Diabetologia (2018) 61:977–984
https://doi.org/10.1007/s00125-017-4510-1

ARTICLE

Plasma concentrations of 8-hydroxy-2′-deoxyguanosine and risk of kidney disease and death in individuals with type 1 diabetes

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Received: 11 August 2017 / Accepted: 1 November 2017 / Published online: 28 November 2017
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Abstract

Aims/hypothesis Oxidative stress is involved in the pathogenesis of diabetic kidney disease. We evaluated the association between 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of DNA oxidative damage, and end-stage renal disease (ESRD) or death in individuals with type 1 diabetes.

Methods Plasma 8-OHdG concentrations were measured at baseline in participants with type 1 diabetes from GENEDIAB ($n = 348$) and GENESIS ($n = 571$) cohorts. A follow-up was conducted in 205 and 499 participants for a mean ± SD duration of 8.9 ± 2.3 years and 5.2 ± 1.9 years, respectively. We tested associations between 8-OHdG concentrations and urinary albumin concentration (UAC) or eGFR at baseline, and the risk of ESRD or all-cause mortality during follow-up. Analyses were performed in pooled cohorts.

Results The highest UAC (geometric mean [95% CI]) was observed in the third 8-OHdG tertile (tertile 1, 9 [6, 13] mg/l; tertile 2, 10 [7, 16] mg/l; tertile 3, 16 [10, 25] mg/l; $p = 0.36$ for tertile 1 vs tertile 2 and $p = 0.003$ for tertile 3 vs tertile 1) after adjustment for potential confounding covariates. The lowest eGFR (mean [95% CI]) was observed in the third tertile (tertile 1, 87 [82, 93] ml min^{-1} 1.73 m^{-2}; tertile 2, 88 [82, 94] ml min^{-1} 1.73 m^{-2}; tertile 3, 74 [68, 80] ml min^{-1} 1.73 m^{-2}; $p = 0.61$ for tertile 1 vs tertile 2; $p < 0.001$ for tertile 3 vs tertile 1). ESRD and death occurred in 48 and 64 individuals, respectively. The HR for ESRD, but not death, was higher in the third tertile than in the first (tertile 2 vs tertile 1, 1.45 [0.45, 5.04], $p = 0.54$; tertile 3 vs tertile 1, 3.05 [1.16, 9.60], $p = 0.02$) after multiple adjustments.

Conclusions/interpretation Higher plasma concentrations of 8-OHdG were independently associated with increased risk of kidney disease in individuals with type 1 diabetes, suggesting that this marker can be used to evaluate the progression of diabetic kidney disease.

Keywords Albuminuria · End-stage renal disease · Glomerular filtration rate · Kidney disease · Mortality · Oxidative stress · Type 1 diabetes

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00125-017-4510-1) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

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Abbreviations

AOPP	Advanced oxidation protein products
ARB	Angiotensin receptor blocker
ESRD	End-stage renal disease
GENEDIAB	Génétique de la Néphropathie Diabétique study
GENESIS	The GENESIS France–Belgium study
IQR	Interquartile range
8-OHdG	8-Hydroxy-2′-deoxyguanosine
ROS	Reactive oxygen species
UAC	Urinary albumin concentration

Introduction

Kidney disease is a common and severe complication seen in individuals with type 1 diabetes mellitus. It is associated with a high risk of end-stage renal disease (ESRD) and reduced life

expectancy [1–4]. Chronic hyperglycaemia activates numerous biochemical pathways through mitochondrial overproduction of reactive oxygen species (ROS) [5]. Increased ROS production with decreased antioxidant capacity leads to oxidative stress, which plays an important role in the pathogenesis of kidney disease in individuals with diabetes [6–11]. Oxidative stress affects lipids, proteins and DNA and leads to changes in cell structure and function [12, 13]. DNA oxidation is associated with a wide range of damage, including cellular senescence and apoptosis [12, 14, 15].

