Urinary Isoprostane Levels and Age-Related Macular Degeneration

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PURPOSE. Oxidative stress, characterized by an excessive production of reactive oxygen intermediates has been suggested to play a role in the pathogenesis of age-related macular degeneration (AMD). We examined the association of urinary F2-isoprostanes (F2-IsoPs), a marker of lipid peroxidation and the most reliable marker of oxidative damage with AMD.

METHODS. We included 238 adults with AMD and 390 age- and sex-matched controls without AMD who participated in a population-based cross-sectional study in Singapore (Singapore Chinese Eye Study, 2009–2011). AMD was graded from retinal photographs using the Wisconsin Age-Related Maculopathy Grading System. Urinary-free F2-IsoPs (pmol/mmol of creatinine) were measured by gas chromatography mass spectrometry (GC-MS). The association between F2-IsoPs and AMD was examined using unconditional logistic regression models adjusted for potential confounders including smoking, body mass index (BMI), blood pressure, total and high-density lipoprotein cholesterol, and history of cardiovascular disease.

RESULTS. Higher levels of F2-IsoPs were associated with AMD independent of potential confounders. Compared to quartile 1 (Q1) of F2-IsoPs, the multivariable odds ratio (95% confidence interval) of AMD in quartiles 2, 3, and 4 were 2.05 (1.26–3.32), 1.80 (1.10–2.94), and 1.76 (1.06–2.94), respectively. In subgroup analyses comparing Q4 to Q1, this association was stronger in women, those with BMI less than 25 kg/m² and those with hypertension, but no significant interaction was found (P interaction > 0.1 for each strata).

CONCLUSIONS. Higher levels of urinary F2-IsoPs levels were associated with AMD independent of potential confounders in Chinese adults.

Keywords: age-related macular degeneration, Asians, isoprostane, oxidative stress

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in older adults worldwide.[1,2] Globally, 170 million adults have AMD, exerting a significant socioeconomic burden on healthcare systems.[2,3] Despite significant research over decades, the pathogenesis of AMD is still not clear, and robust systemic biomarkers of AMD have not been identified yet.[4]

Oxidative stress, characterized by an imbalance between excessive generation of reactive oxygen species (ROS) and their degradation by antioxidants, has recently been proposed to play a significant role in the pathogenesis of AMD.[5–7] With advancing age, the retina becomes more susceptible to oxidative stress and damage to the retinal pigment epithelium (RPE) occurs early in the natural history of AMD.[8] At physiological concentrations, ROS are involved in cell signaling and regulation of immune responses.[9] However, disproportionate levels of ROS with concurrent impairment of enzymatic and nonenzymatic antioxidants damage cellular proteins, membrane lipids, and DNA that eventually lead to tissue injury, cellular death[10] resulting in accumulation of detrimental products including intracellular lipofuscin, and extracellular drusen.[6]

Oxidation of membrane lipids or lipid peroxidation has been strongly linked to atherosclerosis[11,12] and inflammation,[13] two of the major mechanisms underlying AMD. Isoprostanes, the most sensitive and stable marker of lipid peroxidation and oxidative stress are lipid peroxidation products formed via the free-radical mediated oxidation of arachidonic acid.[14] Isoprostanes have been shown to be associated with atherosclerosis[15–17] and inflammation[18–22] in several previous studies. However, the association of isoprostanes with AMD is not clear. Two previous studies that examined association between isoprostanes measured using immunoassays and AMD[23,24] did not find any significant association.[25,26]

In the current study, we evaluated the association of AMD with oxidative stress as indicated by urinary F2-isoprostanes (F2-IsoPs) measured by gas chromatography tandem mass spectrometry (GC-MS), the gold standard method for measuring isoprostanes[25,26] in a population-based sample of adults in Singapore

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METHODS

We designed a case-control study using data from the Singapore Chinese Eye Study (SCES; 2009–2011, $n = 3353$), a population-based cross-sectional study aimed to assess the prevalence and risk factors of age-related eye diseases in Chinese adults in Singapore. Study design and methodology of SCES has been published elsewhere.[27] In brief, 3353 adults aged 40 to 80 years, living in the southwestern part of Singapore were recruited into the study (2009–2011). They were identified by an age-stratified random sampling method from a computer-generated random list of 12,000 ethnic Chinese residents provided by Singapore's Ministry of Home Affairs. The study followed Tenets of the Declaration of Helsinki and ethics approval was obtained from the Singapore Eye Research Institute institutional review board. Written informed consent was obtained from all participants.

