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Research: Complications

Higher skin autofluorescence in young people with Type 1 diabetes and microvascular complications

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- Higher skin autofluorescence (a non-invasive measure of advanced glycation end products) is significantly associated with early retinopathy and abnormal cardiac autonomic function in adolescents with Type 1 diabetes.
- Skin autofluorescence reflects up to 10 years of glycaemic history in adolescents with Type 1 diabetes, and may provide a measure of 'metabolic memory'.

Abstract

Aim To test the hypothesis that non-invasive skin autofluorescence, a measure of advanced glycation end products, would provide a surrogate measure of long-term glycaemia and be associated with early markers of microvascular complications in adolescents with Type 1 diabetes.

Methods Forearm skin autofluorescence (arbitrary units) was measured in a cross-sectional study of 135 adolescents with Type 1 diabetes [mean $\pm$ SD age 15.6 ± 2.1 years, diabetes duration 8.7 ± 3.5 years, HbA_{1c} 72 ± 16 mmol/mol ($8.7 \pm 1.5\%$)]. Retinopathy, assessed using seven-field stereoscopic fundal photography, was defined as ≥ 1 microaneurysm or haemorrhage. Cardiac autonomic function was measured by standard deviation of consecutive RR intervals on a 10-min continuous electrocardiogram recording, as a measure of heart rate variability.

Results Skin autofluorescence was significantly associated with age ($R^2=0.15$; $P<0.001$). Age- and gender-adjusted skin autofluorescence was associated with concurrent HbA_{1c} ($R^2=0.32$; $P<0.001$) and HbA_{1c} over the previous 2.5–10 years ($R^2=0.34$ – 0.43 ; $P<0.002$). Age- and gender-adjusted mean skin autofluorescence was higher in adolescents with retinopathy vs those without retinopathy [mean 1.38 (95% CI 1.29, 1.48) vs 1.22 (95% CI 1.17, 1.26) arbitrary units; $P=0.002$]. In multivariable analysis, retinopathy was significantly associated with skin autofluorescence, adjusted for duration ($R^2=0.19$; $P=0.03$). Cardiac autonomic dysfunction was also independently associated with skin autofluorescence ($R^2=0.11$; $P=0.006$).

Conclusions Higher skin autofluorescence is associated with retinopathy and cardiac autonomic dysfunction in adolescents with Type 1 diabetes. The relationship between skin autofluorescence and previous glycaemia may provide insight into metabolic memory. Longitudinal studies will determine the utility of skin autofluorescence as a non-invasive screening tool to predict future microvascular complications.

Introduction

The physiological accumulation of advanced glycation end products (AGEs) in long-lived proteins, such as skin collagen and lens crystallins, occurs throughout life and is accelerated in Type 1 diabetes [1]. AGEs formed as a result of chronic hyperglycaemia and oxidative stress have been

implicated in the development and progression of the macrovascular and microvascular complications of diabetes [2]. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) showed that skin AGE accumulation was significantly associated with complication outcomes mostly independent of HbA_{1c} levels (recent and cumulative up to 6 years) [3], and made a greater contribution than HbA_{1c} levels to risk of complications over the subsequent 10 years [4]. For these studies, AGE accumulation was measured by complex biochemical analyses on full thickness skin biopsies [3,5], but it can now be measured non-invasively using a device suitable for the clinical setting. Skin autofluorescence correlates well with both fluorescent and non-fluorescent AGEs in biopsied skin collagen [6].

Most studies examining the utility of skin autofluorescence to date have been in adults with Type 1 diabetes [7–11], Type 2 diabetes [12] or chronic kidney disease. As serum AGEs are renally excreted, impairment of renal function can lead to AGE accumulation [13] and may be a confounder in adult studies; however, there are limited published data on AGE accumulation and their microvascular associations in young people with Type 1 diabetes before the onset of clinically significant renal impairment. The relative contribution of current HbA_{1c} vs glycaemic history [9,14] in this age group, with relatively short diabetes duration, has not been described previously.

We therefore evaluated the relationship between skin autofluorescence and microvascular complications in young people with Type 1 diabetes, and assessed the relationship of skin autofluorescence to mean HbA_{1c} over time. We hypothesized that skin autofluorescence would (1) provide a surrogate measure of cumulative glycaemic burden in Type 1 diabetes over 10 years and (2) be associated with early markers of microvascular complications in adolescents.