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage in which oxidised guanosine is excised in mitochondrial and nuclear DNA by the base excision and repair system [16]. Upon DNA repair by nuclease activity, 8-OHdG is excreted in the plasma and urine where it can easily be measured. Thus, 8-OHdG is used as a biomarker of oxidative DNA damage [17]. Previous studies have shown that individuals with type 2 diabetes exhibit higher plasma and urinary concentrations of 8-OHdG compared with healthy individuals [18–22]. However, the relationship between 8-OHdG concentrations and kidney disease has not been fully investigated in individuals with type 1 diabetes. In the present study, we measured concentrations of 8-OHdG in plasma samples collected at baseline in two prospective cohorts of type 1 diabetes, and tested for the association of this biomarker with the risks of diabetic kidney disease and death during follow-up. We also tested the independence between plasma concentrations of 8-OHdG and advanced oxidation protein products (AOPP), another biomarker of oxidative stress, on the risk of outcomes.

Methods

Study participants The Génétique de la Néphropathie Diabétique study (GENEDIAB) was a multicentre, binational (Belgium and France) study conducted from 1994 to 2006 in 490 individuals with type 1 diabetes [23]. Participants were selected based on a diagnosis of type 1 diabetes before the age of 35 years and past or present pre-proliferative (19%) or proliferative diabetic retinopathy (81%). The GENESIS France–Belgium study (GENESIS) was a family-based study conducted from 1999 to 2007 in 578 first-degree relatives and 662 probands with type 1 diabetes [24]. Participants were selected based on a diagnosis of type 1 diabetes before the age of 35 years, with initial ketosis and requirement for permanent insulin treatment within 1 year of diagnosis and past or present diagnosis of diabetic retinopathy. In the present investigation, we studied 348 participants from GENEDIAB and 571 participants with type 1 diabetes from GENESIS for whom plasma samples were available at baseline. In the prospective study, 205 GENEDIAB and 499 GENESIS participants who had attended outpatient clinics at least once during the follow-up period were followed until an endpoint was reached or until February 2007. The mean ± SD duration of follow-up was 8.9 ± 2.3 and 5.2 ± 1.9 years for the two respective cohorts. All participants gave written informed consent and the Ethics Committee of Angers University Hospital (Angers, France) approved the two study protocols.

Definition of clinical variables and outcomes Diabetic retinopathy was staged according to Kohner's classification as non-proliferative, pre-proliferative or proliferative retinopathy [25]. The main outcome of interest during follow-up was the incidence of ESRD, defined as new occurrences of renal replacement therapy (haemodialysis or kidney transplantation) requirement among participants free of ESRD at baseline. We also studied all-cause mortality, defined as death from any cause during follow-up.

Laboratory procedures Plasma concentrations of 8-OHdG were measured in duplicate on plasma samples collected at baseline and kept frozen at −80°C. We used an immunoassay method (ADI-EKS-350 DNA Damages EIA; Enzo LifeSciences, Lausen, Switzerland) and optical density reader (Nanoquant Infinite M200 Pro; Tecan, Zurich, Switzerland). The sensitivity of the assay to determine 8-OHdG concentration was 0.59 ng/ml and the intra-assay and inter-assay precision was determined to be less than 10%. Specificity analysis found a proportion of cross-reactivity inferior to 0.016% with intact guanosine or other compounds derived from guanosine except for 8-mercaptoguanosine (3.5% of cross-reactivity). In the present study, correlation between duplicates was 96%. Plasma concentrations of AOPP were measured by spectrophotometry using a microplate reader (MR 5000; Dynatech, Paris, France) [26].

Statistical analyses Categorical variables are expressed as the number of participants with corresponding percentage. Continuous variables are expressed as mean ± SD, or as median (interquartile range [IQR]) for those with skewed distribution. Urinary albumin concentration (UAC) was log-transformed for the cross-sectional analysis. Participants were categorised into three groups of equal size corresponding to increasing tertiles (1, 2 and 3) of plasma 8-OHdG concentrations.

