

Autofluorescence of Skin Advanced Glycation End Products as a Risk Factor for Open Angle Glaucoma: The ALIENOR Study

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Submitted: May 29, 2017

Accepted: December 1, 2017

Citation: Schweitzer C, Cougnard-Gregoire A, Rigalleau V, et al. Autofluorescence of skin advanced glycation end products as a risk factor for open angle glaucoma: the ALIENOR study. *Invest Ophthalmol Vis Sci*. 2018;59:75-84. DOI:10.1167/iov.17-22316

PURPOSE. To analyze the association between skin autofluorescence (sAF), estimating tissue accumulation of advanced glycation end-products (AGEs), and open angle glaucoma (OAG) in an elderly population.

METHODS. The Antioxydants, Lipides Essentiels, Nutrition and maladies OculaiRes (ALIENOR) study is an on-going epidemiologic population-based study on age-related eye diseases. In 2009 to 2010, 624 subjects, aged 74 years or older, were recruited. All subjects underwent a complete eye examination, including optic disc color photography and spectral-domain optical coherence tomography (SD-OCT) examination. Sociodemographic and medical history data were collected using standardized questionnaires. Glaucoma diagnosis was made using optic nerve head retinophotography and International Society for Epidemiologic and Geographical Ophthalmology criteria. sAF was measured with a noninvasive autofluorescence reader in 467 subjects.

RESULTS. Of subjects, 455 had complete data, 424 were classified as controls, and 31 classified as OAG. Mean age was 82.3 ± 4.3 years, mean and median sAF were 2.8 ± 0.7 and 2.7 arbitrary units (AU), respectively. In a multivariate analysis, higher sAF values (≥ 2.7 AU) were associated with OAG (odds ratio [OR] = 2.28, 95% Confidence Interval [CI]: 1.03; 5.04). Other variables significantly associated with OAG were age (OR = 1.10, 95%CI: 1.00; 1.21), glaucoma family history (OR = 2.83, 95%CI: 1.14; 7.01) and smoking (1-20 pack-years [OR = 3.31, 95%CI: 1.18; 9.26]; ≥ 20 pack-years [OR = 3.85, 95%CI: 1.42; 10.46]).

CONCLUSIONS. Higher level of sAF, which may act as a long-term biomarker of metabolic memory, and smoking are independently associated with an increased risk of glaucoma. Long-term accumulation of AGEs, a marker of oxidative stress, could play a role in the pathogenesis of glaucomatous chronic optic neuropathy.

Keywords: glaucoma, advanced glycation end-products, AGEs, skin autofluorescence, glaucoma risk factors

Glaucoma is a neurodegenerative disease defined by a progressive loss of optic nerve axons and retinal ganglion cells resulting in a characteristic enlargement of the optic nerve head cup and associated visual field defects.¹ It remains the most common cause of irreversible blindness worldwide by affecting more than 60.5 million people in 2010 and with an estimated increasing prevalence up to 111.8 million in 2040.^{2,3} Although IOP is an important contributing factor in neuroretinal rim thinning observed in POAG, the pathophysiologic process leading to a progressive degeneration of retinal ganglion cells may be more complex. Local microvascular blood flow dysfunction, neurotrophic factors, modified biomechanical response of lamina cribrosa to an increase in IOP, or oxidative stress mechanisms can be associated.⁴⁻⁷

Advanced glycation end-products (AGEs) represent a group of various proteins, lipids, or nucleic acids that become glycosylated as a result of a chronic exposure to glucose or other

reducing sugars and have been described as one component of oxidative stress generation in tissues. AGEs result from a nonenzymatic reaction between reducing sugars and amino groups of proteins, lipids, or nucleic acids; thus, giving rise to a Schiff base and glycation of these molecules. This early modification is reversible but glycosylated molecules slowly rearrange by rehydration, fragmentation, or cyclization reaction in irreversible Amadori products leading to the formation of AGEs, which accumulate in long-lived proteins, such as collagen.⁸⁻¹⁰ Formation and accumulation of AGEs are not only associated with diabetes mellitus, characterized by a higher concentration of carbonyl substrates in tissue, but are also generated in physiologic conditions, and are thus involved in the natural ageing process.⁸ Furthermore, they can also influence the incidence or progression of several age-related chronic diseases, such as chronic kidney disease, cardiovascular disease, heart failure, and dementia or other neurodegenerative



diseases.¹¹⁻¹⁶ Additionally, some experimental studies have demonstrated that AGEs accumulate in ocular tissues and may be involved in the pathophysiologic process of eye diseases, such as cataract, diabetic retinopathy, or AMD.¹⁷⁻¹⁹

Accumulation of AGEs can be estimated with skin autofluorescence (sAF) measurements using a noninvasive device.^{20,21} This method uses fluorescence properties of AGEs and has been validated with specific AGEs analyzed in skin biopsies obtained from the same site and showing a high correlation between sAF and skin AGEs. Additionally, blood or urine circulating AGEs measurements do not necessarily reflect their tissue level and sAF has been proposed as a better biomarker of cumulative metabolic stress by evaluating AGEs accumulation in tissues and particularly in skin collagen having a mean half-life of 15 years.^{10,21} Several studies have also demonstrated an association between higher level of sAF and some chronic diseases as diabetes mellitus and associated complications, chronic kidney disease, some cardiovascular diseases, arterial stiffness, and peripheral arterial disease or some neurologic diseases.²²⁻³² Furthermore, as sAF reflects tissue accumulation of AGEs in long-lived proteins, sAF has also been described as a marker of metabolic memory, defined as the cumulative effect of metabolic activity over a long period, and a strong predictive factor of cardiovascular or end-stage renal disease complications.³³⁻³⁷