Patients and Methods

Participants were recruited through the Diabetes Complications Assessment Service at the Children's Hospital at Westmead: those aged ≤18 years with Type 1 diabetes duration ≥2 years were eligible for the study. Clinical data collected included age, diabetes duration, anthropometry, biochemistry and complication outcomes.

Blood pressure was measured by auscultation after a 5-min rest in the seated position, using an appropriate sized cuff, with age- and gender-related SD scores calculated according to published standards [15]. Height was measured using a Harpenden stadiometer and weight using electronic scales. Current HbA_{1c} was measured at the clinic visit and all past HbA_{1c} values recorded for the total diabetes duration for each participant were retrieved from the medical records. HbA_{1c} was

assessed by high-performance liquid chromatography (Variant analyser; Bio-Rad Laboratories, Hercules, CA, USA until December 2009, then Adams Arkray Inc., Kyoto, Japan from January 2010 onwards; Adams = 1.0566x Variant, where $R^2 = 0.98$). Lipid profiles and creatinine were measured by routine laboratory methods. Estimated GFR (eGFR) was calculated from the formula: $\text{eGFR (ml/min/1.73m}^2\text{)} = 42 \times \text{height (cm)} / \text{plasma creatinine (}\mu\text{mol/l)}$.

Skin autofluorescence was measured using the AGE Reader (Diagnoptics, Groningen, the Netherlands) and results reported in arbitrary units (AU) as the mean of three readings on the volar surface of each arm, corrected for skin pigmentation [16]. Data on control subjects without diabetes data were obtained using the same equipment. Based on published data on skin autofluorescence (same equipment) in adults with diabetes [mean (SD) 0.016 (0.004) AU vs age-matched controls 0.018 (0.005) AU] [6], we calculated that 40 participants would be required in each group to detect a mean difference between the diabetes groups.

Retinal assessment was performed using seven-field stereoscopic fundal photography. Early retinopathy was defined as the presence of at least one microaneurysm or haemorrhage (≥ 21) graded according to modified Airlie House criteria [17].

Urinary albumin was measured with an Immulite 1000 immunoassay analyser (Siemens, Los Angeles, CA, USA). Early indicators of nephropathy were defined as (1) elevated mean albumin excretion rate (AER) $\geq 7.5 \mu\text{g/min}$ (above 95th percentile of the normal adolescent population) [18] and (2) albuminuria (AER $\geq 20 \mu\text{g/min}$ in at least two of three samples from timed overnight urine collections).

Peripheral nerve function was assessed by thermal threshold at the dorsum of the left foot and vibration threshold at the left malleolus and left great toe (Neurosensory TSA-II and Vibratory Sensory Analyser; Medoc Ltd, Ramat Yishai, Israel), and compared with reference ranges derived from age- and gender-adjusted controls [19].

Autonomic function was assessed by pupillary reaction to light stimulus using an infrared pupillometer (Pupilsan; Fairville Medical Optics, Amersham, UK) [20]. Heart rate variability, as a measure of cardiac autonomic function, was derived using LabChart Pro (AD Instruments, Sydney, NSW, Australia), based on a 10-min continuous electrocardiogram recording at rest, and included standard deviation of mean NN intervals, where NN indicates successive RR intervals on the electrocardiogram. Normal reference ranges were derived from heart rate variability of local contemporary controls measured using the same equipment. Abnormal standard deviation of mean

NN intervals was defined as above the 5th percentile for age and gender.

The study was approved by the Human Research Ethics Committee at the Children's Hospital, Westmead. Written informed consent was obtained from participants and their families.