Characteristics of participants at baseline were compared using χ^2, ANOVA, Wilcoxon or Kruskal–Wallis tests. A linear regression analysis was used to test the association between 8-OHdG tertiles (tertile 2 vs tertile 1; tertile 3 vs tertile 1) and UAC or eGFR at baseline.

To assess the nonlinearity in the relationship between plasma 8-OHdG and outcomes, we performed cubic splines regression with knots at 2, 10, 20, 25 and 40 ng/ml and a reference value at 5 ng/ml.

Kaplan–Meier curves were used to plot survival (outcome-free) rates during follow-up, and compared using the log-rank

test. Cox proportional hazards regression models were fitted to estimate HRs, with associated 95% CIs, for ESRD or all-cause mortality during follow-up by 8-OHdG tertile at baseline (tertile 2 vs tertile 1; tertile 3 vs tertile 1). Analyses were adjusted for potential confounding covariates at baseline: age, sex and cohort membership (model 1); plus duration of diabetes, HbA_{1c}, number of injections and dose of insulin therapy per day, systolic blood pressure, use of antihypertensive drugs, ACE inhibitors or angiotensin receptor blockers (ARBs), diabetic retinopathy stages and use of lipid-lowering drugs (model 2); plus eGFR (computed by the Chronic Kidney Disease–Epidemiology Collaboration equation [27]) and UAC (model 3). The Schoenfeld residuals method was used to check the proportional hazards assumption for ESRD ($p = 0.89$) or death ($p = 0.16$).

Sensitivity analyses were performed for the following purposes: (1) to evaluate the association between 8-OHdG tertiles and ESRD after treating death as a competing risk using the Fine and Gray method [28] and (2) to compare the risk of outcomes by plasma concentration of 8-OHdG using a threshold defined on the basis (above vs below) of the median. To test the independence between 8-OHdG and AOPP on the risk of outcomes, we computed the following: (1) HRs for outcomes by baseline plasma AOPP using cubic splines regression (knots at 27, 38, 79 and 107 μmol/l and reference at 53 μmol/l) after adjustment as in model 3 plus plasma 8-OHdG; (2) association of 8-OHdG tertiles with outcomes after adjustment for plasma AOPP (further to model 3) and (3) multiplicative interaction between plasma concentrations of 8-OHdG and AOPP on the risk of outcomes.

A complete case method was used to handle missing data (HbA_{1c} [$n = 28$], blood pressure [$n = 14$], use of antihypertensive treatment [$n = 11$], use of ACE inhibitors or ARBs [$n = 14$], eGFR [$n = 5$], UAC [$n = 22$], diabetic retinopathy [$n = 2$]). Thus, 73 participants with at least one missing value were omitted in the present study and 919 participants (704 in the prospective analyses) were included in the complete case study.

To increase statistical power, GENEDIAB and GENESIS cohorts were pooled, with adjustment for cohort membership as shown above. Statistics were performed using JMP Pro 13 software (SAS Institute, Cary, NC, USA) and Stata software version 13 (StataCorp, www.stata.com). Two-sided p values <0.05 were considered significant.

Results

Characteristics of participants by plasma concentrations of 8-OHdG at baseline Of the 919 participants, 53.8% were men. The mean ± SD age and duration of diabetes was 43.1 ± 11.5 and 27.5 ± 9.4 years, respectively, and the mean HbA_{1c} was 70 ± 17 mmol/mol (8.5 ± 1.5%). GENEDIAB participants, when compared with GENESIS participants, were older and had a longer duration of diabetes, higher daily dose of insulin therapy, higher systolic and diastolic blood pressure and lower eGFR and had more severe retinopathy (ESM Table 1). The median (IQR) plasma concentration of 8-OHdG at baseline was 9.1 (5.8–13.6) ng/ml, with a higher concentration seen in men than in women: 9.5 (6.5–14.7) vs 8.7 (5.2–12.7) ng/ml ($p = 0.001$). Characteristics of participants by 8-OHdG tertiles at baseline are summarised in Table 1. Briefly, systolic and diastolic blood pressure, proportion of individuals treated with antihypertensive medications, ACE inhibitors or ARBs and severity of diabetic retinopathy increased with increasing tertiles (Table 1).

