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Abstract
Schizophrenia is associated with a substantially increased somatic morbidity and mortality, which may partly be caused by accelerated cellular aging. Oxidative stress is an established mediator of aging and a suggested aetiological mechanism in both schizophrenia and age-related medical disorders such as cardiovascular disease, type 2 diabetes and dementia. We determined the urinary excretion of markers of systemic Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) oxidation, 8-oxo-7,8-dihydro-2’-deoxyguanosine and 8-oxo-7,8-dihydroguanosine, respectively, in 40 schizophrenia patients and 40 age- and sex-matched controls, using ultra-performance liquid chromatography with tandem mass spectrometry. Measures of psychopathology, perceived stress and cortisol secretion were collected. Patients were re-examined after four months. We found a 20% increase in the median excretion of both markers in schizophrenia patients vs. healthy controls ($P = 0.003$ and $o.001$, respectively). This difference persisted after the adjustment for multiple demographical, lifestyle and metabolic factors. In patients, the marker excretion was not influenced by medication load, and was not driven by symptom severity, perceived stress or cortisol secretion, neither at baseline nor in relation to changes at follow-up. We conclude that schizophrenia is associated with increased systemic nucleic acid damage from oxidation, which could constitute a molecular link between schizophrenia and its associated signs of accelerated aging.

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1. Introduction

Schizophrenia has been suggested to constitute a “syndrome of accelerated aging” (Kirkpatrick et al., 2008). Life expectancy is shortened with as much as 15–20 years, mainly due to increased mortality from cardiovascular disease (Hoang et al., 2011; Wahlbeck et al., 2011). Furthermore, schizophrenia is associated with mildly accelerated cognitive decline across the lifespan, paralleled by structural brain alterations such as ventricular enlargement and hippocampal volume reduction (Jeste et al., 2011; Shenton et al., 2001; Velakoulis et al., 2006). Finally, some studies have found telomere length, a molecular correlate of aging, to be reduced in schizophrenia patients in comparison to healthy controls (Kao et al., 2008), although others have found no difference (Mansour et al., 2011).

Recent evidence has converged on mitochondrial dysfunction and oxidative stress as potential pathogenetic mechanisms in schizophrenia, with findings of both cerebral and peripheral perturbations in these systems (Arvindakshan et al., 2003b; Gysin et al., 2007; Kunz et al., 2008; Prabakaran et al., 2004; Regenold et al., 2009; Rosenfeld et al., 2011), as well as beneficial clinical effects of antioxidant therapy (Arvindakshan et al., 2003a; Chengappa et al., 2012; Dakhale et al., 2005). Similarly, administration of N-acetyl cystein (NAC) – a precursor of the key brain antioxidant glutathione – lead to improvement of clinical symptoms and auditory sensory processing in schizophrenia (Berk et al., 2008; Lavoie et al., 2007). Interestingly, preclinical experiments have indicated that glutathione deficits produce neuroanatomical and cognitive deficits similar to those observed in schizophrenia, and that these effects are counteracted by NAC administration (Cabungcal et al., 2012; Choy et al., 2010).
2. Methods

2.1. General study outline

Recruitment took place from September 2008 through May 2011. Patients were recruited by psychiatrists from the Psychiatric Centre Copenhagen, which provides mental health services to the citizens of the central, northern and north-western area of Copenhagen. Physicians at the centre were informed of the study in writing and by word of mouth, and received regular reminders to refer patients. Both inpatients as well as patients from the affiliated outpatient clinics were recruited. Investigations were carried out at baseline (“T0”) and repeated after 4 months (“T4”). In the intermediate period, patients received treatment as usual, including medication.

The inclusion criterion for patients was an ICD-10 diagnosis of either schizophrenia or acute schizophreniform psychosis, confirmed by a structured interview at referral. Exclusion criteria were: (1) somatic disease and somatic medication. A non-regular use of e.g. painkillers or asthma medication was allowed, (2) abuse of alcohol, marihuana or other drugs of abuse, (3) coercion of any kind, (4) severely disorganised thinking, making it impossible to obtain an informed consent, (5) use of dietary supplements, and (6) pregnancy or breast-feeding. Of forty-five patients referred to the study and accepting to participate, 40 were included. In the rejected patients, the diagnosis of schizophrenia was considered uncertain after the inclusion interview (N=4), or the biochemical screening revealed a medical disorder (N=1).

