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Markers of DNA/RNA damage from oxidation as predictors of a registry-based diagnosis of psychiatric illness in type 2 diabetic patients

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ABSTRACT

Oxidative stress is a potential biological mediator of the higher rates of psychiatric illness (PI) observed after the onset of type 2 diabetes (T2DM). We investigated validated urinary markers of systemic DNA/RNA damage from oxidation (8-oxodG/8-oxoGuo respectively) as predictors of incident PI in a cohort of 1381 newly diagnosed T2DM patients, who were followed prospectively for a total of 19 years after diagnosis. Psychiatric diagnoses were from Danish national registries. Patients were examined at the time of diagnosis and at a 6-year follow-up. At baseline, 8-oxodG was slightly lower in PI vs. non-PI patients, while at 6-year follow-up, 8-oxoGuo was significantly higher in PI patients. Using Cox proportional hazard models, we found that higher levels of 8-oxodG at 6-year follow-up significantly predicted lower incidence of PI after the adjustment for confounders. In a subgroup analysis, this association was most predominant in minor PIs (unipolar depression and anxiety) compared to major PIs such as schizophrenia and bipolar disorder. These observations indicate that higher levels of systemic oxidative stress are not associated with a higher risk of PI after T2DM onset. Only PI patients treated in hospital care were included in the registries, and the conclusion thus only applies to these individuals.

1. Introduction

Substantial epidemiological evidence supports an association between psychiatric illness (PI) and type 2 diabetes mellitus (T2DM) (Vancampfort et al., 2016). In schizophrenia, bipolar disorder, and major depressive disorder, there is clear evidence of a severely increased prevalence of T2DM. Lifestyle, insufficient health care, and side effects of medications are the main factors thought to increase the occurrence of T2DM in PI across different diagnostic subgroups (Vancampfort et al., 2016). There are also indications that the primary pathophysiology of some psychiatric disorders may include impaired glucose tolerance and increased diabetes risk (Fernandez-Egea et al., 2009; Rajkumar et al., 2017). With respect to unipolar depression and anxiety, there is evidence of a bidirectional relationship, in which the presence of T2DM increases the risk of depression (Golden et al., 2008; Mezuk et al., 2008; Pan et al., 2010) and anxiety disorders (Meurs et al., 2016; Smith et al., 2013; Trento et al., 2015), as well as vice versa (however, for a recent negative report see Samaan et al., (2015)).

The mechanism by which T2DM increases the risk of these PIs is unknown, and may involve both psychological factors (e.g. the psychological strain of living with a chronic medical disorder) and biological ones. These have been hypothesized to involve e.g. metabolic dysregulation, systemic inflammation, reduced central nervous system (CNS) neurotrophic factors, and oxidative stress (Ernst et al., 2013; Forbes and Cooper, 2013; Moulton et al., 2015; Schmitz et al., 2016); mechanisms which are not mutually exclusive. It is clinically important to be able to predict which patients are at greater risk of developing a PI after a T2DM diagnosis, not only due to the suffering associated with having a mental disorder, but also because the co-occurrence of T2DM and a PI - such as depression - is associated with a range of poor health outcomes, including an increased need for hospitalization (Davydow et al., 2015), poor cognitive function (Danna et al., 2016), increased risk of dementia (Katon et al., 2011), and increased mortality (Pan et al., 2011).

Oxidative stress is increased in T2DM (Robertson et al., 2004), and we have recently found a marker of RNA damage from oxidation to be predictive of mortality in newly diagnosed T2DM patients (Broedbaek et al., 2011). Furthermore, we have found increased levels of DNA/RNA

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Table 1
Demographics, clinical characteristics and biochemistry at baseline and 6-year follow up respectively, separately for those with and without prevalent PI at these time points. Data are given as median (interquartile range) or n (%), and analyzed with Wilcoxon test or χ^2-test for continuous or categorical characteristics, respectively.