Plasma 8-OHdG concentration, UAC and eGFR at baseline The median (IQR) UAC increased throughout 8-OHdG tertiles: 13 (6–66) mg/l; 22 (6–117) mg/l; 104 (10–857) mg/l; $p < 0.001$. The highest UAC (geometric mean [95% CI]) was observed in the highest 8-OHdG tertile (tertile 1, 9 [6, 13] mg/l; tertile 2, 10 [7, 16] mg/l; tertile 3, 16 [10, 25] mg/l; $p = 0.36$ for tertile 1 vs tertile 2 and $p = 0.003$ for tertile 3 vs tertile 1) after adjustment for potential confounding covariates (Table 2, model 3). The lowest mean ± SD eGFR was observed in the highest 8-OHdG tertile (tertile 1, 89 ± 26 ml min^{-1} 1.73 m^{-2}; tertile 2, 89 ± 28 ml min^{-1} 1.73 m^{-2}; tertile 3, 66 ± 33 ml min^{-1} 1.73 m^{-2}; $p < 0.001$). These associations remained significant after multiple adjustments (mean [95% CI]: tertile 1, 87 [82, 93] ml min^{-1} 1.73 m^{-2}; tertile 2, 88 [82, 94] ml min^{-1} 1.73 m^{-2}; tertile 3, 74 [68, 80] ml min^{-1} 1.73 m^{-2}; $p = 0.61$ for tertile 1 vs tertile 2 and $p < 0.001$ for tertile 3 vs tertile 1; Table 2, model 3).

Risk of ESRD during follow-up by plasma concentrations of 8-OHdG at baseline Among 659 individuals free from ESRD at baseline, new cases of ESRD occurred in 48 (7.3%) during a mean ± SD duration of follow-up of 6.3 ± 2.8 years. The incidence rate of ESRD was 1.37 per 100 person-years. Participants who developed ESRD during follow-up, compared with those who did not, were younger, had higher HbA_{1c}, systolic and diastolic blood pressure and UAC, had lower daily doses of insulin and lower eGFR, were more likely to use antihypertensive drugs, ACE inhibitors or ARBs and had a more prevalent proliferative retinopathy at baseline (ESM Table 2).

Median (IQR) plasma concentrations of 8-OHdG at baseline were higher in individuals who developed ESRD than in those who did not: 17.3 (11.5–23.2) vs 8.5 (5.4–11.9) ng/ml ($p < 0.001$). The association between plasma 8-OHdG at baseline and the risk of ESRD during follow-up was not linear ($p < 0.001$; ESM Fig. 1a). The Kaplan–Meier estimate of 10 year cumulative incidence of ESRD was higher in the third 8-OHdG tertile than in the others: 4.3% (tertile 1), 4.1% (tertile 2) and 22.1% (tertile 3), $p < 0.001$ (Fig. 1a). Cox analyses confirmed the association between the third 8-OHdG

Table 1 Characteristics of participants by tertiles of plasma concentrations of 8-OHdG at baseline