Statistical analysis

Descriptive data are summarized as mean $\pm$ SD for parametric data. Skin autofluorescence was multiplied by 10 (AU*10) and analysed as a continuous variable. The presence of any retinopathy, abnormal albumin excretion, peripheral nerve abnormalities and pupillary abnormalities were analysed as categorical outcomes. Heart rate variability (as measured by standard deviation of mean NN intervals) was analysed as both continuous and categorical outcomes. Mean differences between groups were compared using independent-samples *t*-tests (diabetes vs controls, retinopathy vs retinopathy-free). Multivariable linear regression was used to model the association between skin autofluorescence and clinical explanatory variables (age, diabetes duration, HbA_{1c}, cumulative mean HbA_{1c} over the past 10 years in 2.5-year time blocks). Multivariable linear and logistic regression analyses were used to examine the association between complications outcomes and clinical explanatory variables (with or without skin autofluorescence). The prevalence of retinopathy and abnormal standard deviation of mean NN intervals in adolescents with Type 1 diabetes divided according to HbA_{1c} groups (above and below clinical target of 58 mmol/mol (7.5%) and skin autofluorescence groups (highest tertile ≥ 13.3 AU vs lower two tertiles) was compared using Fisher's exact test. All statistical analyses were conducted using SPSS version 22 (IBM SPSS Statistics, Chicago, IL, USA).

Results

The characteristics of the Type 1 diabetes group and control group at the first study visit are shown in Table 1. The mean ($\pm$ SD) age of the adolescents with Type 1 diabetes was 15.6 ($\pm$ 2.1) years with mean diabetes duration 8.7 ($\pm$ 3.5) years. Diabetes duration was ≥ 5 years in 113 (84%), ≥ 7.5 years in 83 (61%) and ≥ 10 years in 46 participants (34%). The majority of participants were intensively managed using insulin pump therapy (56%) or multiple daily injections (30%), while the remainder were treated with fewer than four injections per day.

The clinical characteristics of the diabetes group, according to complication status, are described in Table 2. Retinopathy was found in 16% (22/135), elevated AER in 23% (29/127) and albuminuria in 2% of the participants (2/124). eGFR ranged from 78 to 200 ml/min/1.73m², and no participant had renal impairment, as defined by eGFR < 60 ml/min/1.73m². Pupillary abnormalities were found

in 65% (79/121), peripheral nerve dysfunction in 25% (30/122) and cardiac autonomic abnormality (standard deviation of mean NN intervals, a measure of overall heart rate variability) in 11% of participants (19/128).

In adolescents with diabetes, higher skin autofluorescence was significantly associated with older age, which explained 15% of the variation ($B = 0.52$, 95% CI 0.31–0.72; $P < 0.001$; $R^2 = 0.15$), longer duration ($B = 0.26$, 95% CI 0.13–0.38; $P < 0.001$; $R^2 = 0.11$), female gender ($B = 1.6$, 95% CI 0.7–2.5; $P < 0.001$; $R^2 = 0.08$), concurrent HbA_{1c} ($B = 0.58$, 95% CI 0.28–0.88; $P < 0.001$; $R^2 = 0.10$) and HbA_{1c} over the previous 10 years ($B = 0.99$, 95% CI 0.27–1.73; $P = 0.008$; $R^2 = 0.15$). Skin autofluorescence adjusted for chronological age and gender was significantly associated with concurrent HbA_{1c} ($B = 0.51$, 95% CI 0.25–0.77; $P < 0.001$; $R^2 = 0.32$), mean HbA_{1c} over the past 2.5 years ($B = 0.65$, 95% CI 0.36–0.93; $P < 0.001$; $R^2 = 0.34$), 5 years ($B = 0.67$, 95% CI 0.31–1.02; $P < 0.001$; $R^2 = 0.40$), 7.5 years ($B = 0.74$, 95% CI 0.24–1.24; $P = 0.004$; $R^2 = 0.42$) or 10 years ($B = 0.75$, 95% CI 0.12–1.39; $P = 0.02$; $R^2 = 0.40$). Skin autofluorescence was not significantly associated with total cholesterol levels, triglycerides or eGFR.

Age- and gender-adjusted skin autofluorescence was significantly higher in adolescents with early diabetic retinopathy (mean 1.38, 95% CI 1.29–1.48 AU) compared with those without retinopathy (mean 1.22, 95% CI 1.17–1.26 AU; $P = 0.002$) and to controls (mean 1.16, 95% CI 1.10–1.24 AU; $P < 0.001$; Fig. 1). There was no statistically significant difference in mean skin autofluorescence (unadjusted) in the adolescents with Type 1 diabetes compared with controls (Table 1).