Identification of Cases and Controls

Cases were defined by the presence of any AMD (early or late AMD) and controls as those without any AMD determined by grading from fundus photography. One to two controls frequency matched on age (within 5-year age group) and sex to cases were chosen. We identified 238 cases and 390 controls. In quartile analysis, our study sample size had 80% power to detect a minimum odds ratio (OR) of 1.65 for AMD.

Assessment and Definition of AMD

Digital retinal photographs taken using a nonmydriatic retinal camera (Canon CR-DGi with a 10 dioptre SLR backing; Canon, Tokyo, Japan) were used to assess the presence of AMD. After pupillary dilation, two $45°$ retinal images corresponding to Early Treatment for Diabetic Retinopathy Study (ETDRS) standard field 1 (centered on the optic disc) and ETDRS standard field 2 (centered on the fovea) were taken. Images were sent to Centre for Vision, University of Sydney, Australia, where trained graders masked to participant characteristics graded the images according to a modified Wisconsin Age-related Maculopathy Grading System. Any AMD was defined as the presence of either early or late AMD.[28,29] Early AMD was defined by the presence of soft drusen or RPE abnormalities or a combination of both,[30] while late AMD was defined by the presence of neovascular AMD or geographic atrophy.

Measurement of F2-IsoPs

Urinary F2-IsoPs, albumin, and creatinine were measured from stored (−80°C) urine samples collected at baseline. F2-IsoPs levels were measured at the University of Sydney while albumin and creatinine were measured at the National University Hospital Reference laboratory, Singapore. Urinary samples were kept frozen until shipped on dry ice to Sydney. F2-IsoPs were measured by GC-MS electron capture negative chemical ionization using stable isotope dilution method as described by Mori et al.[31] and Tsai et al.[32] The intra- and interassay coefficient of variation (CV) were 0.9% and 7.4%, respectively, with a lower limit of detection of 254 pM. Urinary albumin and creatinine were measured using commercial assays (for urinary albumin and creatinine, respectively; Immulite, DPC; Roche Diagnostics GmbH, Mannheim, UK). As the levels of F2-IsoPs in urine are affected by the hydration status of the individual, F2-IsoPs levels were normalized to urinary creatinine (pmol/mmol of creatinine) for analysis.[21,33,34]

Assessment of Covariates

Covariate information was obtained from standardized questionnaires, physical, and laboratory examinations. Questionnaire data included information on age, sex, education level, history of smoking and alcohol consumption, and medical history. Physical examination included height, weight, and blood pressure (BP) measurements. Laboratory examination included measurement of blood glucose, HbA1c, total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol from nonfasting venous blood samples. Cigarette smoking was categorized into never, former, and current smoking. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters squared (kg/m^2). BMI was categorized into two categories: less than 25 (normal) and greater than or equal to 25 kg/m^2 (overweight and obesity). Hypertension was defined as systolic BP greater than or equal to 140 mm Hg or diastolic BP greater than or equal to 90 mm Hg or self-reported previously diagnosed hypertension. Diabetes mellitus was defined as a casual plasma glucose greater than or equal to 200 mg/dL (11.1 mM) or self-reported physician-diagnosed diabetes or use of glucose-lowering medication(s). Cardiovascular disease (CVD) was defined as self-reported myocardial infarction, angina, or stroke.

Statistical Analysis

F2-IsoPs were analyzed in quartiles (quartile 1 [Q1], 107–738; Q2, 738–999; Q3, 999–1364; and Q4, 1364–5970) and also as a continuous variable (per SD increase). We compared the characteristics of the study participants by AMD status using the χ^2 test for categorical and analysis of variance for continuous variables. We also compared participant characteristics by quartiles of F2-IsoPs levels. We examined the association between F2-IsoPs levels and any AMD using two unconditional logistic regression models: model 1 adjusted for age and sex, and model 2 adjusted for age, sex, current smoking, BMI, systolic BP, diabetes, total cholesterol, HDL cholesterol, and CVD. In quartile analysis, Q1 was used as the reference group and P for trend was calculated using the quartiles as an ordinal variable. To examine the consistency of the association between F2-IsoPs levels and any AMD, we performed subgroup analysis stratified by potential confounders including sex, BMI, and hypertension status. We examined statistical interaction by stratifying variables by including cross-product interaction terms (strata factor × quartiles of F2-IsoPs) in the corresponding regression models. We performed three supplementary analyses: first we excluded those with late AMD and repeated the analysis using early AMD ($n = 218$) as an outcome; second, we examined the association of F2-IsoPs with AMD stratified by lesion type, drusen ($n = 301$) and pigmentary lesion ($n = 242$) in separate models; and third, to demonstrate that the associations of AMD observed with F2-IsoPs normalized to urinary creatinine were not driven by urinary creatinine, we evaluated the association of 1/urinary creatinine with AMD in unconditional logistic regression models. In addition, we also examined the association of F2-IsoPs without normalization to urinary creatinine (absolute F2-IsoPs) with AMD in multivariable logistic regression models. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Participant characteristics in terms of demographic, lifestyle, or clinical factors were similar (Table 1) between those with (cases) and without AMD (controls), except that controls had