	Diabetes diagnosis			6-year follow-up		
	No PI (n = 1312)	PI (n = 69)	P-value	No PI (n = 913)	PI (n = 57)	P- value
Demographic/clinical						
Age (years)	65.7 (55.9–73.7)	61.3 (53.6–66.7)	0.005	69.4 (60.1–77.4)	62.9 (56.6–72.2)	0.01
Sex			0.10			0.11
Male gender	703 (53.6)	30 (43.5)		469 (51.4)	23 (40.3)	
Education			0.6			0.84
Basic	980 (78.7)	53 (80.3)		687 (78.1)	42 (79.3)	
Higher	265 (21.3)	13 (19.7)		193 (21.9)	11 (20.7)	
Living alone			0.005			0.06
Living alone	402 (31.4)	32 (47.8)		297 (34.7)	23 (47.9)	
Physical activity			0.04			0.004
Sedentary	346 (27.1)	26 (38.8)		242 (28.6)	23 (47.9)	
Smoking			< 0.0001			0.003
Smoker	433 (33.2)	39 (58.2)		254 (29.9)	24 (50.0)	
BMI (kg/m2)	29.1 (26.0–32.6)	30.5 (26.4–34.3)	0.08	28.4 (25.5–31.9)	28.4 (25.8–32.8)	0.49
Hypertension			0.72			0.41
Present	976 (74.4)	50 (72.5)		670 (73.4)	39 (68.4)	
Treatment						
Randomization arm			0.99			0.45
Structured	723 (55.1)	38 (55.1)		514 (56.3)	35 (61.4)	
Anti-diabetes treatment			–			0.62
Diet alone	–	–		271 (29.7)	19 (33.9)	
Oral agents	–	–		525 (57.5)	32 (57.1)	
Insulin	–	–		117 (12.8)	5 (8.9)	
Biochemistry						
Diagnostic plasma glucose (mM)	13.7 (10.7–16.9)	11.0 (10.0–17.3)	0.08	–	–	–
HbA1c (%)	10.2 (8.7–11.8)	9.4 (8.0–11.7)	0.10	8.6 (7.8–9.8)	8.6 (7.4–9.8)	0.58
Total cholesterol (mM)	6.2 (5.4–7.1)	6.4 (5.5–7.2)	0.33	6.0 (5.3–6.9)	6.1 (5.0–6.8)	0.67
Triglycerides (mM)	1.97 (1.40–2.89)	2.03 (1.53–3.67)	0.21	1.79 (1.24–2.60)	1.94 (1.26–3.13)	0.54
Microalbuminuria			0.2034			0.65
Yes	526 (41.9)	32 (50.0)		337 (39.5)	23 (42.6)	
Serum creatinine (μM)	89 (80–101)	90 (78–105)	0.76	90 (80–104)	90 (80–100)	0.83
Urinary 8-oxodG (nmol/mmol creatinine)	2.11 (1.61–2.77)	1.89 (1.39–2.45)	0.040	2.06 (1.59–2.68)	2.06 (1.45–2.95)	0.83
Urinary 8-oxoGuo (nmol/mmol creatinine)	3.64 (2.86–4.76)	3.64 (2.90–5.01)	0.91	3.63 (2.93–4.61)	4.21 (3.38–6.10)	0.002

damage from oxidation in several major PIs, including schizophrenia (Jorgensen et al., 2013a), severe depression (Jorgensen et al., 2013b), and bipolar disorder (Munkholm et al., 2014). A study of genetically modified mice recently showed that reduced insulin sensitivity in the brain lead to mitochondrial dysfunction and oxidative damage to proteins and lipids, altered turnover of neurotransmitters involved in emotional processing, as well as depressive and anxiety-like behaviour (Kleinridders et al., 2015). Some studies have indicated that antioxidant interventions given to animals can reverse brain oxidative stress and depressive behaviour induced by experimental diabetes (de Morais et al., 2014; Reus et al., 2016).

In the present study, we investigated systemic DNA/RNA damage from oxidation as a possible predictor of PI in a cohort of 1381 newly diagnosed T2DM patients. The patients were reexamined six years after diabetes diagnosis and followed for a total of 19 years, during which period detailed diagnostic information on psychiatric morbidity was obtained from Danish national registries. The markers used (urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo)) are among the most validated and sensitive markers of systemic oxidative stress (Barregard et al., 2012; Poulsen et al., 2014).