In univariate analysis, retinopathy was associated with higher skin autofluorescence [odds ratio (OR) 1.32, 95% CI 1.11–1.57; $R^2 = 0.13$], older age (OR 1.35, 95% CI 1.03–1.77; $P = 0.03$; $R^2 = 0.07$), longer diabetes duration (OR 1.26, 1.09–1.45; $P = 0.002$; $R^2 = 0.14$), higher concurrent HbA_{1c} (OR 1.50, 95% CI 1.11–2.04; $P = 0.009$; $R^2 = 0.09$), higher mean HbA_{1c} over 2.5 years (OR 1.71, 95% CI 1.22–2.39; $P = 0.002$; $R^2 = 0.13$), 5 years (OR 1.77, 95% CI 1.18–2.64; $P = 0.006$; $R^2 = 0.11$), 7.5 years (OR 2.10, 95% CI 1.21–3.64; $P = 0.008$; $R^2 = 0.15$) or 10 years (OR 2.15, 95% CI 1.06–4.34; $P = 0.03$; $R^2 = 0.17$). Retinopathy was not associated with gender, eGFR, elevated AER, systolic or diastolic blood pressure, total cholesterol or triglyceride levels. In multivariable analysis, retinopathy remained significantly associated with skin autofluorescence, in separate models adjusted for age ($R^2 = 0.15$), duration ($R^2 = 0.19$), concurrent HbA_{1c} ($R^2 = 0.16$), mean HbA_{1c} over 2.5 years ($R^2 = 0.19$) or mean HbA_{1c} over 5 years ($R^2 = 0.18$; all $P \leq 0.03$; Table 3). Retinopathy was not associated with skin autofluorescence when adjusted for both the time factor (age or duration) and measure of glycaemia (concurrent HbA_{1c} or mean HbA_{1c} of previous years) in the same multivariable model.

Retinopathy was significantly more prevalent among cases in the highest tertile of skin autofluorescence compared with those in the lower two tertiles, regardless of whether their current HbA_{1c} was below or above the clinical target of 58 mmol/mol (7.5%; Fig. 2a).

Cardiac autonomic dysfunction, defined as abnormal standard deviation of mean NN intervals for age and gender, was associated with higher skin autofluorescence (OR 1.29, 95% CI 1.08–1.54; $P=0.006$; $R^2=0.11$) and higher triglyceride levels (OR 2.09, 95% CI 1.20–3.65; $P=0.01$; $R^2=0.09$). Abnormal standard deviation of mean NN intervals was not associated with diabetes duration, HbA_{1c}, eGFR or AER in univariate analyses. In a multivariable model, abnormal standard deviation of mean NN intervals was associated with higher skin autofluorescence (OR 1.32, 95% CI 1.09–1.59; $P=0.005$), triglycerides (OR 1.93, 95% CI 1.06–3.52; $P=0.03$) and eGFR (OR 1.02, 95% CI 1.00–1.05; $P=0.045$; $R^2=0.23$). Standard deviation of mean NN intervals as a continuous variable was not associated with skin autofluorescence.

Abnormal standard deviation of mean NN intervals was also significantly more prevalent among those in the highest tertile of skin autofluorescence compared with those in the lower two tertiles of skin autofluorescence, for the diabetes group with current HbA_{1c} above 58 mmol/mol (7.5%; Fig. 2b).

Higher skin autofluorescence was also associated with greater number of complications in the adolescents with Type 1 diabetes. There was no association between skin autofluorescence and the other complication outcomes (peripheral nerve dysfunction, pupillometry abnormalities or elevated AER).

Discussion

In this cross-sectional study in adolescents with Type 1 diabetes, higher skin autofluorescence was significantly associated with early retinopathy and cardiac autonomic dysfunction. The association with retinopathy remained after adjusting for age and mean HbA_{1c} up to 5 years. Whereas age- and gender-adjusted skin autofluorescence reflected the previous 10-year glycaemic history, a large proportion of the variability may be related to individual variations in metabolic handling of hyperglycaemia and resulting oxidative stress.

