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Original Contribution

Genetic and environmental influences on oxidative damage assessed in elderly Danish twins

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ABSTRACT

Previous studies have shown an association between oxidative stress and various diseases in humans including cancer, cardiovascular disease, diabetes, and chronic respiratory disease. To what extents this damage is determined by genetic and environmental factors is unknown. In a classical twin study with 198 elderly twins we examined the contributions of genetic versus environmental factors to nucleic acid oxidation and lipid peroxidation. Urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), 8-oxo-7,8-dihydroguanosine (8-oxoGuo), and dinor,dihydro F_2-isoprostane metabolites (F_2-IsoP-M) was measured using liquid chromatography–tandem mass spectrometry. The environmental influence on nucleic acid oxidation and lipid peroxidation was predominant, leaving only little influence from genetic factors, as evidenced by no differences in intraclass correlations between monozygotic (MZ) and dizygotic (DZ) twins, neither for 8-oxodG ($r_{MZ}=0.55$, $r_{DZ}=0.47$; $P=0.43$), F_2-IsoP-M ($r_{MZ}=0.33$, $r_{DZ}=0.22$; $P=0.42$), nor 8-oxoGuo ($r_{MZ}=0.45$, $r_{DZ}=0.58$; $P=0.21$). Accordingly, heritability estimates for the three markers of oxidative damage were low ($h^2=0.17$–0.22). The three urinary markers of oxidative stress were closely correlated ($r=0.60$–0.84). In conclusion, we demonstrated in a large population of elderly Danish twins that "whole-body" oxidative damage to nucleic acids and lipids is predominantly determined by potentially modifiable nongenetic factors.

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Oxidative stress is associated with damage to a wide range of macromolecules including nucleic acids, proteins, and lipids. Among these, DNA is regarded as a crucial target. DNA oxidation is thought to be important in a number of cancer, precancerous, and noncancerous conditions [1]. A large number of base- and sugar-derived DNA lesions have been identified, but the most thoroughly studied is the guanine modification 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) [2,3]. 8-oxodG and the RNA ribonucleoside counterpart termed 8-oxo-7,8-dihydroguanosine (8-oxoGuo) are excreted in urine and represent rate estimates of the "whole-body" DNA and RNA oxidation [4].

Lipids are subject to oxidation, and F_2-isoprostanes have been used extensively as clinical markers of lipid peroxidation. Associations between F_2-isoprostane levels and a wide variety of human diseases, including cardiovascular, pulmonary, neurological, renal, and liver diseases, have been found in many studies [5,6]. The excretion of dinor, dihydro F_2-isoprostane metabolites (F_2-IsoP-M) in urine is considered to be a useful measure of whole-body lipid peroxidation [7].

It has been shown that the rate of DNA and lipid oxidation is influenced by environmental factors, e.g., tobacco smoke [8,9], exercise [10,11], and exposure to air pollution and wood-smoke particulates [12,13], but less is known about the genetic contribution to oxidative damage. The contributions of genetic versus environmental factors to the rate of oxidative damage remain to be determined.

It is important to estimate the relative importance of genetic and nongenetic factors for the variation in a trait, because nongenetic factors are more readily subject to prevention or intervention than genetic factors. To our knowledge no studies have addressed the genetic versus the environmental contribution to whole-body

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; MZ, monozygotic; DZ, dizygotic; h^2, heritability; F_2-IsoP-M, dinor,dihydro-F_2-isoprostane metabolites.

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oxidative damage. The study of twins provides an opportunity to evaluate the relative contributions of genetic and environmental factors to complex traits. The classical twin design uses the fact that monozygotic (MZ) twins are almost genetically identical, whereas dizygotic (DZ) twins, like siblings, share only approximately 50% of their genes. Based on the assumption of equal environments for MZ and DZ twins, a comparison of similarity gives an estimate of the relative importance of genetic and environmental effects for the variation in a trait [14].