We hypothesized 1) that markers of oxidative stress on nucleic acids are higher in T2DM patients diagnosed with a PI at baseline and at 6-year follow-up compared to T2DM patients without PI; and 2) that higher levels of systemic DNA/RNA damage from oxidation at T2DM diagnosis and at 6-year follow-up predict incident PI in individuals diagnosed with T2DM. Finally, due to the epidemiological evidence mentioned above, and the fact that major PIs are generally more determined by genetics and less by environmental factors (Bienvenu et al., 2011), we hypothesized 3) that the association between T2DM, OXS and PI is more pronounced in “minor” PIs, e.g. depression and anxiety disorders, than in other psychiatric disorders. To our knowledge, this is the first study to investigate biomarkers of oxidative stress as potential predictors of psychiatric illness after a T2DM diagnosis.

2. Methods

2.1. Patients

The participants in this study were from the Diabetes Care in General Practice (DCGP) trial. DCGP was a pragmatic, open, cluster-randomized, controlled trial (ClinicalTrials.gov NCT01074762) (Olivarius et al., 2001). 474 general practitioners volunteered and were randomly allocated to give patients either structured personal diabetes

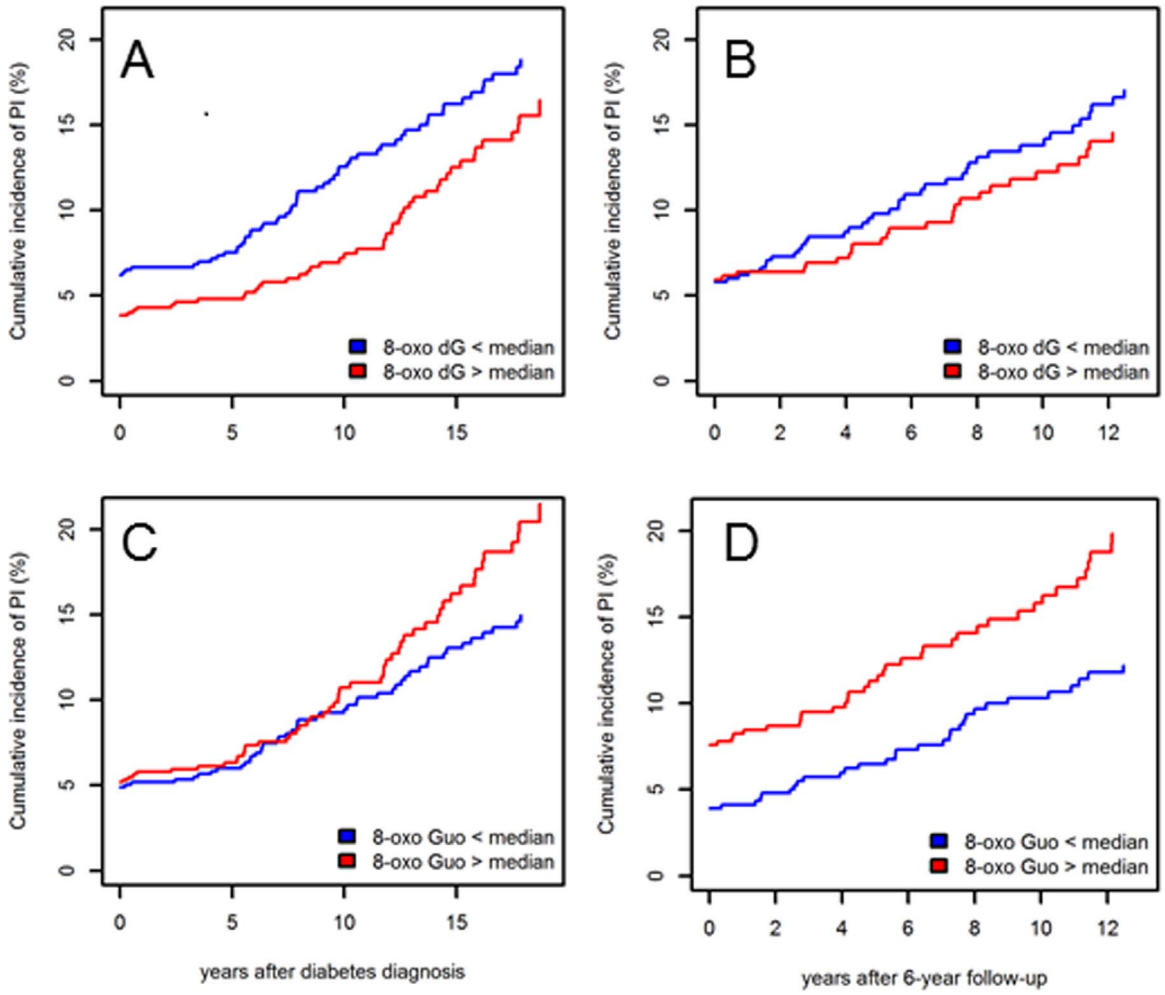

Fig. 1. Cumulative incidence of PI in the high (> median) and low (< median) 8-oxodG and 8-oxoGuo groups after baseline and 6-year follow-up, respectively (A–D).

Table 2
Urinary 8-oxodG and 8-oxoGuo as predictors of subsequent PI after diabetes diagnosis and after 6-year follow-up. "Low" and "High" groups of 8-oxodG/8-oxoGuo are defined by the median of the data. Data are analyzed with Cox proportional hazards regression models. Model 1: Unadjusted. Model 2: Age, sex, and randomization arm. Model 3: Model 2 + BMI, education, living alone, physical activity, smoking, hypertension, anti-diabetes treatment, HbA1c, cholesterol, triglycerides, microalbuminuria, and serum creatinine.

	Absolute risk (1000 patient years) (95% CI)	**Model 1** HR (95% CI)	P-value	**Model 2** HR (95% CI)	P-value	**Model 3** HR (95% CI)	P-value
	Diabetes diagnosis						
Low 8-oxodG	7.07 (5.28–9.28)	1.00		1.00		1.00	
High 8-oxodG	5.77 (4.16–7.81)	0.83 (0.56–1.22)	0.34	0.73 (0.50–1.07)	0.11	0.79 (0.50–1.22)	0.28
Low 8-oxoGuo	5.57 (4.06–7.46)	1.00		1.00		1.00	