TABLE 1. Characteristics of Study Participants Stratified by AMD Status

Characteristics	Controls ($n = 390$)	Cases ($n = 238$)	P Value*
Age, y	67.1 (9.2)	66.2 (9.4)	0.3
Male, %	251 (64.4)	150 (63.0)	0.7
Diabetes, %	79 (20.4)	60 (25.2)	0.2
Hypertension, %	291 (74.6)	177 (74.4)	0.9
Current smoker, %	61 (15.6)	25 (10.5)	0.07
Ever drinker, %	34 (8.7)	30 (12.7)	0.1
History of CVD, %	42 (10.8)	19 (8.0)	0.3
Blood glucose, mM	6.5 (2.4)	6.9 (3.4)	0.2
Systolic BP, mm Hg	141.5 (19.6)	141.1 (18.9)	0.8
Diastolic BP, mm Hg	76.9 (9.1)	77.6 (8.7)	0.4
BMI, kg/m²	23.9 (3.5)	23.3 (3.6)	0.05
Total cholesterol, mM	5.3 (1.1)	5.3 (1.0)	0.7
HDL cholesterol, mM	1.25 (0.41)	1.32 (0.41)	0.03
F2-IsoPs, pmol/mmol Cr	1117.4 (695.9)	1203.6 (716.0)	0.1

Data presented are numbers and (proportions) or mean and (SD). BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; F2-IsoPs, F2-isoprostanes; HDL, high-density lipoprotein.

* *P* value represents difference in characteristics by case-control status based on χ^2 or ANOVA as appropriate.

lower levels of HDL cholesterol than cases. Table 2 presents the characteristics of study participants by quartiles of F2-IsoPs. Compared with those in Q1 of F2-IsoPs, those in Q3 and Q4 were more likely to be females and current smokers, had lower prevalence of hypertension and CVD, higher levels of blood glucose and HDL cholesterol, and lower levels of BMI.

Table 3 shows the association between F2-IsoPs and any AMD. Compared with those in Q1 of F2-IsoPs, Q2 to Q4 showed significant positive association with any AMD in both age, sex-adjusted, and multivariable models. But, the positive association across the quartiles was not linear, rather, all quartiles above the first showed similar OR estimates. Furthermore, no significant association was observed when F2-IsoPs were analyzed as continuous variables. In subgroup analyses (Fig.), although the association of F2-IsoPs with any AMD was stronger in women, those with BMI less than 25 kg/m² and those with hypertension, no significant interactions were found (*P* interaction > 0.1 for each strata).

In supplementary analyses, when we repeated the analyses using early AMD as an outcome, the pattern of associations observed was similar to that of any AMD. Compared with Q1, the ORs (95% confidence interval [CI]) of early AMD in Q2, Q3, Q4 were: 2.32 (1.40–3.85), 1.98 (1.19–3.31), and 1.81 (1.06–3.09), *P* trend = 0.3 (data not shown). We could not test the association with late AMD due to small number of cases with late AMD ($n = 20$). In analyses stratified by lesion types, association of F2-IsoPs were significant with drusen (2.06 [1.26–3.37] comparing Q4 versus Q1) but not with pigmentary lesion (1.41 [0.86–2.33] comparing Q4 versus Q1). Finally, in analyses evaluating the association of urinary creatinine alone (1/creatinine) with AMD, no significant associations were observed either in the univariate (*P* = 0.08) or multivariate analyses (*P* = 0.2). In addition, no significant associations were detected in analyses including absolute F2-isoPs without normalization to urinary creatinine (1.29 [0.67–2.46] comparing Q4 versus Q1).