Healthy controls were recruited from the blood donation corps at Rigshospitalet by personal contact, as they were scheduled for donating blood. Exclusion criteria for the healthy controls were: (1) any psychiatric or somatic disease, (2) abuse of alcohol, marihuana or other drugs, (3) use of any medication including dietary supplements, and (4) non smoking. A total of 175 healthy controls meeting none of the exclusion criteria were asked to participate, and of these, 40 accepted to be included. They underwent the same examinations as the patients (except for schizophreniform psychopathology), but were only examined at T0.

The study schedule was as follows: on day 1, blood was drawn in the fasting state at 9 AM. In a few cases (two controls and six patients), blood was not drawn in the morning, and these cases were omitted from analyses including plasma cortisol. A spot urine sample was obtained from the first voided urine after blood sampling. Sociodemographic and clinical data were recorded. Ratings and questionnaires were completed. On day 2 (or no more than one week from the examination), the participants made salivary samples for cortisol determination. At T4, the sequence was repeated.

The study protocol complied with the Declaration of Helsinki, and was approved by the Research Ethics Commitee (ID-2008-064) and the National Data Protection Agency (2008-41-2052). All participants gave a written informed consent before inclusion.

2.2. Interviews and questionnaires

A Schedules in Clinical Neuropsychiatry (SCAN)-interview (Wing et al., 1999) was applied at T0 to ensure that the ICD-10 diagnostic criteria for schizophrenia (F20.0–F20.9) were met, and that no lifetime psychiatric morbidity was present in the healthy controls. Medical doctors with previous clinical experience in psychiatry performed all the SCAN ratings. The severity of psychopathology was measured with the Positive and Negative Syndrome Scale (PANS) (Kay et al., 1987). Regular co-ratings with a certified expert PANSS-rater (AFJ) were performed, yielding an IntraClass Correlation Coefficient of 0.91 for positive items, 0.76 for negative items, 0.79 for general items and 0.83 for all items, indicating very good agreement across all subscales. The level of perceived stress was assessed with the Perceived Stress Scale 10-item (PSS) (Cohen and Williamson, 1988).

2.3. General biochemical screening

This comprised a full blood count, hepatic enzymes, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides, C-reactive protein (CRP), thyroid stimulating hormone (TSH), sodium, potassium, creatinine, glucose, glycated haemoglobin (HbA1c), and cortisol.

2.4. Urinary 8-oxodG and 8-oxoGuo

The urinary sample was obtained with a standard sampling kit without any additives. Samples were kept on ice and transferred within hours to storage at −20°C until analysis. The urinary content of the oxidatively modified guanine nucleosides was assayed using ultraperformance liquid chromatography and tandem mass spectrometry (UPLC–MS/MS). The chromatographic separation was performed on an Acquity UPLC system (Waters, Milford, MA, USA). The column was an Acquity UPLC BEH Shield RP18 column (1.7 µm, 2.1 x 100 mm²) protected with an in-line filter (4 x 2 mm², 0.2 µm) both obtained from Waters. The MS–MS detection was performed on an API 3000 triple quadrupole mass spectrometer (Sciex, Toronto, Canada) equipped with an ESI ion source (TurboSpray) operated in the positive mode. Details of the analysis are described elsewhere (Henriksen et al., 2009). 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 (Evans et al., 2010a).

2.5. Plasma markers

Plasma was obtained from EDTA-coated tubes which were centrifuged at 4°C and 1590 g for 10 min. Samples were stored at −80°C within 30 min from venipuncture. 3-aminodialdehyde (MDA, a marker of lipid peroxidation), as well as...
the two plasma antioxidants uric acid and vitamin C were also determined, using previously described chromatographic methods (Lykkesfeldt, 2001, 2007a).