High 8-oxoGuo	7.50 (5.55–9.92)	1.45 (0.94–2.22)	0.09	1.11 (0.71–1.74)	0.65	1.25 (0.78–2.01)	0.35
	6-year follow-up						
Low 8-oxodG	9.41 (6.66–12.93)	1.00		1.00		1.00	
High 8-oxodG	6.76 (4.37–9.99)	0.72 (0.43–1.20)	0.20	0.66 (0.40–1.10)	0.11	0.47 (0.22–0.97)	0.042
Low 8-oxoGuo	6.73 (4.50–9.67)	1.00		1.00		1.00	
High 8-oxoGuo	9.93 (6.87–13.89)	1.51 (0.88–2.57)	0.13	1.10 (0.64–1.91)	0.73	0.76 (0.39–1.49)	0.42

care or routine diabetes care. A detailed description of the study design, the intervention and definition of study parameters has previously been published (Olivarius et al., 2001). The GPs were asked to include all patients on their practice lists who were aged 40 or over and diagnosed with diabetes mellitus between March 1, 1989 and February 28, 1991. In the third year, 71 doctors in the structured personal care group volunteered to recruit patients for a further year. These patients received the same intervention as the other patients in the structured personal care group. Following recruitment, diagnosis was confirmed by a single fasting whole-blood/plasma glucose concentration ≥7.0/8.0 mmol/l, measured at a major laboratory.

The GPs were allowed to exclude patients with PI, if the GPs found them too ill to comply with the procedures of the study. In total, 50 patients with severe PI were excluded: 41 due to dementia, six due to schizophrenia or other psychoses, and three for other reasons. Other causes of exclusion were severe somatic disease (n = 50), declined consent (n = 62), or that the diagnosis was not confirmed (n = 47) (Olivarius et al., 2001). Of 1381 patients in the final study population, 1369 (99.1%) were of Western European descent. A clinical follow-up examination was completed for 970 (93.4%) of 1039 surviving patients

Table 3
Baseline levels of urinary 8-oxodG and 8-oxoGuo as predictors of subsequent subgroups of PI after diabetes diagnosis and 6-year follow-up. "Low" and "High" groups of 8-oxodG/8-oxoGuo are defined by the median of the data. The number of patients diagnosed with a PI within each subgroup after diabetes diagnosis and 6-year follow-up, respectively, are given in parentheses. No patients were diagnosed with abuse after 6-year follow so this subgroup has been left out. Data are analyzed with Cox proportional hazards regression models. Model 1: Unadjusted. Model 2: Age, sex, and randomization arm.