Many structures of the eye, including conventional and uveoscleral aqueous outflow pathways or some structures of the optic nerve head, are mainly composed of collagen or other extracellular matrix molecules with a slow turnover time. Whereas glaucoma is an age-related chronic neurodegenerative disease and oxidative stress may contribute to its pathophysiologic process, the association between AGEs and glaucoma received little attention.^{5,38-40} Hence, only a few experimental studies have reported a higher accumulation of AGEs in the optic nerve head, in the retinal ganglion cell layer, in the glia and in the retinal distribution of Müller cells or around blood vessels, thus suggesting a potential role in the pathogenesis of glaucoma.^{41,42} However, to date, AGEs have never been evaluated *in vivo* and in a general population as a potential risk factor for glaucoma.

We therefore investigated the associations between sAF and glaucoma in a population-based study of elderly subjects having a long history of accumulation of AGEs in skin collagen and in which metabolic activity had been recorded over a long period.

MATERIALS AND METHODS

The Antioxydants, Lipides Essentiels, Nutrition and maladies OculaiRes (ALIENOR) Study is an on-going population-based epidemiologic study on age-related eye diseases performed at the University Hospital of Bordeaux. The complete methodology of this study was published previously.⁴³⁻⁴⁵

Study Population

The main objective of the ALIENOR study was to analyze the associations of age-related eye diseases with nutritional, environmental, and vascular factors, or gene polymorphisms.⁴³ Participants were recruited from an on-going population-based study analyzing risk factors for dementia, the Three-City (3C) Study.⁴⁴ In 1999 to 2001, the 3C study randomly recruited participants aged 65 years or older on electoral rolls from three French cities (Bordeaux, Dijon, and Montpellier). Participants were individually contacted and 9294 participants were recruited, including 2104 from Bordeaux. Since then, all participants were followed for approximately every 2 years. The ALIENOR Study consists of eye examinations offered to all

participants of the 3C cohort in Bordeaux since the third follow-up (2006-2008). Among the 1450 participants enrolled at this follow-up, 963 (66.4%) accepted the ALIENOR study's baseline eye examination. Delcourt et al.⁴³ published demographic characteristics of participants included at baseline, and their comparison with nonparticipants.

At the fourth follow-up (2009-2010), a measurement of sAF was included. Among the 963 participants, 624 (59 deaths, 265 refusals, 15 moves) participated in the ALIENOR study's second eye examination. They were aged 74 years or more. At this follow-up, representativeness of our population sample has been tested using the database of the National Institute for Statistics and Economics Studies INSEE (Institut National de la Statistique et des Etudes Economiques) for the Bordeaux area. There was no significant difference in age or sex distribution, residency location, and loneliness index between our population sample and data of Bordeaux area population.⁴⁶

This research was approved by the Ethical committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III) in May 2006 and followed the declaration of Helsinki's tenets. All participants provided informed written consent for enrollment in the study.

Eye Examination

All participants underwent an ophthalmologic examination including a best-corrected visual acuity measurement, a tear break-up time test, a central corneal thickness measurement using Pachpen (Accutome, Inc., Malvern, PA, USA), after local anesthesia with oxybuprocaine eye drops, and an IOP measurement using a noncontact tonometer (KT 800; Kowa, Aichi Japan). Nonstereoscopic color photography of the macula and the optic disc were performed using a nonmydriatic retinograph (TRC NW6S; Topcon, Inc., Tokyo, Japan). Macula and optic disc measurements were assessed by spectral-domain optical coherence tomography (SD-OCT; Spectralis; Heidelberg Engineering, Germany). All measurements were performed between 2 PM and 6 PM, including IOP measurements.

Retinal photographs were interpreted in duplicate by two specially trained technicians. Inconsistencies between the two interpretations were adjudicated by a glaucoma specialist for classification of glaucoma and by a retina specialist for classification of AMD or other retinal diseases. All cases of glaucoma and retinal disease were reviewed and confirmed by specialists.

Clinical and Lifestyle Determinants

All clinical and lifestyle determinants were collected at baseline (1999-2001) and at the fourth follow-up (2009-2010) of the 3C study. Sociodemographic, lifestyle, and medical history data were collected during a face-to-face interview using a standardized questionnaire administered by a trained psychologist or nurse. It included age, sex, educational level, smoking, hypertension (systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or antihypertensive treatment), body mass index (BMI, [weight (kg)/height² (m²)] and an inventory of all drugs used during the preceding month. Pack-years of smoking were calculated as numbers of smoking years \times (mean number of cigarettes per day) \div 20.

Plasma glucose and lipids were determined from fasting blood samples performed at baseline and in 2010. Diabetes was defined as fasting blood glucose greater than or equal to 7.0 mM or current use of antidiabetic treatment.

Estimated glomerular filtration rate (eGFR) derived from serum creatinine based on modification of diet in renal disease equation and chronic kidney disease was defined as an eGFR inferior to 60 mL/min/1.73 m².⁴⁷

Glaucoma Diagnosis

The methods chosen to diagnose glaucoma in the ALIENOR study were published in details and used the classification provided by Foster et al.^{43,45,48} Briefly, the vertical cup:disc ratio (VCDR) and the minimal rim:disc ratio were estimated from optic disc photographs. Participants were classified as suspects for glaucoma when, in at least one eye, VCDR was greater than or equal to 0.65 and/or minimal rim:disc ratio less than or equal to 0.1 or when an asymmetry of VCDR between eyes greater than or equal to 0.2 was observed. For glaucoma suspect cases, an additional eye examination was performed by a glaucoma specialist including a white-on-white visual field test (Octopus 101; Haag-Streit, Koniz, Switzerland), an examination of the optic disc by slit-lamp and of the irido-corneal angle by gonioscopy, and an IOP measurement.