DISCUSSION

We demonstrated that urinary F2-IsoPs were positively associated with any AMD independent of age, sex, smoking, BMI, systolic BP, total and HDL cholesterol, and CVD. The positive association was not linear; with a ceiling effect observed with higher levels, which implied conversely that lower levels of F2-IsoPs were protectively associated with AMD. In subgroup analyses, this association was stronger in women, those with BMI less than 25 kg/m² and those with hypertension. The positive association between F2-IsoPs and AMD remained significant when early AMD was used as an outcome. Within lesion subtype, the association was significant among those with drusen, but not in those with pigmentary lesion. F2-IsoPs are considered to be the best available markers of oxidative stress and lipid peroxidation in vivo.[35] F2-IosPs have been shown to be reliable indicators of oxidative stress both in vitro[36] and in vivo[35,37] in that they are specific products of lipid peroxidation, stable, their levels increase considerably in several animal models of oxidant injury, and their formation is modulated by antioxidant agents,[38] besides being not affected by lipid content of the diet.[39]

TABLE 2. Characteristics of Study Participants by Quartiles of Urinary F2-IsoPs

Characteristics	F2-IsoPs (pmol/mmol Cr)				
	Quartile 1 (<738.4, $n = 155$)	Quartile 2 ($738–999$, $n = 159$)	Quartile 3 ($999–1364$, $n = 157$)	Quartile 4 ($1364–5970$, $n = 157$)	P Value*
Age, y	67.9 (9.3)	66.7 (8.9)	66.1 (9.8)	66.3 (9.2)	0.6
Male, %	102 (65.8)	114 (71.7)	98 (62.4)	87 (55.4)	0.02
Diabetes, %	38 (24.7)	32 (20.1)	36 (22.9)	33 (21.2)	0.8
Hypertension, %	134 (86.5)	110 (69.2)	108 (68.8)	116 (73.9)	<0.001
Current smoker, %	8 (5.2)	18 (11.3)	22 (14.0)	38 (24.2)	<0.001
Ever drinker, %	10 (6.5)	18 (11.3)	18 (11.5)	18 (11.5)	0.4
History of CVD, %	26 (16.8)	11 (6.9)	14 (8.9)	10 (6.4)	0.006
Blood glucose, mM	6.5 (2.4)	6.3 (2.4)	6.9 (3.3)	6.8 (3.1)	0.005
Systolic BP, mm Hg	144.0 (19.0)	139.6 (18.5)	139.8 (19.4)	142.1 (20.1)	0.7
Diastolic BP, mm Hg	77.9 (9.7)	77.4 (8.5)	76.8 (8.7)	76.5 (8.9)	0.4
BMI, kg/m²	24.6 (3.3)	23.8 (3.5)	23.2 (3.6)	23.1 (3.4)	<0.001
Total cholesterol, mM	5.3 (1.1)	5.3 (1.0)	5.4 (1.1)	5.2 (1.2)	0.4
HDL cholesterol, mM	1.2 (0.4)	1.2 (0.4)	1.3 (0.4)	1.4 (0.5)	<0.001
F2-IsoPs, pmol/mmol Cr	537.1 (157.0)	855.3 (70.4)	1152.5 (100.0)	2051.2 (821.6)	<0.001

Data presented are numbers and (proportions) or mean and (SD). BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; F2-IsoPs, F2-isoprostanes; HDL, high-density lipoprotein.

* The *P* value represents the difference in characteristic by quartile of F2-IsoPs, using analysis of variance or χ^2 test.

TABLE 3. Association Between Urinary F2-IsoPs and Any AMD

F2-IsoPs Level (pmol/mmol Cr)	Control, n = 390	Cases, n = 238	Age, Sex-Adjusted OR (95% CI)	Multivariable Adjusted OR (95% CI)*
Quartile 1 (107–738)	113	42	1.00 (Reference)	1.00 (Reference)
Quartile 2 (738–999)	91	68	2.00 (1.24–3.21)	1.96 (1.21–3.19)
Quartile 3 (999–1364)	92	65	1.87 (1.16–3.01)	1.76 (1.08–2.88)
Quartile 4 (1364–5970)	94	63	1.77 (1.10–2.85)	1.72 (1.03–2.88)
P trend			0.6	0.6
Per SD increase (SD = 704.26)			1.12 (0.95–1.32)	1.09 (0.92–.30)

CI, confidence interval; F2-IsoPs, F2-isoprostanes; OR, odds ratio.

* Adjusted for age, sex, BMI, current smoking, systolic BP, diabetes, total cholesterol, HDL cholesterol, and history of cardiovascular disease.