		Absolute risk (1000 patient years)	Model 1		Model 2	
		(95% CI)	HR (95% CI)	P-value	HR (95% CI)	P-value
		Diabetes diagnosis				
Minor PI	**Low 8-oxodG**	2.58 (1.55–4.04)	1.00		1.00	
(n = 27)	**High 8-oxodG**	1.10 (0.47–2.18)	0.43 (0.19–0.97)	0.041	0.40 (0.18–0.91)	0.023
	Low 8-oxoGuo	1.73 (0.94–2.91)	1.00		1.00	
	High 8-oxoGuo	1.99 (1.05–3.41)	1.17 (0.50–2.72)	0.72	0.99 (0.40–2.49)	0.99
Major PI	**Low 8-oxodG**	1.09 (0.46–2.15)	1.00		1.00	
(n = 10)	**High 8-oxodG**	0.27 (0.03–1.01)	0.26 (0.05–1.27)	0.10	0.25 (0.05–1.13)	0.07
	Low 8-oxoGuo	0.74 (0.27–1.63)	1.00		1.00	
	High 8-oxoGuo	0.61 (0.16–1.58)	0.83 (0.26–2.66)	0.76	0.74 (0.17–3.20)	0.69
Organic PI	**Low 8-oxodG**	3.13 (1.98–4.70)	1.00		1.00	
(n = 55)	**High 8-oxodG**	4.12 (2.78–5.89)	1.34 (0.78–2.31)	0.28	1.10 (0.64–1.90)	0.73
	Low 8-oxoGuo	2.85 (1.80–4.28)	1.00		1.00	
	High 8-oxoGuo	4.59 (3.09–6.56)	1.83 (1.06–3.16)	0.03	1.15 (0.64–2.06)	0.65
Abuse	**Low 8-oxodG**	0.27 (0.03–1.00)	1.00		1.00	
(n = 5)	**High 8-oxodG**	0.27 (0.03–1.01)	0.98 (0.14–7.02)	0.99	1.18 (0.16–8.77)	0.87
	Low 8-oxoGuo	0.25 (0.02–0.91)	1.00		1.00	
	High 8-oxoGuo	0.31 (0.03–1.13)	1.07 (0.16–7.24)	0.94	5.06 (0.45–56.63)	0.19
		6-year follow-up				
Minor PI	**Low 8-oxodG**	3.47 (1.89–5.83)	1.00		1.00	
(n = 18)	**High 8-oxodG**	0.81 (0.15–2.40)	0.23 (0.07–0.82)	0.024	0.22 (0.06–0.75)	0.015
	Low 8-oxoGuo	2.78 (1.43–4.88)	1.00		1.00	
	High 8-oxoGuo	1.46 (0.46–3.44)	0.50 (0.18–1.44)	0.20	0.36 (0.13–0.99)	0.048
Major PI	**Low 8-oxodG**	0.74 (0.14–2.20)	1.00		1.00	
(n = 4)	**High 8-oxodG**	0.27 (0.00–1.55)	0.37 (0.04–3.56)	0.39	0.38 (0.04–3.45)	0.39
	Low 8-oxoGuo	0.46 (0.04–1.71)	1.00		1.00	
	High 8-oxoGuo	0.58 (0.06–2.15)	1.31 (0.20–8.74)	0.78	1.55 (0.29–8.21)	0.60
Organic PI	**Low 8-oxodG**	5.20 (3.21–7.97)	1.00		1.00	
(n = 44)	**High 8-oxodG**	5.68 (3.51–8.69)	1.09 (0.59–2.03)	0.79	0.99 (0.52–1.88)	0.97
	Low 8-oxoGuo	3.48 (1.94–5.75)	1.00		1.00	
	High 8-oxoGuo	7.89 (5.19–11.49)	2.37 (1.23–4.58)	0.01	1.68 (0.82–3.47)	0.16

after a median (inter quartile range, IQR) of 5.57 (4.96–6.16) years in the structured personal care group and after 5.85 (5.30–6.45) years in the routine care group.