Glaucoma was defined according to three levels of evidence and all glaucoma cases were classified according to category 1 criteria, except one case with category 2 criteria. The final classification was reviewed and confirmed by a glaucoma specialist. All cases of glaucoma were classified as open angle glaucoma (OAG).

Skin Autofluorescence Measurement

In 2009 to 2010, sAF measurements were performed using the AGE Reader (DiagnOptics Technologies B.V., Groningen, the Netherlands) and were expressed in arbitrary units (AU). This noninvasive device estimates AGEs accumulation by using their fluorescence properties. A skin surface of approximately 4 cm² guarded against surrounding light is illuminated on the forearm with an excitation light source. Autofluorescence is calculated as the ratio of the emission light intensity per nanometer (wavelength 420–600 nm) and the reflected excitation light intensity per nanometer (wavelength 300–420 nm) measured with a spectrometer. All measurements were performed on a normal skin site of the volar side of the forearm and without visible vessels, scars, abnormal pigmentation, or lichenification of the skin and without any use of skin creams or products. As recommended by the manufacturer, all measurements were also performed in a dark environment and at room temperature.

Meerwaldt et al.²¹ reported a high correlation between sAF measurements and specific AGEs analyzed in skin biopsies taken from the same site on the volar side of the forearm. They also reported that sAF values obtained from the AGE reader were reproducible with intraindividual measurements performed on one day or with seasonal variation showing Altman error percentages of 5.03% and 5.87%, respectively.²¹ Furthermore, reproducibility of sAF measurements was evaluated in other studies with intraobserver and interobserver coefficients of variations ranging from 6.2% to 7% and from 3.9% to 8%, respectively.^{49,50} Finally, sAF measurements were reliable for a wide range of native skin colors including Fitzpatrick skin phototypes from I to IV.⁵¹

Additionally, sAF evaluation was performed in triplicate and the mean of the three values was recorded. Participants with Fitzpatrick skin phototype V and VI could not be evaluated by the AGE reader due to darker skin pigmentation causing ultraviolet reflectance below 10%. Fitzpatrick skin phototype classification is a standard grading scale on skin pigmentation and skin response to sun exposure, and ranges from I (very pale skin, always burns) to V (dark brown skin, very rarely burns), and VI (darkest brown skin, never burns).

Median sAF value was calculated and participants were allocated in two groups, those with a sAF value lower than the median and those with a sAF value superior than or equal to the median.

Statistical Analysis

Results were presented using means \pm SDs for continuous variables, and *n* and percentage for noncontinuous variables.

Participants included in the analysis were compared with nonincluded participants for demographic characteristics to assess potential selection bias in the final statistical analysis.

Firstly, associations of demographic and medical characteristics (independent parameters) with glaucoma (dependent parameter) were examined with logistic generalized estimating equations (GEE) models, adjusted for age and sex. GEE models allow taking into account data from both eyes and their intraindividual correlations (using an unstructured correlation matrix).

Secondly, all variables, which were significantly associated with glaucoma in the age- and sex-adjusted models at *P* less than 0.15 were included in a multivariate model. In addition, age and sex, were forced into all analyses. The associations are presented as odds ratios (OR) and 95% confidence intervals (CI). All statistical tests were two-sided and *P* values less than 0.05 were considered as significant. All statistical analyses were performed using SAS version 9.3 (procedure GENMOD for the GEE analysis; SAS Institute, Inc., Cary, NC, USA).

RESULTS

There were 624 participants evaluated at the second follow-up of the ALIENOR study. Of participants, 169 were excluded from the analysis: 157 had missing sAF measurements or a Fitzpatrick skin phototype V or VI, and 12 could not have a diagnosis of glaucoma due to poor quality of the retinophotography and optic disc measurements could not be performed. Finally, 455 participants were included for analysis.

Table 1 shows comparison of demographic characteristics between included and nonincluded participants. There was no significant difference in the distribution of sex or mean age, family history of glaucoma, IOP, VCDR, medical characteristics, and smoking between the 2 groups. Prevalence of glaucoma was similar in the two groups (*P* = 0.64). At eye examination visit performed for all glaucoma suspect participants, the mean defect of the visual field test was 8.91 \pm 5.50 dB (minimum–maximum [min–max]: 0.58–24.30) for participants with glaucoma.

Demographic Characteristics and sAF Comparison Between Glaucoma and Control Groups

Table 2 shows the associations of demographic characteristics, family history and sAF with glaucoma using GEE models adjusted for age and sex. Of participants, 424 were included in the control group and 31 in the glaucoma group. The mean age was 82.32 \pm 4.23 years and was not significantly different between the group with and the group without glaucoma. Male subjects represented 38.24% (*n* = 174) of the sample and there was no significant difference between the two groups.

Mean and median sAF values were 2.08 \pm 0.7 and 2.7 AU (min–max: 1.2–5.1), respectively. sAF values superior than or equal to 2.7 AU are significantly associated with a 2.71-increased risk of having glaucoma (odds ratio [OR] = 2.71; 95% confidence interval [CI]: 1.23; 5.93). The Figure illustrates the distribution of sAF values in the group with and in the group without glaucoma. We observed a symmetry of distribution of sAF values with the median line in the two groups and the glaucoma group exhibited higher sAF values particularly for first, second, and third interquartiles of the boxplot.