### 2.6. Salivary cortisol

Samples were obtained by the "Salivette" system immediately upon awakening, at 15, 30, 45 and 60 min after awakening, at 6 PM and at 11 PM. Samples were made either in the participant's own home and mailed to the laboratory (controls and out-patients) or at the ward (in-patients). Salivary cortisol is preserved for exercise and oral contraceptive use). Model 2 additionally included as independent variables all the clinical and metabolic factors showing differences between the groups (waist–hip ratio, pulse, triglycerides, HbA1c, CRP, TSH and cortisol), as well as the plasma antioxidants (Table 1). For all parametric tests, the dependent variables in question deviating from normal distribution (8-oxodG/8-oxoGuo and plasma cortisol) were transformed by the natural logarithm. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 20.0 (IBM Corporation, NY, USA). Statistical significance was defined as P < 0.05. All statistical tests were two sided.

### 3. Results

Basic data of patients and controls are presented in Table 1. The urinary excretion of both 8-oxodG and 8-oxoGuo was significantly increased in the schizophrenia vs. the control group (8-oxodG: 1.7 (1.4–2.4) vs. 1.4 (1.2–1.6) nmol/mmol urinary creatinine, Mann–Whitney U-test, Mann–Whitney U (MWU) = 4960.0, P = 0.003. 8-oxoGuo: 2.2 (1.9–2.7) vs. 1.8 (1.4–2.1) nmol/mmol, MWU = 3480.0, P < 0.001) (Fig. 1). The effect size (Cohen's d) was 0.69 for 8-oxodG and 1.13 for 8-oxoGuo, indicating a moderate and large effect, respectively. The median excretion of 8-oxodG was 29% higher than 8-oxodG in both groups, and the two markers were highly correlated (Pearson’s r = 0.52, P < 0.001). In contrast to the nucleic acid oxidation markers, MDA did not
significantly differ between the groups (controls: 0.89 (± 0.23) µmol/L, schizophrenia: 0.78 (± 0.33) µmol/L, independent samples t-test, t = 1.53, d.f. = 74, P = 0.13).

The difference in 8-oxodG/8-oxoGuo excretion between the groups persisted after the adjustment for demographical and lifestyle variables (Model 1) (8-oxodG: β = 0.52, P < 0.001, 8-oxoGuo: β = 0.68, P < 0.001). After the additional adjustment for multiple clinical and metabolic factors (model 2), group was still significantly associated with 8-oxodG/8-oxoGuo excretion (8-oxodG: β = 0.61, P = 0.002, 8-oxoGuo: β = 0.64, P = 0.002). In a separate analysis, we found no difference in neither marker between smokers vs. non-smokers in neither the control (8-oxodG: MWU = 99.0, P = 0.33, 8-oxoGuo: MWU = 98.0, P = 0.31) nor in the patient group (8-oxodG: MWU = 146.0, P = 0.16, 8-oxo-Guo: MWU = 160.0, P = 0.3).

The clinical baseline characteristics of schizophrenia patients are shown in Table 2. All patients received second generation antipsychotics (SGA). The primary antipsychotic agent used was risperidone (N = 9), quetiapine (N = 9), aripiprazole (N = 6), olanzapine (N = 5) or clozapine (N = 5). Six patients received other SGAs. None of the markers showed associations with the number of antipsychotics used (8-oxodG: one-way ANOVA, F(2,37) = 0.52, P = 0.60, 8-oxoGuo: F(2,37) = 1.26, P = 0.30), the Defined Daily Dose (DDD) of antipsychotics (8-oxodG: Pearson’s r = −0.18, P = 0.26, 8-oxoGuo: r = 0.11, P = 0.5) or the total DDD of all medications (8-oxodG: r = −0.26, P = 0.1, 8-oxoGuo: r = 0.06, P = 0.73). The individual agents were not associated with significant differences in the marker excretion, except for a higher 8-oxodG excretion in the five clozapine users compared to risperidone users (one-way ANOVA, F(5,34) = 3.3, P = 0.02, post-hoc Tukey test of clozapine vs. risperidone: P = 0.006). The 8-oxodG/8-oxoGuo excretion was not associated with the duration of illness (even after adjusting for age), number of admissions, or admission status (in- or out-patient) at the time of inclusion.