In this study we applied a classical twin approach to determine the contributions of genetic versus nongenetic factors to the variation in whole-body oxidative damage using urinary markers of nucleic acid oxidation and lipid peroxidation.

Materials and methods

The subjects were identified through the Danish Twin Registry as previously described [15–17]. A total of 198 elderly twins (ages 62–83 years), 46 MZ and 53 DZ same-sex twin pairs, were included in this study [16,17]. Zygosity was determined by a questionnaire concerning phenotypic similarities [16]. The study was approved by the regional ethical committees and conducted in accordance with the Helsinki Declaration.

Spot urine samples were assayed for 8-oxodG, 8-oxoGuo, and F_2-IsoP-M. 8-oxodG and 8-oxoGuo were determined using ultraperformance liquid chromatography (UPLC) and tandem mass spectrometry (MS). Chromatographic separation was performed on an Acquity UPLC system (Waters, Milford, MA, USA). The column used was an Acquity UPLC BEH Shield RP18 column (1.7 μm, 2.1×100 mm) protected with an inline filter (4×2 mm, 0.2 μm), both obtained from Waters. The MS detection was performed on an API 3000 triple-quadrupole mass spectrometer (Sciex, Toronto, ON, Canada) equipped with an electrospray ionization ion source (Turbospray) operated in the positive-ion mode. Details of the analysis are described elsewhere [18].

F_2-IsoP-M were determined by high-performance liquid chromatography coupled to electrospray ionization–tandem mass spectrometry as described previously [19]. 8-oxodG, 8-oxoGuo, and F_2-IsoP-M were normalized against urinary creatinine concentration.

Pearson's correlation coefficients were calculated, and because of deviation from normal distribution the variables 8-oxodG, 8-oxoGuo, and F_2-IsoP-M were log-transformed before calculation. The total phenotypic variance is the sum of the variance attributable to effects of both genetic and environmental factors [20]. The heritability (h^2) expresses the proportion of variance attributable to genetic variance and was calculated from intraclass correlations in MZ and DZ twins [$h^2=(r_{MZ}-r_{DZ})\times 2$] [20,21]. Intraclass correlation coefficients were calculated using 2*n*. A test for equality of two correlation coefficients was conducted using Fisher's Z transformation.

The relationships between the various measured markers of oxidative stress and the relationships between these markers and selected sociodemographic and lifestyle variables were assessed by Pearson's or Spearman's correlation coefficients. Pairwise comparisons were performed using Student's *t* test.

All statistical analyses were performed using the SAS software version 9.1 (SAS Institute, Inc., Cary, NC, USA). Statistical significance was defined as $P<0.05$.

Results

Characteristics of the study population are presented in Table 1. MZ and DZ twins were balanced with respect to baseline demographic, clinical, and biochemical characteristics. Similar levels of 8-oxodG, 8-oxoGuo, and F_2-IsoP-M were seen in MZ and DZ twins.

Table 1
Characteristics of elderly twin population ($n=198$).

Variable	MZ twins	DZ twins	P[a]
n (male/female)	92 (54/38)	106 (36/70)	
Age (years)	74.0±4.9	72.6±5.5	0.07
Smoking			
Current smoker (%)	20.5	16.7	0.5
Concordant for smoking (%)	9.3	4.0	0.4
Concordant for nonsmoking (%)	69.8	74.0	0.8
Body mass index (kg/m^2)	26.0 (23.9–28.1)	25.9 (23.5–27.7)	0.6
Systolic blood pressure (mm Hg)	144±21	141±22	0.4
Glucose (mmol/L)	5.6 (5.3–6.1)	5.6 (5.3–5.9)	0.8
Total cholesterol (mmol/L)	5.5±1.0	5.7±0.8	0.2
Triglyceride (mmol/L)	1.2 (0.9–1.6)	1.1 (0.8–1.4)	0.1
Hemoglobin (mmol/L)	8.6±0.9	8.6±0.8	0.8
C-reactive protein (mg/L)	3 (3–5)	3 (3–3)	0.3
Urinary 8-oxodG (nmol/mmol creatinine)	2.04 (1.64–2.56)	2.04 (1.57–2.85)	0.8
Urinary 8-oxoGuo (nmol/mmol creatinine)	2.92 (2.29–3.73)	2.93 (2.35–3.76)	0.8
Urinary F_2-IsoP-M (ng/mg creatinine)	38.3 (32.0–56.1)	40.5 (30.2–60.6)	0.6
Urinary creatinine (mmol/L)	9.7 (6.3–13.7)	8.7 (5.8–13.4)	0.6