Family history of glaucoma was associated with a significant increased risk of having glaucoma with an OR of 2.87 (95%CI: 1.18; 7.01).

TABLE 1. Demographic Characteristics Between Included and Nonincluded Participants

	Total (N = 624)	Included Participants (N = 455)	Nonincluded Participants (N = 169)	P Value
Age, y (SD)	82.19 (4.25)	82.32 (4.23)	81.82 (4.32)	0.19
Sex: male subjects (%)	233 (31.34)	174 (38.24)	59 (34.91)	0.45
Glaucoma, n (%) (n = 606)	43 (7.1)	31 (6.8)	12 (7.95)	0.64
Family history of glaucoma, n (%) (n = 622)	83 (13.34)	57 (12.56)	26 (15.48)	0.34
Vertical cup disc ratio (SD) (n = 613)	0.43 (0.09)	0.43 (0.08)	0.42 (0.09)	0.14
Minimal rim ratio (SD) (n = 613)	0.21 (0.04)	0.21 (0.04)	0.21 (0.05)	0.63
IOP, mm Hg (SD) (n = 617)	13.67 (2.18)	13.68 (2.21)	13.69 (2.08)	0.90
Hypertension* (n = 603)	508 (84.25)	371 (84.70)	137 (83.03)	0.62
Chronic kidney disease† (n = 506)	208 (41.11)	149 (40.49)	59 (42.75)	0.65
HbA1c ≥7.5% (n = 501)	15 (2.99)	11 (3.01)	4 (2.94)	1.00
Diabetes mellitus‡ (n = 519)	86 (16.57)	68 (17.99)	18 (12.77)	0.16
Fasting blood glucose ≥7.0 mM (n = 495)	34 (6.87)	25 (6.93)	9 (6.72)	0.94
Smoking, pack/y (n = 619) no	400 (64.62)	294 (65.19)	106 (63.10)	0.28
1–20	113 (18.26)	76 (16.85)	37 (22.02)	
≥20	106 (17.12)	81 (17.96)	25 (14.88)	

* Systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or antihypertensive medication.

† eGFR <60 mL/min/1.73 m².

‡ Diabetes: fasting plasma glucose ≥7 mM and/or use of antidiabetic medication.

Finally, smokers had a significant higher risk to have glaucoma than nonsmokers (OR = 2.99 [95%CI: 1.19; 7.58] for 1 to 20 pack-years exposure and OR = 3.23 [95%CI: 1.27; 8.23] for >20 pack-years exposure).

Medical Characteristics at Baseline (1999–2001) and at Ophthalmologic Examination (2009–2010) Between Glaucoma and Controls Groups

Table 3 shows medical characteristics at baseline of the 3C Study (1999–2001) and at ophthalmologic examination (2009–2010) using GEE models adjusted for age and sex.

Hypertension was not significantly associated with an increased risk of glaucoma whether at baseline or at ophthalmologic examination (OR = 0.65 [95%CI: 0.29; 1.42] and OR = 0.64 [95%CI: 0.25; 1.65], respectively).

We did not observe any significant increased risk of glaucoma for subjects with chronic kidney disease whether at baseline or at ophthalmologic examination (OR = 1.78 [95%CI: 0.67; 4.71] and OR = 1.71 [95%CI: 0.66; 4.43], respectively).

Finally, the glycemic status defined either by a diagnostic of diabetes mellitus or glycated hemoglobin (HbA1c) greater than or equal to 7.5 mM or blood glucose greater than or equal to 7.0 mM was not associated with an increased risk of glaucoma either at baseline or during the follow-up.

Multivariate Models of Associations of Glaucoma With Risk Factors

Table 4 shows the multivariate model of determinants associated with glaucoma using GEE models.

TABLE 2. Associations of Demographic Characteristics, Family History, and Skin Autofluorescence With Glaucoma in the ALIENOR Study

	Total (N = 455)	Control Group (N = 424)	Glaucoma Group (N = 31)	OR*	95%CI*	P Value*
Age, y (SD)	82.32 (4.23)	82.26 (4.22)	83.14 (4.35)	1.08	0.99; 1.18	0.08
Sex: male	174 (38.24)	161 (37.97)	13 (41.94)	1.67	0.77; 3.67	0.21
Family history of glaucoma (n = 454)	57 (12.56)	49 (11.58)	8 (25.81)	2.87	1.18; 7.01	0.02
Vertical cup disc ratio						
Mean (SD)	0.43 (0.08)	0.42 (0.07)	0.61 (0.10)	7.56	4.76; 12.00	<0.0001
≥0.65	10 (2.20)	0 (0.00)	10 (100.00)	NA	NA	
≥0.70	7 (1.54)	0 (0.00)	7 (100.00)	NA	NA	
Minimal rim width						
Mean (SD)	0.21 (0.04)	0.21 (0.04)	0.14 (0.05)	0.13	0.06; 0.28	<0.0001
≤0.1	9 (1.98)	0 (0.00)	9 (100.00)	NA	NA	
Smoking, pack/y (n = 451)						
No	294 (65.19)	280 (66.67)	14 (45.16)	1.00 (Ref)		
1–20	76 (16.85)	68 (16.19)	8 (25.81)	2.99	1.19; 7.58	0.02
≥20	81 (17.96)	72 (17.14)	9 (29.03)	3.23	1.27; 8.23	0.01
sAF ≥2.7, AU	243 (53.41)	221 (52.12)	22 (70.97)	2.71	1.23; 5.93	0.01

Ref, reference; NA, not applicable.