As expected, patients had substantially higher perceived stress scores than healthy controls (22.3 (± 6.7) vs. 7.7 (± 4.2) points, independent samples t-test, t = −12.1, d.f. = 78, P < 0.001). There were no significant associations between PSS scores and the nucleic acid oxidation markers in neither the control nor the schizophrenia group. Neither the total nor any subscale PANSS scores of schizophrenia patients correlated with either of the markers.

Fig. 1. Urinary 8-oxodG (left) and 8-oxoGuo (right) excretion (nmol/mmol creatinin) in healthy controls and schizophrenia patients. *P = 0.003, Mann–Whitney test.

<table>
<thead>
<tr>
<th>Diagnosis, ICD-10</th>
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<tbody>
<tr>
<td>Paranoid schizophrenia</td>
<td>55%</td>
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<tr>
<td>Undifferentiated schizophrenia</td>
<td>28%</td>
</tr>
<tr>
<td>Hebephrenic schizophrenia</td>
<td>12%</td>
</tr>
<tr>
<td>Unspecified schizophrenia</td>
<td>5%</td>
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<table>
<thead>
<tr>
<th>Disease course</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset (years)</td>
<td>23.0 (± 5.3)</td>
</tr>
<tr>
<td>Duration of illness (months)</td>
<td>76 (36–189)</td>
</tr>
<tr>
<td>Number of admissions</td>
<td>3 (1–6)</td>
</tr>
<tr>
<td>In-patient at inclusion</td>
<td>60%</td>
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</table>

<table>
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<tr>
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<tbody>
<tr>
<td>Duration of current antipsychotic treatment (months)</td>
<td>20 (3–68)</td>
</tr>
<tr>
<td>Number of antipsychotics used</td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>77%</td>
</tr>
<tr>
<td>Two or three</td>
<td>23%</td>
</tr>
<tr>
<td>Antipsychotics, Defined Daily Dose</td>
<td>1.5 (0.7–2.3)</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>43%</td>
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<td>If antidepressant use, Defined Daily Dose</td>
<td>1.4 (1.0–2.0)</td>
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<tr>
<td>Benzodiazepine use</td>
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</tr>
<tr>
<td>If benzodiazepine use, Defined Daily Dose</td>
<td>0.6 (0.3–1.2)</td>
</tr>
</tbody>
</table>

Psychopathology

| PANSS positive score | 22.2 (± 4.3) |
| PANSS negative score | 22.3 (± 5.6) |
| PANSS general score | 44.6 (± 8.5) |
| PANSS total score | 89.1 (± 16.5) |

As defined by the World Health Organization.

Table 2

Clinical characteristics of schizophrenia patients at baseline. Numbers are in mean (± standard deviation) or median (interquartile range).

<table>
<thead>
<tr>
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As defined by the World Health Organization.
In the control group, both markers were positively associated with the log-transformed 9 AM plasma cortisol, while being non-associated or significantly negatively associated with the plasma cortisol in the schizophrenia group. For the correlation to AUCg, a trend of a more negative slope in the schizophrenia group was seen (results are summarized in Fig. 2). There were no significant correlations between plasma MDA and the cortisol measures in neither group (data not shown).

Eighty per cent of the included patients completed the study at T4. Of the non-completers, four refused to participate, two could not be contacted, and two had developed somatic disease. There were no significant differences between the completers and the non-completers with respect to age (independent samples t-test, t = −1.12, d.f. = 38, P = 0.91), DDD of antipsychotic medication (t = −0.7, d.f. = 38, P = 0.49), or total PANSS score (t = −0.45, d.f. = 38, P = 0.65). DDDs of SGAs or all medications were not changed from T0 to T4 (DDD, SGA: Wilcoxon Signed Ranks test, Z = −0.2, P = 0.84, DDD, all medications: Z = −0.3, P = 0.77).