In this group with shorter diabetes duration than in adult studies, long-term glycaemic history contributed to up to 42% of the variability in age- and gender-adjusted skin autofluorescence in adolescents. In contrast to some adult studies, we found that concurrent HbA_{1c} levels also significantly contributed to skin autofluorescence readings. A recent paediatric study also found a

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significant association between skin autofluorescence and both concurrent and mean HbA_{1c} over the preceding year [21], whereas skin AGE accumulation in adults has shown a stronger correlation with long-term HbA_{1c} rather than concurrent HbA_{1c} [9]. In an early study involving skin biopsies, mean HbA_{1c} over the past year correlated more closely with skin AGE levels than the HbA_{1c} value closest in timing to the biopsy [5]. In a more recent study, skin autofluorescence measured using another non-invasive device (SCOUT DS, VeraLight, Albuquerque, NM, USA) was associated with the past 20-year history of HbA_{1c}, but with the weakest magnitude when the most recent 5 years of data were included [14]. Similarly in the DCCT/EDIC cohort at a mean diabetes duration of 30 years, log skin autofluorescence showed stronger correlation with mean HbA_{1c} over the total duration compared with the most recent 5–10 years [22]. The difference between children and adult data might relate to more rapid collagen turnover in children who are younger and growing, or might relate to individual HbA_{1c} levels tracking well during this period.

This is also the first study examining skin AGEs in relation to early markers of microvascular complications in adolescents with Type 1 diabetes. We found that higher skin autofluorescence remained significantly associated with retinopathy outcomes after adjusting for traditional risk factors of age, duration or mean HbA_{1c} up to 5 years. Furthermore, elevated skin autofluorescence was found in adolescents who had evidence of retinopathy despite concurrent HbA_{1c} within target glycaemic range. The independent association between skin autofluorescence and retinopathy in this adolescent cohort provides further evidence that skin autofluorescence reflects a metabolic or epigenetic risk profile beyond current HbA_{1c}, which includes glycaemic history and other biochemical factors involved in metabolic memory in this group with relatively short diabetes duration. Adult studies have shown a strong association between skin autofluorescence and retinopathy, which did not persist after adjustment for HbA_{1c} [7] or total glycaemic exposure [9]. In the present study, skin autofluorescence was no longer independently associated with retinopathy when mean HbA_{1c} over 5–10 years was considered in the model. This suggests that skin autofluorescence is not independent of longer-term glycaemic control beyond 5 years of diabetes duration, which is consistent with data in adults (mean diabetes duration of 30 years), where skin autofluorescence was no longer associated with retinopathy when adjusted for mean HbA_{1c} over time [9].

In the present study, there was a significant difference in age- and gender-adjusted skin autofluorescence between the diabetes group with retinopathy compared with controls. No significant difference in age- and gender-adjusted skin autofluorescence was found between the diabetes group without retinopathy and controls. This further suggests that there are individual

factors beyond diabetes status which lead to AGE accumulation, and protective factors in adolescents with diabetes who are complication-free may relate to their ability to maintain a similar degree of AGE accumulation as adolescents without diabetes.

We also found an independent association between skin autofluorescence and cardiac autonomic dysfunction. In the Epidemiology of Diabetes Complications cohort with mean diabetes duration >35 years, skin autofluorescence (measured non-invasively by the SCOUT system) showed stronger association with autonomic neuropathy than cumulative 18-year mean HbA_{1c} level [10]. The findings in our adolescent study group with a mean diabetes duration of <10 years support the existing hypothesis of AGE accumulation as pathogenic in autonomic dysfunction, even at shorter diabetes duration, and the potential role of autonomic dysfunction in perpetuating inflammation and further build-up of pathogenic AGEs [23].

In contrast to the adult studies [11,12], we did not find a significant association between skin autofluorescence and peripheral nerve abnormalities on thermal and vibration threshold testing. These differences in the findings may relate to the varying definitions and methods of assessment of peripheral neuropathy, in addition to the differences in clinical cohorts (adult vs adolescence) as well as study sample size not powered to detect these differences.