Characteristic	8-OHdG concentration			*p* value
	Tertile 1	Tertile 2	Tertile3	
N	306	306	307	
Male sex, %	47.4	54.9	59.0	0.01
Age, years	42.2 ± 11.5	42.7 ± 11.4	44.2 ± 11.5	0.09
Age at diabetes onset, years	14.5 ± 8.5	15.9 ± 8.5	16.1 ± 9.0	0.05
BMI, kg/m^2	24 ± 3	24 ± 3	24 ± 4	0.17
Duration of diabetes, years	27.7 ± 9.5	26.9 ± 9.3	28.1 ± 9.5	0.26
HbA_{1c}, mmol/mol	70 ± 15	71 ± 16	69 ± 19	0.45
HbA_{1c}, %	8.5 ± 1.4	8.6 ± 1.5	8.5 ± 1.7	0.45
No. of insulin injections per day	3.2 ± 0.9	3.2 ± 0.9	3.2 ± 0.9	0.94
Dose of insulin, U/day	44 ± 15	45 ± 15	47 ± 18	0.05
Systolic blood pressure, mmHg	131 ± 18	133 ± 17	140 ± 21	<0.001
Diastolic blood pressure, mmHg	75 ± 10	76 ± 10	79 ± 12	<0.001
Use of antihypertensive medication, %	40.2	48.4	67.8	<0.001
Use of ACE inhibitors or ARBs, %	34.3	37.9	53.8	<0.001
Use of lipid-lowering drugs, %	6.9	8.8	10.4	0.29
Diabetic retinopathy stage, %	31/18/51	28/18/54	18/16/66	<0.001
History of current smoking, %	29.2	29.7	28.4	0.94

Quantitative and qualitative variables are expressed as % or means ± SD, respectively

Comparisons performed using χ^2 and ANOVA tests; $p < 0.05$ was significant

Diabetic retinopathy stage: non-proliferative/pre-proliferative/proliferative

tertile at baseline and the risk of ESRD during follow-up: tertile 2 vs tertile 1 HR (95% CI) 1.45 (0.45, 5.04), $p = 0.54$; tertile 3 vs tertile 1 HR (95% CI) 3.05 (1.16, 9.60), $p = 0.02$ (Table 3, model 3).

Risk of all-cause mortality by plasma concentrations of 8-OHdG at baseline Sixty-four individuals (9.1%) died during 6.3 ± 2.8 years of follow-up, with an incidence rate of 1.47 per 100 person-years. Participants who died during follow-up,

Table 2 Associations between plasma concentrations of 8-OHdG and UAC or eGFR at baseline

Variable	Model 1[a]		Model 2[b]		Model 3[c]	
	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value
UAC, mg/l						
Tertile 1	27 (20, 37)		8 (5, 12)		9 (6, 13)	
Tertile 2	40 (30, 55)	0.03	9 (6, 13)	0.42	10 (7, 16)	0.36
Tertile 3	122 (81, 164)	<0.001	16 (11, 25)	<0.001	16 (10, 25)	0.003
eGFR, ml min^{-1} 1.73 m^{-2}						
Tertile 1	80 (75, 84)		95 (89, 101)		87 (82, 93)	
Tertile 2	78 (74, 82)	0.51	96 (90, 102)	0.83	88 (82, 94)	0.61
Tertile 3	57 (53, 62)	<0.001	79 (73, 85)	<0.001	74 (68, 80)	<0.001

Data are presented as arithmetic (eGFR) or geometric means (UAC) and their 95% CIs; eGFR was computed by the Chronic Kidney Disease–Epidemiology Collaboration (CKD-EPI) equation; UAC was log-transformed for these analyses

[a] Model 1: comparison performed using linear regression analyses adjusting for age, sex and cohort membership

[b] Model 2: model 1 plus duration of diabetes, HbA_{1c}, no. of injections and dose of insulin per day, systolic blood pressure, use of antihypertensive drugs, ACE inhibitors or ARBs, diabetic retinopathy stage and use of lipid-lowering drugs

[c] Model 3: model 2 plus UAC (for eGFR analyses) and eGFR (for UAC analyses)