Results in bold are statistically significant ($P < 0.05$).

* Estimated using GEE logistic regression adjusted for age and sex.

TABLE 3. Associations of Medical Characteristics With Glaucoma at Baseline and at Ophthalmologic Examination

	Total (N = 455)	Control Group (N = 424)	Glaucoma Group (N = 31)	OR*	95%CI*	P Value*
Medical characteristics at baseline (1999–2001)						
Hypertension†	335 (73.63)	314 (74.06)	21 (67.74)	0.65	0.29; 1.42	0.28
Chronic kidney disease‡ (n = 423)	57 (13.48)	52 (13.20)	5 (17.24)	1.78	0.67; 4.71	0.25
HbA1c ≥7.5% (n = 104)	12 (11.54)	10 (10.42)	2 (25.00)	2.74	0.49; 15.40	0.25
Diabetes mellitus§ (n = 422)	32 (7.58)	29 (7.38)	3 (10.34)	1.41	0.40; 5.02	0.60
Fasting blood glucose ≥7.0 mM (n = 420)	16 (3.81)	13 (3.32)	3 (10.34)	3.28	0.86; 12.48	0.08
Medical characteristics at ophthalmologic examination (2009–2010)						
Hypertension† (n = 438)	371 (84.70)	347 (85.05)	24 (80.00)	0.64	0.25; 1.65	0.35
Chronic kidney disease‡ (n = 368)	149 (40.49)	137 (39.60)	12 (54.55)	1.71	0.66; 4.43	0.27
HbA1c ≥7.5% (n = 365)	11 (3.01)	10 (2.91)	1 (4.76)	1.68	0.20; 14.23	0.63
Diabetes mellitus§ (n = 378)	68 (17.99)	63 (17.75)	5 (21.74)	1.32	0.47; 3.68	0.59
Fasting blood glucose ≥7.0 mM (n = 361)	25 (6.93)	24 (7.04)	1 (5.00)	0.59	0.08; 4.52	0.61

Results in bold are statistically significant ($P < 0.05$)

* Estimated using GEE logistic regression models adjusted for age and sex.

† Systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or antihypertensive medication.

‡ eGFR < 60 mL/min/1.73 m².

§ Diabetes: fasting plasma glucose ≥7 mM and/or use of antidiabetic medication.

After adjustment with age, sex, family history of glaucoma, smoking, chronic kidney disease, and blood glucose greater than or equal to 7.0 mM, sAF values greater than or equal to 2.7 AU remained strongly associated with glaucoma and an odds ratio of 2.28 (95%CI: 1.03; 5.04).

Age and family history of glaucoma were significantly associated with glaucoma (OR = 1.1 [95%CI: 1.00; 1.21] and OR = 2.83 [95%CI: 1.14; 7.01], respectively), whereas the association with sex was not statistically significant (OR = 0.97 [95%CI: 0.42; 2.22]).

In this model, association between smoking and glaucoma remained significant with an increased risk up to 3.31 (95%CI: 1.18; 9.26) for the group 1 to 20 pack-years exposure and to 3.85 (95%CI:1.42; 10.46) for the group of 20 or more pack-years exposure.

Finally, subjects with blood glucose greater than or equal to 7.0 mM tended to have an increased risk of having glaucoma with an OR of 2.82 (95%CI: 0.81; 9.84) without reaching statistical significance.

DISCUSSION

The current study is the first population-based study showing that higher levels of sAF are independently associated with

TABLE 4. Multivariate Models of Associations of Glaucoma With Risk Factors (N = 800 eyes)

	OR*	95%CI*	P Value*
Age	1.10	1.00; 1.21	0.04
Sex	0.97	0.42; 2.22	0.94
Family history of glaucoma	2.83	1.14; 7.01	0.03
Smoking, pack/y			
No	Ref	Ref	
1–20	3.31	1.18; 9.26	0.02
≥20	3.85	1.42; 10.46	0.008
sAF ≥2.7 AU	2.28	1.03; 5.04	0.04
Chronic kidney disease† at baseline	1.24	0.45; 3.39	0.67
Fasting blood glucose at baseline ≥7.0 mM	2.82	0.81; 9.84	0.10

Results in bold are statistically significant ($P < 0.05$).

* Estimated using GEE regression logistic models.

† eGFR < 60 mL/min/1.73 m².

glaucoma in a large and unselected population of elderly people. By reflecting accumulation of AGEs in long-lived proteins of the skin, this result also suggests that long-term accumulation of AGEs may contribute to the pathogenic process of glaucomatous neuropathy.