The present study also explored the role of renal function on skin AGE accumulation, although we did not find a statistically significant association between skin autofluorescence and eGFR in this study group. In the DCCT/EDIC cohort (mean diabetes duration 30 years), eGFR <60 ml/min/1.73m² was a significant determinant of higher skin autofluorescence [9], but in another cohort (mean diabetes duration 36 years), this eGFR threshold eliminated any association between skin autofluorescence and mean HbA_{1c} level [14]. In adolescents, GFR (and eGFR) can be paradoxically elevated in early diabetic nephropathy, which could lead to falsely lowered skin autofluorescence, and may be followed by reduced GFR in more advanced renal disease. Skin autofluorescence may therefore not show a linear relationship with eGFR or AER in this younger age group.

The persistent benefit of intensive diabetes management was evident in the DCCT/EDIC cohort for many years after the defined period of study intervention. It was initially demonstrated from the follow-up of that cohort that HbA_{1c}, measured 2–3 years earlier, had the greatest relative risk contribution to current retinopathy progression, whilst values up to 8 years ago continued to have an impact [24]. This concept, whereby prior glycaemic control contributes to the development of microvascular and macrovascular complications, has been described as 'metabolic memory' [25],

with increasing recognition of the contribution of factors beyond glycaemia such as AGE-related protein modification or even prolonged memory of past lifestyle factors [26]. Skin autofluorescence may therefore provide a quantification of the contribution of metabolic memory on chronic diabetes complications. In addition, the presence of microalbuminuria related to previous glycaemic control, has been shown to have independent risk for future cardiovascular disease, and if associated with lower GFR, may have secondary effects through altering the renal clearance of AGEs.

We previously showed the predictive role of plantar fascia thickness, another non-invasive measure of collagen glycation, on microvascular complications independent of HbA_{1c} over a 3-year follow-up period [27]. The present study provides further evidence of early detectable accumulation of AGEs, which probably contribute to the early pathophysiology of diabetes complications over a relatively short period, using a non-invasive clinically feasible tool.

The limitations of this study include the sample size and relatively low frequency of complication events in adolescents, which preclude analysis with all of the explanatory variables in the same multivariable model, and the cross-sectional nature, preventing evaluation of the predictive value of skin autofluorescence on the development and progression of these early markers of complications. Furthermore, we did not have data on makers of oxidative stress or inflammation which may impact AGE formation, as well as genetic [28] and environmental determinants of skin AGEs, including smoking, caffeine consumption [29] and dietary AGE burden [30]. Technical aspects that may theoretically impact on the skin AGE reading include skin pigmentation and topical agents on skin surface. The model of AGE Reader used in the present study incorporates an algorithm to correct skin autofluorescence readings for skin pigmentation [16]. We also ensured there were no skin lesions or sun-blocking agents on the skin surface of the testing region at the study visit. Study strengths include the detailed clinical characterization and historical data in a young population with Type 1 diabetes. This age group may also provide an opportunity to assess metabolic memory with fewer confounding factors than usually found in the adult population in which there are higher rates of impaired renal function, concurrent medication use and smoking history.

In conclusion, higher skin autofluorescence in adolescents with Type 1 diabetes is associated with early retinopathy and cardiac autonomic dysfunction independent of traditional risk factors of older age, longer diabetes duration or worse glycaemic control, and provides a simple and strong composite measure of glycaemic history up to 10 years. Skin autofluorescence may have future clinical value as it reflects long-term glycaemic control over several years. Further studies are required to determine whether skin autofluorescence may also reflect other aspects of glycaemia not captured by a clinic HbA_{1c} value, such as glycaemic variability, in addition to non-glycaemic

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factors. Furthermore, longitudinal studies examining skin autofluorescence will help determine the early role of AGEs on retinopathy and autonomic dysfunction in adolescents with Type 1 diabetes.

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Competing interests

None declared.

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FIGURE 1 (a) Age- and gender-adjusted skin autofluorescence for the control and diabetes groups. (b) Age- and gender-adjusted skin autofluorescence for control group; age- and gender-adjusted abnormal/normal standard deviation of mean NN intervals (SDNN; standard deviation of consecutive RR intervals on a 10-min continuous ECG recording). AU, arbitrary units. Data are mean and 95% CI.

FIGURE 2 (a) Xxx. (b) Xxx.