p values are shown for comparisons with tertile 1; $p < 0.05$ was significant

Tertiles 1, 2 and 3, first, second and third 8-OHdG tertiles

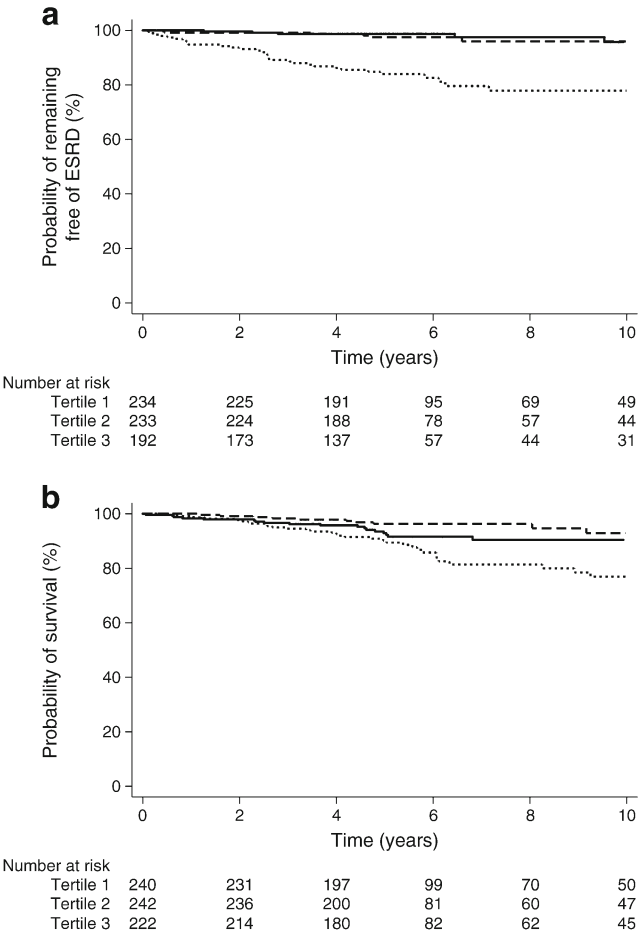

Fig. 1 ESRD and all-cause mortality during follow-up by tertiles of plasma concentrations of 8-OHdG at baseline. (**a**) Probability of remaining free of ESRD ($p < 0.001$) or (**b**) probability of survival ($p < 0.001$) in tertile 3 (dotted line) and tertile 2 (dashed line) compared with tertile 1 (solid line)

compared with those who survived, were more likely to be men, were older, had a longer duration of diabetes, higher HbA_{1c}, systolic and diastolic blood pressure and UAC and had a lower eGFR. They were also more likely to use antihypertensive drugs, and had more severe diabetic retinopathy (ESM Table 2).

Median (IQR) plasma concentrations of 8-OHdG at baseline were higher in participants who died during follow-up compared with those who were still alive: 12.2 (6.1–20.8) vs 8.7 (5.7–12.6) ng/ml ($p = 0.001$). The association between plasma 8-OHdG at baseline and all-cause mortality was not linear ($p < 0.001$; ESM Fig. 1b). The Kaplan–Meier estimate of 10 year cumulative incidence of all-cause mortality was higher in tertile 3 (Fig. 1b): 9.6% in tertile 1, 7.1% in tertile 2 and 23.0% in tertile 3 ($p < 0.001$). The HR for all-cause mortality tended to be lower in tertile 2 and higher in tertile 3 compared with tertile 1 (Table 3, model 1), although these associations did not remain significant after multiple adjustments (Table 3, model 3): tertile 2 vs tertile 1 HR (95% CI) 0.49 (0.22, 1.03), $p = 0.06$; tertile 3 vs tertile 1 HR (95% CI) 1.14 (0.61, 2.17); $p = 0.69$.

Sensitivity analyses The association of the highest 8-OHdG tertile with the risk of ESRD during follow-up remained significant after treating death as a competing risk: tertile 2 vs tertile 1 subdistribution HR (95% CI) 1.48 (0.42, 5.28), $p = 0.54$; tertile 3 vs tertile 1 subdistribution HR (95% CI) 3.57 (1.41, 9.01), $p = 0.007$ (ESM Table 3, model 3).

The risk of ESRD was higher in individuals with plasma 8-OHdG above the median (9.1 ng/ml) than in those with concentrations below this threshold (HR [95% CI] 4.13 [1.18, 26.30], $p = 0.02$; model 3). No significant association was observed between the risk of death and plasma 8-OHdG using this threshold (HR [95% CI] 0.68 [0.35, 1.42], $p = 0.29$; model 3).