Several studies have also demonstrated that increased sAF values increased the incidence or progression of some age-related chronic diseases.^{22–32} In diabetic patients, sAF was described as a marker of chronic hyperglycemia for a longer lifetime period than HbA1c and was also independently associated with micro- and macrovascular complications.^{27,30,52} In nondiabetic patients, increased sAF was also associated with increased arterial stiffness, peripheral arterial disease, and coronary artery calcifications.^{25,25,37} sAF was also associated with brain atrophy using magnetic resonance imaging, impaired cognitive functions, or some neurologic diseases.^{26,29,32} Finally and more interestingly, sAF was proposed as a long-term metabolic marker and a predictive factor of diseases or complications.^{35–37} Indeed, we showed that sAF levels reflect glycemic and renal status 10 years before, and other prospective studies found that sAF predicts 5-year amputation in patients with peripheral arterial disease and is associated with a higher risk of mortality in patients with

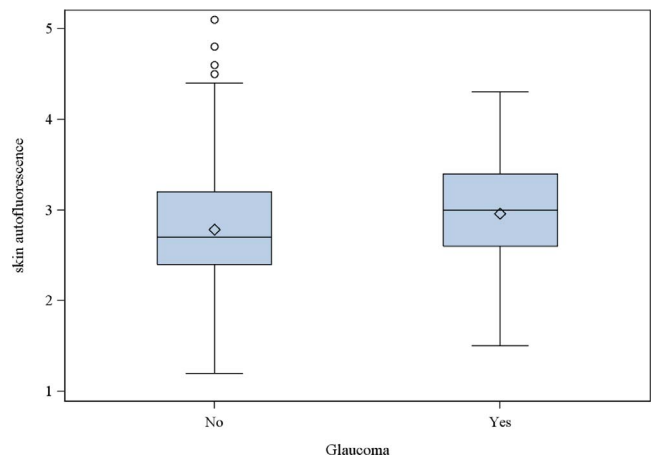


FIGURE. Boxplot showing the distribution of skin autofluorescence values in the group of participants without glaucoma and the group of participants with glaucoma.

end-stage renal disease.^{33,34,37} All these findings emphasize that long-term accumulation of AGEs in skin collagen, as measured with sAF, is associated with the ageing process and could also lead to several age-related chronic diseases for increased accumulation of AGEs.

Several factors influence the progressive accumulation of AGEs in tissues with increasing age, including blood concentration of carbonyl substrates, the glycation reactivity of some carbonyl intermediates, the permeability of some cells, and the ability of the biological system to degrade AGEs or the rate of glomerular filtration.⁹ But one of the most important factors is probably the turnover of proteins with a higher accumulation rate of AGEs observed in long-lived proteins as in crystalline lens, extracellular matrix, or collagen of tissues; thus, potentially leading to their modified structure and function or oxidative stress reaction and tissular damage.^{10,53,54} Indeed, some AGEs are able to crosslink with adjacent proteins of the extracellular matrix as collagen IV or elastin and can stiffen elastic tissues especially vascular tissue and can also affect cell adhesion.⁵⁵ Furthermore, AGEs could also be responsible for intracellular glycation of proteins leading to modified intracellular enzymatic pathways or altered proteins of mitochondrial chain transport.^{56,57} Finally, AGEs can interact with specific protein binding of cellular membranes and can activate intracellular signal pathways inducing the expression of proinflammatory cytokines and chemokines, and thus oxidative stress.^{58,59} An important extracellular AGE receptor is receptor for advanced glycation end products (RAGE) and is expressed at the surface of different tissues and cell types as smooth muscle cells, endothelial cells, macrophages, and monocytes or neuronal and microglial cells.⁶⁰ Through an activation of the nicotinamide adenine dinucleotide phosphate oxidase system, the interaction of AGEs and RAGE could enhance the generation of reactive oxygen species (ROS), which increases oxidative stress, the formation of AGEs and in turn sustains AGEs-RAGE interaction thus creating a vicious circle leading to a chronic oxidative stress in tissues.⁶¹

Although glaucoma is a chronic age-related disease, the association between AGEs and glaucoma has scarcely been analyzed. To our knowledge, our study is the first to show that higher level of skin AGEs, as measured with sAF, is independently associated with glaucoma in a large and unselected population of elderly people with a long history of metabolic memory. Interestingly, Hondur et al.⁴⁰ reported that the level of AGEs was significantly higher in blood and aqueous humor samples of glaucoma subjects than in samples of age-matched nonglaucoma subjects. Furthermore, the level of AGEs of glaucoma subjects was more than 3 times higher in aqueous humor sample than in blood sample; thus, supporting a potential intraocular generation of oxidative stress in glaucoma subjects. Himori et al.³⁸ recently published a study analyzing the association between sAF and mean deviation values of the visual field test in a Japanese population with OAG and including essentially normal-tension glaucoma cases. They observed that sAF was negatively correlated to mean deviation values of the visual field test; thus, suggesting a role of oxidative stress in the severity of glaucoma and visual field loss. Our results are also in accordance with previously published studies evaluating *in vitro* the accumulation of AGEs on the optic nerve head or the retina from human donors. Interestingly, Tezel et al.⁴² observed that accumulation of AGEs and RAGE were more increased in older rather than younger eyes and higher in glaucomatous eyes than in nonglaucomatous eyes using immunofluorescence labeling on histologic sections of eyes from age-matched glaucomatous and nonglaucomatous donors. Furthermore, accumulation of AGEs and RAGE was particularly observed on lamina cribrosa plates of the optic nerve head, on the retinal ganglion cell layer, in the