In the current study, we found that elevated levels of F2-IsoPs were associated with AMD. Few previous studies have examined the association of isoprostanes in plasma with AMD.[23,24,40] In a case-control study evaluating the role of serum lipids in AMD, Orban et al.[40] analyzed serum lipids from 21 Japanese patients affected by neovascular AMD and 24 controls. Authors found the levels of docosahexaenoic acid, arachidonic acid, and their ratios were significantly different in AMD patients than controls. The increased levels of arachidonic acid in sera of AMD patients compared with controls suggest that AMD patients may have more substrate for generation of F2-isoP than controls.[40] In a clinic-based case-control study involving 77 AMD patients and 75 controls, Brantley et al.[24] found no significant association between plasma levels F2-IsoPs and AMD. Similarly, in the Beaver Dam Eye Study, Klein et al.[23] reported that plasma levels of eight isoprostanes were not associated with 15-year cumulative incidence of AMD. The long follow-up period and selective mortality of those with elevated levels of isoprostanes in this elderly cohort could have contributed to the lack of association in this study. Both the above studies used plasma F2-IsoPs as marker of oxidative stress and used immunoassays, which were shown to correlate poorly with that of GC-MS.[41] F2-IsoPs have been shown to unstable in plasma and plasma samples are prone to artefactual generation of isoprostanes ex vivo by autooxidation if the samples are not processed and stored immediately.[42] In the current study, we used urine samples instead of plasma. F2-IsoPs are quite stable in urine, even when urine samples are left at room temperature for 10 days[43] and their levels are not altered with freeze–thaw cycles. Measurement of F2-IsoPs in a single sample of urine has been shown to

adequately represent the daily isoprostane excretion in humans.[39,44]

Several potential mechanisms have been postulated to explain the association between F2-IsoPs and AMD. Atherosclerosis and inflammation have been identified as the key mechanisms underlying AMD. Increased F2-IsoPs have also been shown to be associated with atherosclerosis,[15–17] atherosclerotic CVD,[21,45,46] and markers of inflammation including serum amyloid A and C-reactive protein (CRP) in previous studies.[18–22] In addition, F2-IsoPs have also been shown to be associated with several proatherogenic factors including smoking, diabetes, and hypertension.[47–49] In the current study, although F2-IsoPs levels were higher in those with current smoking, no significant difference in F2-IsoPs levels across quartiles was found with in those with diabetes and a negative association was found with hypertension and CVD. Although the exact reason is not clear, these null and negative associations could possibly be explained by higher levels of F2-IosPs representing a compensatory metabolic adaptation in response to weight gain. In the Insulin Resistance Atherosclerosis Study (IRAS),[50] authors postulated that urinary levels of F2-IsoPs could represent the level of fat oxidation in an individual, for example, those with higher levels of F2-IosPs may have higher rates of fat oxidation and are protected from weight gain and risk of diabetes. In line with this hypothesis, in the current study, those with higher levels of F2-isoPs had lower levels of BMI. It could also be due to selective mortality of those with higher levels of F2-IsoPs and diabetes, hypertension, or CVD[21,45] given that elevated levels of F2-IsoPs have been shown to predict the extent and severity of coronary artery disease.[51] In addition, CVD in the current study was self-reported and
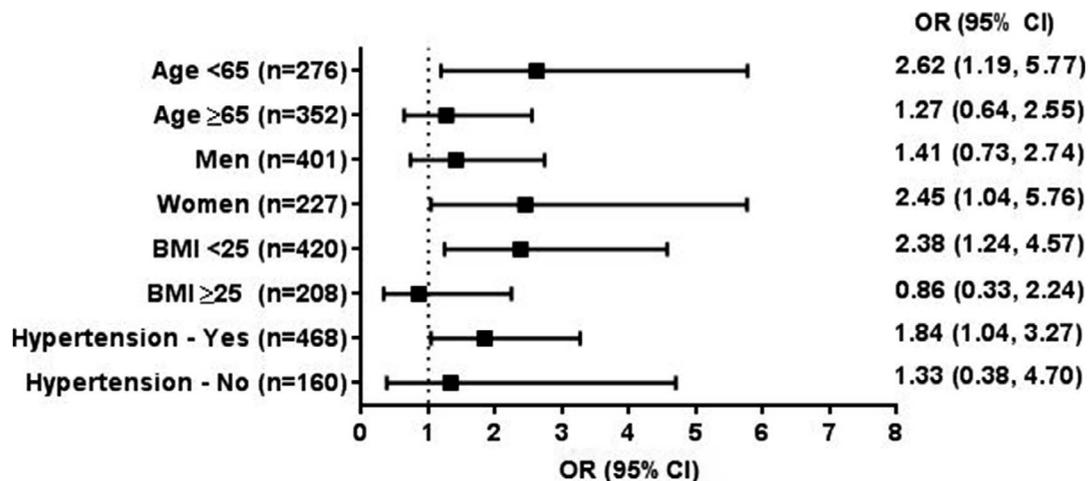