The T4 median excretion of 8-oxodG/8-oxoGuo continued to be elevated in comparison to the T0 levels of healthy controls (8-oxodG: Mann–Whitney U-test, MWU = 460.0, P = 0.04, 8-oxoGuo: MWU = 347.0, P = 0.001), reflecting a strong intra-individual correlation between T0 and T4 values (8-oxodG: Pearson’s r = 0.62, P < 0.001, 8-oxoGuo: r = 0.58, P = 0.001). We found no associations between T0 and T4 changes in 8-oxodG/8-oxoGuo excretion vs. changes in PANSS (range: −28–+23 points), PSS, or cortisol secretion.

### 4. Discussion

Only a few studies have examined levels of DNA damage in schizophrenia. Two previous studies did not find elevated lymphocyte DNA strand breaks, as measured by the comet assay, compared to controls (Psimadas et al., 2004; Young et al., 2007). A recent study found increased levels of phosphorylated histone variant H2AX, an index of DNA double strand breaks, in cultured lymphoblasts from schizophrenia patients compared to healthy controls, and an attenuated activation of the same marker after cell irradiation in patients (Catts et al., 2012). In a post-mortem study, an increased intra-neuronal 8-oxodG immunoreactivity in the hippocampus of elderly, poor-outcome schizophrenia patients was observed (Nishioka and Arnold, 2004). However, to our knowledge, this study provides the first evidence for increased systemic DNA and RNA oxidation in vivo in schizophrenia. In our cohort, the median urinary excretion of both 8-oxodG and 8-oxoGuo was approximately 20% higher in the schizophrenia patients, and the difference persisted after the adjustment for multiple confounders of oxidative stress, indicating that the increased nucleic acid oxidation in patients is not merely an epiphenomenon of lifestyle or dysmetabolism.

Although the data by no means proves a causal relationship between schizophrenia and oxidatively generated DNA/RNA damage, the finding is not inconsistent with the hypothesis that oxidative stress is a pathophysiological mechanism in schizophrenia. In contrast to the finding of others (Arvindakshan et al., 2003b; Zhang et al., 2006), plasma markers of lipid peroxidation and antioxidant status did not differ between the groups. This discrepancy could be due to methodological issues, in that the above-mentioned studies determined MDA by the Thiobarbituric Acid assay, whereas we used HPLC, which is considered a more specific method for the measurement of MDA (Lykkesfeldt, 2007b). Our finding could indicate a specific susceptibility to nucleic acid oxidation in schizophrenia. The mitochondria are the primary source of ROS, and disturbances of mitochondrial function will lead to increased formation of ROS and oxidative stress (Turrens, 2003). If mitochondrial perturbations are a core feature of the biology of schizophrenia as suggested by others (Jablensky et al., 2012; Prakash et al., 2004; Rosenfeld et al., 2011), then molecules located in the proximity of the mitochondria, such as RNA and mitochondrial DNA, could be speculated to be particularly exposed to oxidative damage, whereas markers of extracellular oxidative processes such as plasma MDA would be relatively unaffected.

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**Fig. 2.** Scatterplots of the relation between the excretion of 8-oxodG/8-oxoGuo (Ln-transformed values), 9 AM plasma cortisol (Ln-transformed values, A+B) and the AUC for salivary cortisol (arbitrary units, C+D) in healthy controls (CON, upper panel) and schizophrenia patients (SCZ, lower panel). (A) 8-oxodG vs. 9 AM plasma cortisol. CON: Pearson’s correlation coefficient (r) = 0.46, P = 0.003; SCZ: r = 0.03, P = 0.87. (B) 8-oxoGuo vs. 9 AM plasma cortisol. CON: r = 0.46, P = 0.004; SCZ: r = −0.43, P = 0.02. (C) 8-oxodG vs. the AUCg for salivary cortisol. CON: r = 0.10, P = 0.58; SCZ: r = −0.01, P = 0.62. (D) 8-oxoGuo vs. the AUCg for salivary cortisol. CON: r = −0.07, P = 0.71; SCZ: r = −0.27, P = 0.16.
The markers remained elevated at a 4-month follow-up examination in the patients, reflecting a strong correlation between intra-individual T0 and T4 values, and were not correlated to the duration of illness or any measure of current severity of disease. This indicates that the elevated nucleic acid damage from oxidation is a “trait” rather than a “state” phenomenon, possibly due to a genetically determined increased oxidative stress, as suggested by others (Toxic et al., 2006), or as a result of more persistent and stable metabolic changes developing early in the disease course.