glia, in the retinal distribution of Müller cells, and around blood vessels. Furthermore, in previous studies, while Albon et al.⁶² observed a linear accumulation of AGEs with increasing age, Amano et al.⁴¹ showed a higher accumulation of AGEs in lamina cribrosa plates and around optic nerve head vessels. Although the exact pathophysiologic process associating AGEs and glaucoma is unclear, the higher extracellular accumulation of AGEs in lamina cribrosa plates observed in glaucomatous patients may change its biomechanical compliance and consequently may in part increase the stress on optic nerve axons leading to the characteristic glaucomatous optic nerve head enlargement. Indeed, some studies postulate that biomechanics of the lamina cribrosa and the peripapillary sclera, especially stiffening, may play a significant role in glaucomatous neuropathy by providing significant deformation, stress, and strain on surrounding tissues and structures when pressure is applied.^{6,7,63} Hence, a chronic tissue deformation could induce axoplasmic flow disruption, altered optic nerve head blood flow and finally could lead to connective tissue damage and remodeling. Finally, this chronic injury of surrounding tissues of the lamina cribrosa under the effect of IOP could lead to a progressive loss of optic nerve head axons and glaucomatous neuropathy. Tezel et al.⁴² also observed higher accumulation of AGEs associated with an upregulation of RAGE around the optic nerve head and in the retinal ganglion cell layer. The authors suggested that in addition to direct cytotoxic effects of intracellular and extracellular AGEs, RAGE may activate specific intracellular pathways leading to oxidative stress, glial activation, and ganglion cell dysfunction.⁴² Finally, Takeuchi et al.⁶⁴ demonstrated a direct cytotoxicity of AGEs on neuronal cells that also could induce inflammation, oxidative stress, and generate free radicals. Although the exact association between AGEs and glaucoma would need further evaluations, the results of our study—performed *in vivo*—supports all this experimental evidence suggesting an influence of AGEs in the pathophysiologic process of glaucoma.

Moreover, by estimating the long-term accumulation of AGEs in long-lived proteins, sAF measurements may act as a potential additional biomarker of oxidative stress activity in glaucoma onset or progression. Unlike blood or aqueous humor samples that measure circulating AGEs levels on a short period of time, sAF measurements enable a noninvasive estimation of AGEs accumulation over a long period of time, and could thus represent a better biomarker of cumulative metabolic stress and a potential additional predictive factor for glaucoma. However, although sAF was significantly and independently associated with glaucoma in the present study, our results would need further evaluation of the clinical validity of sAF measurements as a risk factor for glaucoma. Although population-based study are designed to limit the risk of participant selection bias that could lead to an overestimation of results, the number of potential confounding factors in a general population of elderly people, as general diseases, increases and could limit the level of significance of associations in a multivariate model. Thus, an appropriate case-control study including an equivalent number of participants with and without glaucoma, and matched on age and potential confounding factors as general chronic diseases would provide additional information on the discriminating performances of sAF for glaucoma. Additionally, a longer follow-up of our cohort of participants is needed to confirm this association. Finally, the influence of cumulative metabolic stress in glaucoma progression remains unclear and a prospective study with a longitudinal follow-up of glaucoma participants with sAF measurements at baseline could provide valuable clinical information on the role of oxidative stress in glaucoma progression.

In the multivariate analysis, we also observed a strong association between smoking and glaucoma. There was a tendency toward a dose-dependent relationship with a stronger association for the group of 20 or higher pack-years exposure than for the group of 1 to 20 pack-years exposure. Although smoking is an important risk factor for several diseases, the association with glaucoma remains controversial.^{65,66} In a meta-analysis evaluating the association between smoking and glaucoma, Zhou et al.⁶⁵ did not find any significant pooled adjusted relative risk between current smoker and former smoker or between current smoker and never smoker. Inconsistency in determining association between smoking and glaucoma results from limitations mainly related to selective survival biases of the population sample with a lower life expectancy associated to the smoker group. As glaucoma is an age-related eye disease with an increasing incidence with age, the survival difference between participants with smoking exposure and participants without smoking exposure would tend to underestimate a potential association between glaucoma and smoking. Our population sample of elderly people had a long history of smoking exposure and, despite the high mean age of our population, still 34.5% ($n = 157$) of the included participants reported a smoking exposure. Hence, we hypothesize that our study could be less affected by the selective survival bias associated with smoking or that glaucoma could be associated to longer smoking exposure. These findings would need further exploration to be confirmed.

Although we have demonstrated that higher levels of sAF are independently associated with glaucoma in a large and unselected population of elderly people in which metabolic activity had been recorded over a long period, our study may have some limitations that need to be considered. The first limitation is related to the cross-sectional nature of our study that could limit the causality relationship between higher levels of sAF and glaucoma. However, as suggested in previous studies, sAF reflects AGEs accumulation in skin collagen, and could therefore be considered as a long-term metabolic marker or a predictive factor of disease or complications.³³⁻³⁷ Hence, a long-term follow-up of our population of participants is needed to strengthen the association between higher levels of skin AGEs and glaucoma, and could also assess the role of sAF as a potential long-term biomarker for incidence or progression of glaucoma.

Another potential limitation to our results is related to the representativeness of our population sample of elderly people. Indeed, we could have selective survival biases related to the elevated mean age of our sample, which tends to be healthier. However, at this follow-up, the representativeness of our population sample has been compared with the database of INSEE for Bordeaux area population and there was no significant difference in age, sex distribution, residency location, and loneliness index.⁴⁶ Furthermore, demographic characteristics were not significantly different between included and nonincluded participants of the study (Table 1) and the prevalence of glaucoma we found was similar to those observed in other studies.⁶⁷⁻⁶⁹ Hence, our findings need to be replicated in another population sample to confirm all these associations.