FIGURE. Association of urinary F2-IsoPs (Q4 versus Q1) with any AMD within subgroups in multivariable models. BMI, body mass index; CI, confidence interval; OR, odds ratio.

association of F2-IsoPs with CVD could have been offset by the crude measure of the outcome. In the current study, we found that the positive association between F2-IsoPs and AMD was evident from quartile 2 onward with a nonlinear association. The reason for this nonlinear association is not clear. However, we cannot exclude the possibility of survival bias involving selective mortality of those with higher levels of F2-IsoPs and associated comorbidities contributing to this nonlinear association.

Numerous studies have provided evidence supporting the role of lipid peroxidation in AMD.[52-55] The RPE has been shown to be highly vulnerable to oxidative stress by radical-catalyzed lipid peroxidation.[7,8,56] Markers of lipid oxidation including plasma levels of malondialdehyde (MDA),[54,55] and thiobarbituric acid-reactive substance (TBARS), have been shown to be higher in serum of patients with AMD compared with controls without AMD in previous studies.[52,53] However, F2-IsoPs have been shown to have greater utility than these markers. First, urinary F2-IsoPs are very stable because artifactual F_2-IsoPs are not formed by auto-oxidation due to the low lipid content of urine[38] as opposed to MDA and TBARS. Second, MDA and TBARS were less sensitive and specific indicators of lipid peroxidation compared with F2-IsoPs.[35] Third, levels of F2-IsoPs after oxidant injury were found to increase several folds higher compared with that of MDA[35,57] and formation of MDA is affected by oxygen tension[58] in animal models, making it less useful as an oxidative stress marker.

Our study has some limitations. First, the temporal relationship between F2-IsoPs and AMD could not be established due to the cross-sectional design of our study. Second, it is possible that residual confounding due to unmeasured factors, for example, lack of information on inflammation could have affected the study results. Finally, although, inclusion of a homogeneous Chinese population eliminates confounding by ethnicity, we cannot extrapolate our study findings to other race/ethnic groups. Study strengths included selection of cases and controls from a well-characterized population-based cohort with standardized assessment of outcome and covariates and measurement of urinary F2-IsoPs using the gold standard GC-MS method.

CONCLUSIONS

Our study findings suggest that elevated levels of urinary F2-IsoPs were associated with AMD in Chinese adults. These data provide further evidence of a link between oxidative stress and AMD pathogenesis. If confirmed in prospective studies and diverse populations, F2-IsoPs may serve as a potential systemic biomarker for predicting AMD.

References

1. Wong CW, Yanagi Y, Lee WK, et al. Age-related macular degeneration and polypoidal choroidal vasculopathy in Asians. *Prog Retin Eye Res*. 2016;53:107–139.

2. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health*. 2014;2:e106–e116.

3. Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY. Age-related macular degeneration. *Lancet*. 2012;379:1728–1738.

4. Cheung CM, Wong TY. Is age-related macular degeneration a manifestation of systemic disease? New prospects for early intervention and treatment. *J Intern Med*. 2014;276:140–153.

5. Winkler BS, Boulton ME, Gottsch JD, Sternberg P. Oxidative damage and age-related macular degeneration. *Mol Vis*. 1999;5:32.

6. Blasiak J, Petrovski G, Vereb Z, Facsko A, Kaarniranta K. Oxidative stress, hypoxia, and autophagy in the neovascular processes of age-related macular degeneration. *Biomed Res Int*. 2014;2014:768026.

7. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2000;45:115–134.

8. Cai J, Nelson KC, Wu M, Sternberg P Jr, Jones DP. Oxidative damage and protection of the RPE. *Prog Retin Eye Res*. 2000;19:205–221.

9. Swindle EJ, Metcalfe DD. The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. *Immunol Rev*. 2007;217:186–205.

10. Sies H. Oxidative stress: oxidants and antioxidants. *Exp Physiol*. 1997;82:291–295.

11. Shao B, Heinecke JW. HDL, lipid peroxidation, and atherosclerosis. *J Lipid Res*. 2009;50:599–601.

12. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. *Am J Cardiol*. 2003;91:7A–11A.

13. Freigang S. The regulation of inflammation by oxidized phospholipids. *Eur J Immunol*. 2016;46:1818–1825.

14. Musiek ES, Yin H, Milne GL, Morrow JD. Recent advances in the biochemistry and clinical relevance of the isoprostane pathway. *Lipids*. 2005;40:987–994.