The median (IQR) plasma AOPP concentration in the whole study population was 53 (39, 77) μmol/l. It was greater in the highest two 8-OHdG tertiles compared with the lowest (tertile 1, 51 [36–74] μmol/l; tertile 2, 54 [41–80] μmol/l; tertile 3, 53 [39, 80] μmol/l; $p = 0.007$). The multi-adjusted (as in model 3, plus plasma 8-OHdG) HRs for ESRD and all-cause mortality by plasma AOPP at baseline are plotted in ESM Fig. 2. These associations seem to be J-shaped and tended to be log-linear above the median. The increased risk of ESRD in the highest 8-OHdG tertile remained significant after adjustment (further to model 3) for plasma AOPP (tertile 2 vs tertile 1 HR [95% CI] 1.43 [0.44, 5.02], $p = 0.55$; tertile 3 vs tertile 1 HR [95% CI] 3.01 [1.12, 9.57], $p = 0.03$) without evidence for interaction between the two biomarkers ($p = 0.48$). We did not observe an interaction between plasma 8-OHdG and AOPP ($p = 0.87$) or changes after further adjustment for this marker on the risk of death (tertile 2 vs tertile 1 HR [95% CI] 0.48 [0.21, 1.02], $p = 0.06$; tertile 3 vs tertile 1 HR [95% CI] 1.15 [0.60, 2.24], $p = 0.67$).

Discussion

In the current study, we observed association between plasma concentrations of 8-OHdG and kidney disease in individuals with type 1 diabetes. The highest 8-OHdG tertile was associated with a higher UAC and a lower eGFR at baseline, and with increased risk of ESRD during follow-up. The 8-OHdG association was independent of putative confounding variables, including baseline UAC and eGFR, and after treating death as a competing risk. The risk of all-cause mortality tended to decrease in 8-OHdG tertile 2 and increase in tertile 3. However, these tendencies were not reliable and were dependent on cofounding variables, particularly UAC and eGFR, suggesting that the 8-OHdG–death association may be mediated by kidney disease. This observation is consistent with previous findings of attenuation of 8-OHdG–death

Table 3 Associations between plasma concentrations of 8-OHdG at baseline and risk of ESRD or all-cause mortality during follow-up

Variable	Outcome		Model 1[a]		Model 2[b]		Model 3[c]	
	No (*n*)	Yes (*n*)	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
ESRD								
Tertile 1	229	5						
Tertile 2	226	7	1.58 (0.50, 5.35)	0.43	1.41 (0.44, 4.89)	0.56	1.45 (0.45, 5.04)	0.54
Tertile 3	156	36	11.44 (4.88, 33.46)	<0.001	5.88 (2.38, 17.77)	<0.001	3.05 (1.16, 9.60)	0.02
Death								
Tertile 1	221	19						
Tertile 2	232	10	0.52 (0.23, 1.10)	0.09	0.48 (0.21, 1.01)	0.05	0.49 (0.22, 1.03)	0.06
Tertile 3	187	35	1.89 (1.09, 3.39)	0.02	1.63 (0.91, 3.00)	0.10	1.14 (0.61, 2.17)	0.69

[a] Model 1: HRs estimated using Cox proportional hazards regression models adjusting for age, sex and cohort membership

[b] Model 2: model 1 plus duration of diabetes, HbA_{1c}, no. of injections and dose of insulin per day, systolic blood pressure, use of antihypertensive drugs, ACE inhibitors or ARBs, diabetic retinopathy stage and use of lipid-lowering drugs

[c] Model 3: model 2 plus UAC and eGFR

p values are shown for comparisons with tertile 1; $p < 0.05$ was significant

Tertiles 1, 2 and 3, first, second and third 8-OHdG tertiles

association after multiple adjustments including microalbuminuria and serum creatinine in individuals with type 2 diabetes [29].