Our finding could have implications for the signs of accelerated aging observed in schizophrenia. Genotoxic stress caused by oxidants is recognised as a critical event in cellular aging (Finkel and Holbrook, 2000). The oxidatively generated damage to DNA accumulates with age and may induce cell senescence or apoptosis (Moller et al., 2010; Sahin and DePinho, 2010). Furthermore, an increasing attention to RNA oxidation as an instigator of human aging and neuropathology is emerging (Nunomura et al., 2009).

Interestingly, a recent post-mortem study found increased oxidation of RNA, but not of DNA, in the hippocampus of schizophrenia patients (Che et al., 2010). Similarly, we found a more pronounced difference in the RNA marker excretion (effect size (Cohen’s d) = 1.13) compared to the DNA marker excretion (Cohen’s d = 0.69) in patients vs. controls.

The UPLC–MS/MS method for the detection of urinary nucleo-

sides has been extensively validated and allows for a high throughput analysis of samples obtained non-invasively (Evans et al., 2010a), and the determined excretion levels of 8-oxodG/8-oxoGuo are within the reference ranges reported by others (Andreoli et al., 2011). Based on this, 8-oxodG/8-oxoGuo could have utility as biomarkers in larger-scale, prospective studies of aging and mortality in schizophrenia. As an illustration of this potential use, we recently found 8-oxoGuo (but not 8-oxodG) to be an independent predictor of long-term mortality in a large cohort of newly diagnosed type II diabetics (Broedbaek et al., 2011).

We did not find the expected association between the marker excretion and measures of cortisol secretion in schizophrenia patients. In contrast, and in replication of a previous finding (Joergensen et al., 2011), plasma cortisol was positively correlated to both markers in the control group. Corticosteroids impact on mitochondrial function (Du et al., 2009) and tissue DNA oxidation levels (Caro et al., 2007). The lack of an association – or even a negative association – in the schizophrenia patients suggests that the cause of the increased nucleic acid oxidation lies outside of the neurohormonal stress response. It could be speculated that the chronic stress state of suffering from schizophrenia leads to a reduced cellular sensitivity to cortisol, as previously found in a non-pathological human stress condition (being caregiver for a cancer patient) (Miller et al., 2008).

There are several limitations to the study. (1) The cross-sectional comparison of patients and controls does not allow for inferences on the mechanistic relationship between schizophrenia and oxidatively generated nucleic acid damage, and confounding factors which were not determined in the study could contribute to the finding. (2) The sample size is relatively low, and this may explain the null finding with regards to the association between 8-oxodG/8-oxoGuo and the psychometric variables (psychopathology and perceived stress). (3) We found no associations between the markers and any measure of medication load, and this is in line with evidence that only the first generation antipsychotics induce oxidative stress (Krropp et al., 2005). However, an effect of medication cannot entirely be ruled out, and the finding should be replicated in drug-naive patients.

(4) Likewise, while we found no effect of smoking on the markers in neither group, and the adjustment for smoking in multiple regression analysis did not alter the result, we can not rule out an effect of smoking habits, which differed substantially between the patient and control groups.

In conclusion, we found that schizophrenia was associated with a 20% increase in median urinary excretion of DNA and RNA oxidation markers compared to healthy controls. This difference remained significant after the adjustment for multiple socio-demographic, lifestyle, and metabolic variables. Although causal relations cannot be inferred from the study, increased rates of oxidatively generated nucleic acid damage could constitute a molecular link between schizophrenia and the signs of accelerated aging associated with this severe mental disorder.

Acknowledgements

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