15. Gniwotta C, Morrow JD, Roberts LJ II, Kuhn H. Prostaglandin F2-like compounds, F2-isoprostanes, are present in increased amounts in human atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. 1997;17:3236–3241.

16. Morrow JD. Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans. *Arterioscler Thromb Vasc Biol*. 2005;25:279–286.

17. Pratico D, Iuliano L, Mauriello A, et al. Localization of distinct F2-isoprostanes in human atherosclerotic lesions. *J Clin Invest*. 1997;100:2028–2034.

18. Dohi Y, Takase H, Sato K, Ueda R. Association among C-reactive protein, oxidative stress, and traditional risk factors in healthy Japanese subjects. *Int J Cardiol*. 2007;115:63–66.

19. Il'yasova D, Ivanova A, Morrow JD, Cesari M, Pahor M. Correlation between two markers of inflammation, serum C-reactive protein and interleukin 6, and indices of oxidative stress in patients with high risk of cardiovascular disease. *Biomarkers*. 2008;13:41–51.

20. Scholz H, Yndestad A, Damas JK, et al. 8-isoprostane increases expression of interleukin-8 in human macrophages through activation of mitogen-activated protein kinases. *Cardiovasc Res*. 2003;59:945–954.

21. Schwedhelm E, Bartling A, Lenzen H, et al. Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study. *Circulation*. 2004;109:843–848.

22. Sciascia S, Roccatello D, Bertero MT, et al. 8-isoprostane, prostaglandin E2, C-reactive protein and serum amyloid A as markers of inflammation and oxidative stress in antiphospholipid syndrome: a pilot study. *Inflamm Res*. 2012;61:809–816.

23. Klein R, Myers CE, Cruickshanks KJ, et al. Markers of inflammation, oxidative stress, and endothelial dysfunction and the 20-year cumulative incidence of early age-related macular degeneration: the Beaver Dam Eye Study. *JAMA Ophthalmol*. 2014;132:446–455.

24. Brantley MA Jr, Osborn MP, Sanders BJ, et al. Plasma biomarkers of oxidative stress and genetic variants in age-related macular degeneration. *Am J Ophthalmol*. 2012;153: 460–467.

25. Wiswedel I. F(2)-isoprostanes: sensitive biomarkers of oxidative stress in vitro and in vivo: a gas chromatography-mass spectrometric approach. *Methods Mol Biol*. 2009;580: 3–16.

26. Liu W, Morrow JD, Yin H. Quantification of F2-isoprostanes as a reliable index of oxidative stress in vivo using gas chromatography-mass spectrometry (GC-MS) method. *Free Radic Biol Med*. 2009;47:1101–1107.

27. Lavanya R, Jeganathan VS, Zheng Y, et al. Methodology of the Singapore Indian Chinese Cohort (SICC) eye study: quantifying ethnic variations in the epidemiology of eye diseases in Asians. *Ophthalmic Epidemiol*. 2009;16:325–336.

28. Cheung CM, Li X, Cheng CY, et al. Prevalence, racial variations, and risk factors of age-related macular degeneration in Singaporean Chinese, Indians, and Malays. *Ophthalmology*. 2014;121:1598–1603.

29. Seshasai S, Liao J, Toh QC, et al. Serum leptin and age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56: 1880–1886.

30. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1995;102:1450–1460.

31. Mori TA, Croft KD, Puddey IB, Beilin LJ. An improved method for the measurement of urinary and plasma F2-isoprostanes using gas chromatography-mass spectrometry. *Anal Biochem*. 1999;268:117–125.

32. Tsai IJ, Croft KD, Mori TA, et al. 20-HETE and F2-isoprostanes in the metabolic syndrome: the effect of weight reduction. *Free Radic Biol Med*. 2009;46:263–270.

33. Barocas DA, Motley S, Cookson MS, et al. Oxidative stress measured by urine F2-isoprostane level is associated with prostate cancer. *J Urol*. 2011;185:2102–2107.

34. Milne GL, Sanchez SC, Musiek ES, Morrow JD. Quantification of F2-isoprostanes as a biomarker of oxidative stress. *Nat Protoc*. 2007;2:221–226.

35. Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med*. 2000;28:505–513.

36. Morrow JD, Roberts LJ II. The isoprostanes. Current knowledge and directions for future research. *Biochem Pharmacol*. 1996;51:1–9.

37. Milne GL, Musiek ES, Morrow JD. F2-isoprostanes as markers of oxidative stress in vivo: an overview. *Biomarkers*. 2005; 10(suppl 1):S10–S23.