2.2. Registry-based follow-up and subgrouping of psychiatric illness

The vital and emigration status of all patients was certified through the Danish Civil Registration System with a permanent and unique personal identification number, which enables linkage between study populations and all Danish national registries (Pedersen et al., 2006), and surviving patients were censored on December 31, 2008. For one patient, the vital status could not be assessed because this person emigrated in 1992. Psychiatric diagnoses were retrieved from The Danish Psychiatric Central Research Register (PCRR) (Mors et al., 2011), as described previously (Larsen et al., 2016). This register contains information on all psychiatric hospital admissions in Denmark since 1969; and from 1995, data on outpatient treatment and emergency room contacts are also included in the register. There are no private psychiatric hospitals in Denmark, and the PCRR does not contain data from privately practicing psychiatrists or general practitioners (GPs).

Patients diagnosed with a PI were analyzed as a whole, as well as in four pre-defined subgroups, which were based on the World Health Organization International Classification of Diseases (ICD)-10 diagnoses, as described previously (Larsen et al., 2016). The subgroups were: 1) *Minor PI* (F32 + F33 (unipolar depression) + F4 (anxiety disorders) + F6 (personality disorders)); 2) *Major PI* (F2 (schizophrenia and related psychotic disorders) + F30 + 31 (mania, bipolar disorder)); 3) *Organic PI* (F0 (organic psychiatric disorders) + F7 (mental retardation); and 4) *Abuse* (F1). Patients diagnosed with PI after ICD-8, which was used in Denmark until 1995, were allocated into one of the subgroups based on the ICD-8 code. If the psychiatric diagnoses changed during follow-up period, the patient was classified according to the most severe diagnosis (e.g. a diagnosis of an anxiety disorder followed by a diagnosis of schizophrenia was classified as schizophrenia). Patients with a primary PI and comorbid abuse (F1) were classified according to the primary PI. In analyses on the incidence of diagnoses from one of the subgroups, patients were censored if a diagnosis from one of the other subgroups occurred. For details, please see Larsen et al. (2016).

2.3. Determination of urinary 8-oxodG and 8-oxoGuo

At baseline and at 6-year follow up, patients gave a morning urine sample and the urinary markers of DNA and RNA damage from oxidation (8-oxodG and 8-oxoGuo, respectively) were determined by Ultra-Performance Liquid Chromatography with tandem mass spectrometry (UPLC-MS/MS) (Henriksen et al., 2009). The chromatographic separation was performed on an Acquity UPLC system (Waters Corp., Milford, USA) using an Acquity UPLC BEH Shield RP18 column (1.7 μm, 2.1 × 100 mm) protected with an in-line filter (4 × 2 mm, 0.2 μm) both from Waters. The column temperature was 1 °C. The mass spectrometry detection was performed on a API 3000 triple quadrupole mass spectrometer (Sciex, Toronto, Canada) using electrospray ionisation (Turbospray®) operated in the positive mode. The urinary creatinine concentration was determined by Jaffe's reaction. The 8-oxodG/8-oxoGuo excretion is defined as the urinary concentration of the nucleoside normalised to urinary creatinine concentration (Barregard et al., 2012).

2.4. Statistical analysis

A detailed analysis plan was defined before any statistical analyses were performed, and no additional exploratory analyses or adjustments were made before submission of the manuscript. Baseline and 6-year follow-up demographics, clinical characteristics and biochemistry were compared between patients with and without PI with a Wilcoxon test or a χ^2-test for continuous and categorical characteristics, respectively. Analyses of 8-oxodG and 8-oxoGuo as predictors of incident PI were performed from baseline and from 6-year follow-up. The 8-oxodG/8-oxoGuo data was dichotomized into a "high" vs. "low" group, as defined by the median of the data of each marker at each time point. In the analyses of incident PI after either time point, individuals who were diagnosed with PI before the baseline or 6-year follow-up examinations, respectively, were omitted. The cumulative incidence of PI in the respective 8-oxodG/8-oxoGuo groups was illustrated with Kaplan–Meier curves. The difference in incidence of PI between the dichotomized 8-oxodG/8-oxoGuo groups from diagnosis or 6 year follow-up was analyzed by hazard ratios (HRs) from Cox proportional hazards regression models (Model 1: unadjusted. Model 2: adjusted for age, sex, and randomization arm. Model 3: Model 2 + BMI, education, living alone, physical activity, smoking, hypertension, anti-diabetes treatment, HbA1c, cholesterol, triglycerides, microalbuminuria, and serum creatinine). In the subgroup analyses, only Model 1 and 2 were used. All variables and endpoints were analyzed with SAS (Version 9.4). The level of statistical significance was $P < 0.05$.