The method we used to identify glaucoma participants could also be another potential limitation. Although Foster et al.⁴⁸ glaucoma diagnosis classification is a validated and well-recognized method to define glaucoma in epidemiologic studies, this method based on the subjective evaluation of VCDR would tend to underestimate glaucoma prevalence by potentially missing mild glaucoma cases having the lowest VCDR values. Furthermore, this definition does not take into account more recent methods of optic nerve head evaluation

including the use of OCT. Indeed, since the Foster et al.⁴⁸ publication, several studies have demonstrated diagnostic performances of OCT for glaucoma that could improve diagnosis classification.^{45,70} However, in our study, VCDR measurements were interpreted in duplicate by two specially trained technicians masked to clinical information to limit the risk of misclassification. Furthermore, the methodology of our study was approved in 2006 and before publications of population-based studies evaluating OCT diagnostic performances for glaucoma. As the aim of our population-based study was to assess the association of age-related eye diseases with nutritional, environmental, genetic, or vascular factors and with a longitudinal follow-up of participants approximately every 2 years, we assume that our constant definition of glaucoma during the study follow-up is the most appropriate method to demonstrate all these associations.

Finally, although the AGE-reader enables a noninvasive *in vivo* measurement of the accumulation of fluorescent AGEs in skin collagen and also shows a strong correlation with several fluorescent and even nonfluorescent AGEs measured on skin biopsy, this device does not provide quantitative information on specific AGEs concentration but estimates a group of several AGEs that have progressively accumulated in the skin. Furthermore, it can also measure some other autofluorescent proteins not related to AGEs.^{21,71,72} Indeed, some other fluorophores such as keratin, vitamin D, or lipofuscin, the latter being involved in the aging process as well as in the formation of AGEs, are within the range of excitation light of the AGE-reader.¹¹ Additionally, some other nonfluorescent chromophores such as melanin or hemoglobin may also have influenced sAF measurements.⁷³ Our population sample of elderly people has a higher mean age than that observed in other published studies with a cumulative exposure to metabolic activity over a longer period.^{22-32,38} Thus, it could have led to a higher accumulation of AGEs in skin collagen and also other fluorophores and chromophores that progressively accumulate with increasing age. Hence, we cannot exclude a higher confounding influence of these non-AGEs components on sAF measurements in our population of elderly people than that observed in younger population sample. However, by showing an association with chronic age-related disease or its complications, the measurement provided by the AGE-reader has demonstrated its clinical value in numerous studies.^{22-31,33-37} Thus, we assume that our results are clinically relevant in glaucoma, but would also probably need further exploration, including longitudinal sAF measurements and an independent assessment of skin content of AGEs and lipofuscin or melanin on skin biopsy, in an attempt to cross-validate these findings in our population sample composed of elderly people. These explorations could confirm these results and determine which AGEs are particularly involved or how they could influence the pathogenesis of the disease. In addition, sAF measurements cannot be performed in the darkest skin phototypes V and VI because of their low reflectance. Moreover, although a specific algorithm has been used to correct for differences among skin phototypes I and IV, the vast majority of studies have been conducted in Caucasian subjects and an effect of skin color on sAF measurements cannot be excluded.²¹ Skin phototype of participants was not collected in our study due to ethical issues related to the record of skin color in France. Although our population sample was mainly composed of subjects from Caucasian origin, future studies should take skin color (including in phototypes I-IV) into account as a potential confounder. Studies are also needed in subjects from other ethnicities, to confirm the interest of sAF measurements in these populations.

In conclusion, in this population-based study of elderly people, increased sAF, as well as smoking, were independently associated with glaucoma. By measuring in vivo and noninvasively long-term accumulation of AGEs in skin collagen, sAF could be a long-term biomarker of glaucoma. Furthermore, several experimental studies reported that formation, accumulation or action of AGEs could be inhibited directly or indirectly and that the binding of AGEs to RAGE could also be inhibited.^{74,75} Thus, our findings associated with other published evidence on the role of AGEs in glaucoma pathogenesis might contribute to enhance glaucoma prevention or treatment strategy, and therefore might improve glaucoma-related visual impairment. Indeed, if this association is confirmed and in addition with other reported risk factors for glaucoma, sAF measurements might contribute to characterize earlier patients with higher risk of glaucoma onset or glaucoma progression. However, further investigations are needed to confirm and replicate this association, to determine the role of sAF as a long-term biomarker or potential predictor of the disease during the follow-up of our study, and to evaluate the correlation between sAF and glaucoma severity in an appropriate case-control study involving more glaucoma patients.

Acknowledgments

Supported by grants from Laboratoires Théa (Clermont-Ferrand, France), Université de Bordeaux (Bordeaux, France), Fondation Voir et Entendre (Paris, France) and CNSA (Caisse Nationale pour la Solidarité et l'Autonomie) (Paris, France). Laboratoires Théa participated in the design of the study, but none of the sponsors participated in the collection, management, statistical analysis and interpretation of the data, or in the preparation, review or approval of the present manuscript.

Disclosure: C. Schweitzer, Alcon (C), Amo (C), Thea (C), Allergan (C); A. Cougnard-Gregoire, Thea (C); V. Rigalleau, None; J.-F. Dartigues, None; F. Malet, None; M.-B. Rougier, Thea (C), Allergan (C), Bayer (C), Novartis (C), Bausch&Lomb (C); M.-N. Delyfer, Thea (C), Allergan (C), Bayer (C), Novartis (C), Bausch&Lomb (C); C. Helmer, None; J.-F. Korobelnik, Thea (C), Allergan (C), Bayer (C), Novartis (C), Zeiss (C), Alcon (C), Alimera (C), Horus (C), Bausch&Lomb (C); C. Delcourt, Thea (C), Allergan (C), Novartis (C), Roche (C), Bausch&Lomb (C)

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