Data are medians (interquartile range) or means±SD unless otherwise stated. MZ, monozygotic; DZ, dizygotic.
[a] Statistics were computed using the two-sample *t* test and the Mann–Whitney test for continuous variables and with the χ^2 test for categorical variables.

Intraclass correlations

The intraclass correlation coefficient for 8-oxodG was $r=0.55$ (95% confidence interval 0.39–0.68) in MZ and $r=0.47$ (95% confidence interval 0.30–0.60) in DZ twins (Table 2). The intraclass correlation coefficient for 8-oxoGuo was $r=0.45$ (95% confidence interval 0.27–0.60) in MZ twins and $r=0.58$ (95% confidence interval 0.44–0.70) in DZ twins. The intraclass correlation coefficient for F_2-IsoP-M was $r=0.33$ (95% confidence interval 0.11–0.52) in MZ twins and $r=0.22$ (95% confidence interval 0.02–0.40) in DZ twins.

No statistically significant differences in intraclass correlation between the two zygosities were found for 8-oxodG, 8-oxoGuo, and F_2-IsoP-M, indicating a relatively small genetic contribution to the variation in these markers (Table 2).

Low heritability estimates were found. The heritability estimates for 8-oxodG and F_2-IsoP-M excretion were $h^2=0.17$ and $h^2=0.22$, respectively (Table 2). A heritability estimate was not calculable for 8-oxoGuo because of a higher intraclass correlation in DZ twins, indicating virtually no contribution of genes to variation in 8-oxoGuo excretion.

Relationship between urinary markers of oxidative stress

The three urinary markers of oxidative damage were closely correlated with one another (Fig. 1). The closest correlation was found between 8-oxodG and 8-oxoGuo ($r=0.84$, $P<0.0001$). F_2-IsoP-M was closely correlated with both 8-oxodG ($r=0.60$, $P<0.0001$) and 8-oxoGuo ($r=0.64$, $P<0.0001$).

Table 2
Intraclass correlations and heritability estimates for urinary markers of nucleic acid oxidation and lipid peroxidation.

	Intraclass correlation		*P*	Heritability (h^2)
	MZ	DZ		
8-oxodG	0.55 (0.39–0.68)	0.47 (0.30–0.60)	0.43	0.17
F_2-IsoP-M	0.33 (0.11–0.52)	0.22 (0.02–0.40)	0.42	0.22
8-oxoGuo	0.45 (0.27–0.60)	0.58 (0.44–0.70)	0.21	Not calculable

Values in parentheses are 95% confidence intervals. MZ, monozygotic; DZ, dizygotic.

