate, ng/mL	-0.01	0.01	0.99	0.96	1.02	0.439
	Homocysteine, umol/L	0.18	0.09	1.19	1.01	1.41	0.036
	ACR, mg/mmol	0.01	0.01	1.01	0.99	1.04	0.310
	Systolic BP, mmHg	0.02	0.01	1.02	0.99	1.04	0.184
	Biguanide (Metformin)	-0.37	0.49	0.69	0.26	1.81	0.450

Abbreviations ACR albumin to creatinine ratio; BP blood pressure

Duration of diabetes and urinary ACR data are log-transformed