38. Montuschi P, Barnes P, Roberts LJ II. Insights into oxidative stress: the isoprostanes. *Curr Med Chem*. 2007;14:703–717.

39. Richelle M, Turini ME, Guidoux R, Tavazzi I, Metairon S, Fay LB. Urinary isoprostane excretion is not confounded by the lipid content of the diet. *FEBS Lett*. 1999;459:259–262.

40. Orban T, Johnson WM, Dong Z, et al. Serum levels of lipid metabolites in age-related macular degeneration. *FASEB J*. 2015;29:4579–4588.

41. Proudfoot J, Barden A, Mori TA, et al. Measurement of urinary F(2)-isoprostanes as markers of in vivo lipid peroxidation-A comparison of enzyme immunoassay with gas chroma-

tography/mass spectrometry. *Anal Biochem*. 1999;272:209–215.

42. Ohashi N, Yoshikawa M. Rapid and sensitive quantification of 8-isoprostaglandin F2alpha in human plasma and urine by liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr B Biomed Sci Appl*. 2000;746:17–24.

43. Pratico D, Barry OP, Lawson JA, et al. IPF2alpha-I: an index of lipid peroxidation in humans. *Proc Natl Acad Sci U S A*. 1998; 95:3449–3454.

44. Wang Z, Ciabattoni G, Creminon C, et al. Immunological characterization of urinary 8-epi-prostaglandin F2 alpha excretion in man. *J Pharmacol Exp Ther*. 1995;275:94–100.

45. LeLeiko RM, Vaccari CS, Sola S, et al. Usefulness of elevations in serum choline and free F2)-isoprostane to predict 30-day cardiovascular outcomes in patients with acute coronary syndrome. *Am J Cardiol*. 2009;104:638–643.

46. Shishehbor MH, Zhang R, Medina H, et al. Systemic elevations of free radical oxidation products of arachidonic acid are associated with angiographic evidence of coronary artery disease. *Free Radic Biol Med*. 2006;41:1678–1683.

47. Devaraj S, Hirany SV, Burk RF, Jialal I. Divergence between LDL oxidative susceptibility and urinary F(2)-isoprostanes as measures of oxidative stress in type 2 diabetes. *Clin Chem*. 2001;47:1974–1979.

48. Keaney JF Jr, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol*. 2003;23: 434–439.

49. Morrow JD, Roberts LJ. The isoprostanes: their role as an index of oxidant stress status in human pulmonary disease. *Am J Respir Crit Care Med*. 2002;166(pt 2):S25–S30.

50. Il'yasova D, Morrow JD, Wagenknecht LE. Urinary F2-isoprostanes are not associated with increased risk of type 2 diabetes. *Obes Res*. 2005;13:1638–1644.

51. Vassalle C, Botto N, Andreassi MG, Berti S, Biagini A. Evidence for enhanced 8-isoprostane plasma levels, as index of oxidative stress in vivo, in patients with coronary artery disease. *Coron Artery Dis*. 2003;14:213–218.

52. Danulescu R, Costin D. Use of blood markers in early diagnosis of oxidative stress in age related macular degeneration. *Rev Med Chir Soc Med Nat Iasi*. 2012;116:1136–1142.

53. Ito T, Nakano M, Yamamoto Y, Hiramitsu T, Mizuno Y. Hemoglobin-induced lipid peroxidation in the retina: a possible mechanism for macular degeneration. *Arch Biochem Biophys*. 1995;316:864–872.

54. Totan Y, Cekic O, Borazan M, Uz E, Sogut S, Akyol O. Plasma malondialdehyde and nitric oxide levels in age related macular degeneration. *Br J Ophthalmol*. 2001;85:1426–1428.

55. Totan Y, Yagci R, Bardak Y, et al. Oxidative macromolecular damage in age-related macular degeneration. *Curr Eye Res*. 2009;34:1089–1093.

56. Fessel JP, Jackson RL. Isofurans: novel products of lipid peroxidation that define the occurrence of oxidant injury in settings of elevated oxygen tension. *Antioxid Redox Signal*. 2005;7:202–209.

57. Lee R, Margaritis M, Channon KM, Antoniades C. Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations. *Curr Med Chem*. 2012;19: 2504–2520.

58. Longmire AW, Swift LL, Roberts LJ II, Awad JA, Burk RF, Morrow JD. Effect of oxygen tension on the generation of F2-isoprostanes and malondialdehyde in peroxidizing rat liver microsomes. *Biochem Pharmacol*. 1994;47:1173–1177.