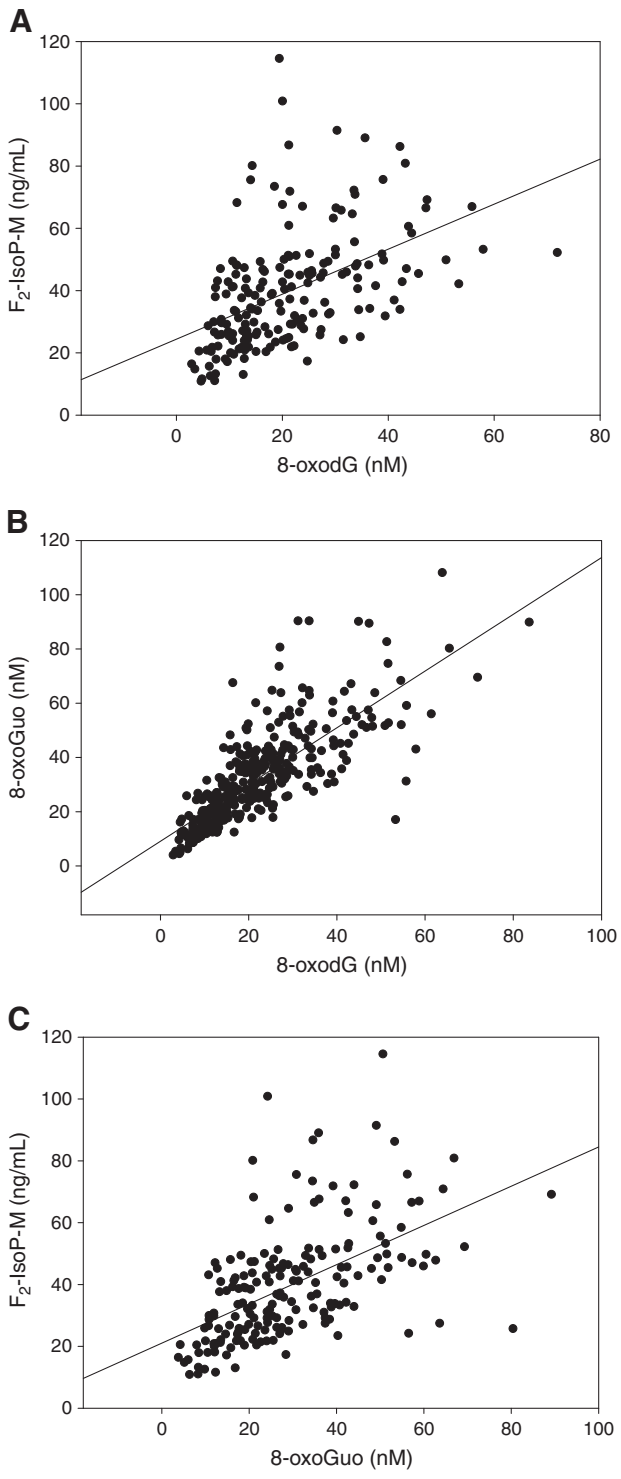

Fig. 1. Relationships between (A) urinary 8-oxodG and F_2-IsoP-M, (B) urinary 8-oxodG and 8-oxoGuo, and (C) urinary 8-oxoGuo and F_2-IsoP-M.

Relationship between urinary markers of oxidative stress and sociodemographic and lifestyle variables

Relations between available sociodemographic and lifestyle variables and the markers of oxidative damage are shown in Table 3. Urinary 8-oxoGuo and F_2-IsoP-M were significantly higher in females and were positively correlated with age. F_2-IsoP-M was significantly positively correlated with cholesterol.

Discussion

This is the first study to investigate the relative contributions of genetic versus environmental factors to nucleic acid oxidation and lipid peroxidation. In a large population of elderly Danish twins we demonstrated that the rates of nucleic acid oxidation and lipid peroxidation are predominantly determined by environmental factors.

Previous studies have shown an association between DNA oxidation/lipid peroxidation and various diseases in humans [1,5]. These conditions include cancer, cardiovascular disease, diabetes, and chronic respiratory disease, which represent the world's leading causes of mortality, causing an estimated 35 million deaths each year—60% of all deaths globally [22].