3. Results

3.1. Baseline and 6-year follow-up levels of 8-oxodG/8-oxoGuo in T2DM patients with and without PI

The data are presented in Table 1. Both at diagnosis and at 6-year follow-up, patients with PI were younger and more likely to be living alone, to be smokers, and to have a more sedentary lifestyle. PI patients did not differ from non-PI patients on key biochemical variables such as glycated hemoglobin, cholesterol, or serum creatinine, neither at diagnosis nor at 6-year follow-up, or on the type of antidiabetic treatment given following diagnosis. At diagnosis, urinary 8-oxodG was slightly lower in PI patients (median (IQR) = 1.89 (1.39–2.45) nmol/mmol creatinine) vs. non-PI patients (2.11 (1.61–2.77) nmol/mmol) ($p = 0.040$). At 6-year follow-up, 8-oxoGuo was significantly higher in PI patients (4.21 (3.38–6.10) nmol/mmol) vs. non-PI patients (3.63 (2.93–4.61) nmol/mmol) ($p = 0.002$).

3.2. 8-oxodG/8-oxoGuo as predictors of incident PI

The risks of incident PI in the high and low 8-oxodG/8-oxoGuo groups at baseline and 6-year follow up are presented in Fig. 1 and Tables 2 and 3. After T2DM diagnosis, 97 (7.4%) patients previously without PI were diagnosed with PI, and after 6-year follow-up, 66 (7.2%) patients previously without PI were diagnosed with PI. At diabetes diagnosis, none of the markers was associated with subsequent PI incidence. However, at 6-year follow-up, high 8-oxodG (i.e. 8-oxodG > median) was associated with a subsequent significantly reduced incidence of PI after adjustment for confounders (HR (95% CI) = 0.47 (0.22–0.97), Model 3, $p = 0.042$).

High 8-oxodG at diagnosis was associated with a reduced incidence of subsequent minor PI, both before and after adjustment (HR (95% CI) = 0.40 (0.18–0.91), Model 2, $p = 0.03$). There was a statistically non-significant tendency that high 8-oxodG also was associated with reduced incidence of subsequent major PI (HR (95% CI) = 0.25 (0.05–1.13), $p = 0.07$). High 8-oxoGuo was associated with an increased incidence of organic PI (HR (95% CI) = 1.83 (1.06–3.16) $p = 0.03$), but this was no longer significant after adjustment. Both high 8-oxodG and high 8-oxoGuo were at 6-year follow-up associated with a reduced incidence of minor PI (HR (95% CI) = 0.22 (0.06–0.75), $p = 0.015$, and 0.36 (0.13–0.99), $p = 0.048$, respectively) after adjustment. High 8-oxoGuo was at 6-year follow-up associated with a subsequent increased incidence of organic PI (HR (95% CI) = 2.37 (1.23–4.58), $p = 0.010$), but this was no longer significant after adjustment (Model 2).

4. Discussion

In the present study, we investigated urinary markers of systemic oxidative stress on nucleic acids as predictors of PI in T2DM patients at diagnosis and on average 6 years later. The cross-sectional comparison of T2DM patients with and without PI revealed a slightly lower urinary excretion of 8-oxodG in PI patients at diagnosis, and an increased excretion of 8-oxoGuo at 6-year follow-up. The last finding was in line with our hypothesis that PI patients with T2DM have higher levels of oxidative stress. The time of diagnosis represents a metabolically unstable state, in which untreated diabetes may be the primary influence of the systemic oxidative stress levels, while the 6-year follow-up represents a more stable condition, in which glycemic control is in steady state. Hence, the reduced 8-oxodG could be a consequence of differences between PI and non-PI patients in the acute diabetic state. For example – although the difference was not statistically significant – diagnostic plasma glucose and glycated hemoglobin was slightly lower in PI patients at T2DM diagnosis, and this could perhaps explain the lower 8-oxodG at this point, whereas the increased 8-oxoGuo at 6-year follow-up is more likely to be a consequence of other factors separating PI and non-PI T2DM patients, such as smoking and sedentary lifestyle. It should be noted that at both time points, PI patients were significantly younger than non-PI patients, and this difference could potentially cause reduced levels of 8-oxodG/8-oxoGuo, because higher age in itself is associated with increased tissue levels of oxidatively generated DNA damage (Moller et al., 2010), and an increased urinary excretion of both markers (Jorgensen et al., 2017).