Increased 8-OHdG concentrations were reported in individuals with chronic kidney disease or ESRD compared with healthy individuals in the general population [30, 31]. Few studies, with limited samples, have examined the relationship between 8-OHdG and kidney disease among people with type 2 diabetes [20–22]. Of these, one of the larger studies, conducted in 396 Japanese participants with type 2 diabetes, reported an association between 8-OHdG urinary concentrations and the progression of albuminuria stages during 5 years of follow-up [32]. However, there were no differences in leucocyte concentrations of 8-OHdG between groups (progressive vs stable). We report here, the first strong and independent associations of high plasma concentrations of 8-OHdG with UAC and eGFR at baseline, and risk of ESRD in two prospective cohorts of participants with type 1 diabetes followed for 6 years.

Our findings, observed in two cohorts of individuals with type 1 diabetes and retinopathy, may not be representative of all populations with type 1 diabetes. Moreover, DNA oxidative stress has been reported to be associated with diabetic retinopathy [33] and this may have biased our results. Indeed, proliferative retinopathy was more frequent at baseline in the highest tertile of 8-OHdG but this association did not persist after adjustment for UAC and eGFR (data not shown), while adjustment for retinopathy stages did not influence the association of 8-OHdG with kidney disease. Another limitation of the present study is the lack of comprehensive urine samples in both cohorts, denying us the opportunity to evaluate the relationship between urinary concentrations of 8-OHdG and outcomes. However, our work has several strengths, including the investigation of participants with long-standing type 1 diabetes from two multicentre binational cohorts, designed to investigate biochemical and genetic factors of diabetic nephropathy, with a comprehensive clinical history of kidney and eye disease at baseline and prespecified renal and survival endpoints during follow-up.

Earlier studies have suggested involvement of AOPP in the pathophysiology of diabetic kidney disease [34, 35]. We previously reported the association of plasma AOPP with diabetic nephropathy stages at baseline and the risk of ESRD during follow-up in the GENEDIAB cohort [11, 36]. In the present analyses of GENEDIAB and GENESIS pooled cohorts, the association of either 8-OHdG or AOPP with ESRD appeared to be independent, with no evidence for interaction.

Our findings do not allow any aetiological conclusion but they are consistent with previous studies suggesting that DNA oxidative damage may play a role in the pathogenesis of diabetic kidney disease. Animal studies showed accumulation of products resulting from oxidative mitochondrial injuries and an increased level of 8-OHdG in kidneys of streptozotocin-induced diabetic rats [37]. Exposure of proximal tubular epithelial cells to high glucose concentration causes a decrease in the expression of oxoguanine glycosylase 1 (a key enzyme involved in DNA base excision repair) and accumulation of 8-OHdG in the kidney cortex of diabetic rats [38]. Recently, in a mouse model of type 1 diabetes, increased mitochondrial DNA products in the glomerular endothelial cells and increased urine secretion of 8-OHdG were found to be responsible for early endothelial injuries [39]. Mitochondrial dysfunction and oxidised mitochondrial DNA in glomerular endothelial cells were associated with diabetes-induced

podocyte depletion. Similarly, kidney biopsies indicated that mitochondrial DNA stress was associated with injury of glomerular endothelial cells in diabetic individuals with progressive kidney disease [39].

In conclusion, we report here a strong and independent association of plasma concentrations of 8-OHdG with kidney disease in individuals with long-standing type 1 diabetes. These findings suggest the potential use of plasma 8-OHdG as a biomarker of kidney disease progression in individuals with type 1 diabetes.

Data availability The datasets analysed during the current study are not publicly available due to consideration of intellectual property, many ongoing active collaborations and to continuing analyses by the study investigators, but are available from the principal investigator on reasonable request.

Funding KM was supported by grants from the 'Fibrosis, Inflammation, Remodeling in cardiovascular, respiratory and renal diseases' hospital and university department (DHU FIRE).

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

Contribution statement MS and KM designed the study, researched data and drafted the manuscript. AM researched data and reviewed/edited the manuscript. RR, SH, MM and GV designed the study, researched data, contributed to the discussion and reviewed/edited the manuscript. KM 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 analyses. All authors approved the current version of the manuscript.

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