A large study of 44,788 twins from Sweden, Finland, and Denmark [23] found that the rates of concordance in twins were generally below 0.10 for most types of neoplasms, which indicates that genetic factors make only a minor contribution to the development of sporadic cancer. Although the exact role of DNA oxidation in most diseases is not fully understood, the role of DNA damage in carcinogenesis is supported by several reports demonstrating the induction of oxidatively generated DNA damage by known carcinogens and elevated levels of oxidatively modified DNA lesions in a wide range of cancers [1,24]. Our results indicate that only a small part (approx 20%) of the total variance in DNA oxidation is due to genetic factors. Accordingly, the majority of the variance is accounted for by environmental factors (e.g., smoking habits, diet, exercise, environmental pollution). These findings provide no direct evidence that oxidative stress is the cause of cancer, but are consistent with the known association between DNA oxidation and cancer and the indications of a small genetic contribution to cancer development.

Another major cause of mortality—type 2 diabetes—is traditionally considered to have a major genetic component, because of concordance rates in the range of 50–92% for MZ twins in various twin studies [25–27]. However, recent studies have shown that environmental factors may be more important, leading to the notion that manifest diabetes mellitus develops when environmental factors such as a Western lifestyle, including a high-caloric diet and physical inactivity, as well as an adverse fetal intrauterine environment, are added to a genetic predisposition [28–32]. Considering the previously shown associations between diabetes and both DNA oxidation and lipid peroxidation [1,5,6], oxidative damage could play a role as a mediator of the nongenetic component of diabetes, but again the issue of causality needs to be examined in more detail.

In some cases genetic factors may be paramount for disease development, but environmental factors may have the dominant role in the pathogenesis/pathophysiology. One such example is hereditary hemochromatosis, a condition characterized by increased iron uptake and deposition in all organs, severe nucleic acid oxidation, and high risk of cancer development [33]. Hereditary hemochromatosis has a genetic predisposition, but disease development requires the exogenic/environmental factor iron. This disease is treated by removal of iron by venesection, which reduces iron load, reduces nucleic acid oxidation and lipid peroxidation [34,35], and returns cancer risk to the same as the population at large. Although primarily a genetic disease, it is a prime example of gene–environment interaction and also a prime example in which prevention most readily is targeted at the environmental component, i.e., iron.

This study was not designed to investigate the influences of various environmental factors, as only limited sociodemographic and lifestyle data were available (Table 3). The contributions from various environmental factors to oxidative damage should be obtained from other studies.

In conclusion, our results indicate that genetic factors have only a minor contribution to the interindividual variability in whole-body oxidative damage to nucleic acids and lipids. One of the implications of this finding is that there seems to be a considerable opportunity for

Table 3
Relationships between urinary markers of oxidative damage and sociodemographic and lifestyle variables.

Variable	8-oxodG		8-oxoGuo		F_2-IsoP-M	
	Median (IQR)	*P*	Median (IQR)	*P*	Median (IQR)	*P*
Sex						
Male	1.88 (1.49–2.56)	0.11	2.71 (2.25–3.32)	0.005	34.3 (25.6–49.0)	<0.001
Female	2.07 (1.71–2.86)		3.06 (2.53–4.32)		42.9 (34.0–61.3)	
Smoking						
Smokers	2.09 (1.57–2.91)	0.63	2.74 (2.24–3.57)	0.17	39.8 (30.2–51.3)	0.46
Nonsmokers	2.03 (1.57–2.73)		2.95 (2.40–3.79)		38.7 (30.0–60.6)	
	r	*P*	*r*	*P*	*r*	*P*
Age (years)	0.14	0.05	0.20	0.006	0.24	<0.001
BMI (kg/m^2)	−0.03	0.64	−0.006	0.93	0.05	0.47
Systolic blood pressure (mm Hg)	0.07	0.34	−0.04	0.59	0.01	0.86
Total cholesterol (mmol/L)	0.008	0.91	0.004	0.95	0.16	0.03

Median and interquartile range values are nmol/mmol creatinine (8-oxodG, 8-oxoGuo) or ng/mg creatinine (F_2-IsoP-M). IQR, interquartile range; BMI, body mass index.

prevention and for the development of therapies for reducing oxidative damage in situations in which this mechanism is involved in the pathogenesis of disease and disease complications.

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