Against our hypothesis, we found that at 6-year follow-up, higher levels of systemic oxidative stress on DNA, as measured by the urinary excretion of 8-oxodG, was associated with a reduced subsequent occurrence of PI. This finding underlines that peripheral markers of oxidative stress are not necessarily a reflection of brain biochemistry or positively linked to psychopathology. For example, we previously found that 8-oxoGuo increased after successful electroconvulsive therapy for severe depression (Jorgensen et al., 2013b), and that an increase of the stress hormone corticosterone caused a *reduction* in the excretion of urinary 8-oxodG (Jorgensen et al., 2017).

The minor PI subgroup was more strongly linked to levels of oxidative stress than the other subgroups. This is in line with the epidemiological evidence indicating that it is only "minor" PI's – such as depression and anxiety – that occur at higher rates after a T2DM diagnosis, as well as the notion that these conditions are generally to a higher degree determined by environmental factors and less by genetic predisposition compared to major PI's such as schizophrenia and bipolar disorder (Bienvenu et al., 2011). However, again the direction of the association was the opposite of the expected, i.e. higher levels of both 8-oxodG and 8-oxoGuo were associated with lower incidence of minor PI, as discussed above. It should be noted that the statistical power of the analyses of 8-oxodG/8-oxoGuo as predictors of major PI was low, because these diagnoses were less frequently made than minor PIs (as would be expected from the age of the participants). However, the trend of the association was the same as for minor PI, namely that higher 8-oxodG predicted lower incidence of major PI (Table 3).

Only patients with so pronounced symptoms that they were referred to a psychiatric hospital were included in the cohort of patients with PI for this study. There are no available data on exactly how many patients are treated for PI outside the hospital sector in Denmark. In general, the majority of non-psychotic cases are considered to be treated in primary care (Mors et al., 2011). With respect major depression, data from other countries indicate that there are surprisingly few clinical differences

between those patients treated in primary care and those treated in hospital care (Gaynes et al., 2007; Vuorilehto et al., 2007).

The major strengths of this study are that it used a large T2DM cohort with a very well defined onset of illness, in which the patients were highly representative of T2DM patients in general practice. The study used detailed diagnostic information from Danish national registries, and the validity of the diagnoses obtained from the register is high (Mors et al., 2011). Finally, we used validated biomarkers of oxidative stress on nucleic acids, which were analyzed with a well-established and highly accurate and specific chromatographic method (Barregard et al., 2012; Henriksen et al., 2009; Weimann et al., 2002). Limitations of the study are that the incidences of PI after the two investigated time points were fairly low, giving relatively low statistical power to the analyses, and that the dichotomization of the 8-oxodG/8-oxoGuo markers (which was chosen due to the low incidence of PI) limits our ability to make statements about a potential dose-response relationship. Hence, further subcategorization of the 8-oxodG/8-oxoGuo datasets (e.g. by quartiles or deciles) was considered, but deemed inappropriate due to the very small number of participants which would be included in each category. Because the diagnostic information was registry-based, only patients with sufficient PI severity to be treated in the hospital sector are included, and the conclusions therefore only apply to this subset of patients. Finally, it should be noted that the present study was not the primary purpose of the DCGP study, and therefore the present study must be considered exploratory and in need of replication.

In conclusion, we found that higher levels of systemic DNA damage from oxidation, measured six years after the diagnosis of T2DM, predicted lower incidence of registry-based PI during the following 13 years, and this was most pronounced for minor PIs such as unipolar depression and anxiety. This observation does not support the hypothesis that a high level of systemic oxidative stress is associated with a higher risk of PI in patients with T2DM.

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Conflicts of interest

None of the authors have any conflicts of interest.

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