Table 1 Characteristics of adolescents with Type 1 diabetes and control subjects

	Diabetes	Controls	P
<i>n</i>	135	40	
Age, years	15.6 ± 2.1	15.4 ± 4.4	0.64
Gender, % male	51	55	0.40
Skin autofluorescence, AU	1.23 ± 0.27	1.14 ± 0.29	0.06
Type 1 diabetes duration, years	8.7 ± 3.5	-	
HbA _{1c}		-	
mmol/mol	72 ± 16		
%	8.7 ± 1.5		
eGFR, mL/min/1.73m ²	127 ± 23	-	

AU, arbitrary units; eGFR, estimated GFR.

Data are mean ± SD, unless otherwise specified.

Table 2 Characteristics of young people with Type 1 diabetes, stratified by complications status

	Retinopathy	No retinopathy	P	Abnormal SDNN	Normal SDNN	P
<i>n</i>	22	113		19	109	
Age ± years	16.5 ± 1.6	15.4 ± 2.1	0.03	16.7 ± 1.6	15.4 ± 2.1	0.007
Gender, % male	50%	51%	1.00	16%	84%	0.002
Skin autofluorescence, AU	1.42 ± 0.27	1.20 ± 0.25	0.001	1.41 ± 0.25	1.21 ± 0.26	0.03
Type 1 diabetes duration, years	11.0 ± 2.4	8.3 ± 3.5	0.001	8.6 ± 4.5	8.4 ± 1.1	0.87
Glycaemia						
Concurrent HbA _{1c}			0.006			0.14
mmol/mol	80 ± 9	70 ± 14		76 ± 24	70 ± 13	
%	9.5 ± 0.8	8.6 ± 1.3		9.1 ± 2.2	8.6 ± 1.2	
Mean HbA _{1c} over 2.5 years			0.001			0.06
mmol/mol	79 ± 19	68 ± 12		75 ± 23	68 ± 12	
%	9.4 ± 1.7	8.4 ± 1.1		9.0 ± 2.1	8.4 ± 1.1	
Mean HbA _{1c} over 5 years			0.002			0.001

mmol/mol	76 ± 18	67 ± 10		78 ± 21	67 ± 10	
%	9.1 ± 1.6	8.3 ± 0.9		9.3 ± 1.9	8.3 ± 0.9	
Mean HbA _{1c} over 7.5 years			0.002			0.004
mmol/mol	75 ± 16	66 ± 8		76 ± 15	66 ± 9	
%	9.0 ± 1.5	8.2 ± 0.7		9.1 ± 1.4	8.2 ± 0.8	
Mean HbA _{1c} over 10 years			0.01			0.01
mmol/mol	76 ± 18	66 ± 7		78 ± 16	66 ± 10	
%	9.1 ± 1.6	8.2 ± 0.6		9.3 ± 1.5	8.2 ± 0.9	
Other risk factors						
Total cholesterol, mmol/l	4.6 ± 0.7	4.3 ± 0.9	0.30	4.6 ± 1.0	4.3 ± 0.9	0.27
Triglycerides, mmol/l	1.3 ± 0.6	1.3 ± 0.8	0.99	1.7 ± 1.0	1.2 ± 0.7	0.004
SBP, SD scores	0.08 ± 1.14	0.04 ± 1.21	0.91	-0.07 ± 1.52	0.11 ± 1.12	0.55
DBP, SD scores	-0.02 ± 0.81	0.00 ± 0.91	0.93	0.23 ± 0.85	-0.02 ± 0.89	0.27
BMI, SD scores	0.82 ± 0.89	0.49 ± 0.96	0.13	0.78 ± 0.79	0.51 ± 1.00	0.48
eGFR, ml/min/1.73m ²	121 ± 23	128 ± 24	0.21	137 ± 25	126 ± 23	0.048
Elevated AER >7.5 mcg/min, n/N (%)	5/21 (24)	24/106 (23)	1.00	3/17 (18)	25/105 (24)	0.76

AU, arbitrary units; AER, albumin excretion ratio; DBP, diastolic blood pressure; eGFR, estimated GFR; SBP, systolic blood pressure; SDNN, standard deviation of mean NN intervals.

Data are mean ± SD, unless otherwise specified.

Table 3 Effect of skin autofluorescence on retinopathy outcomes after adjustment for each potential confounding clinical variable

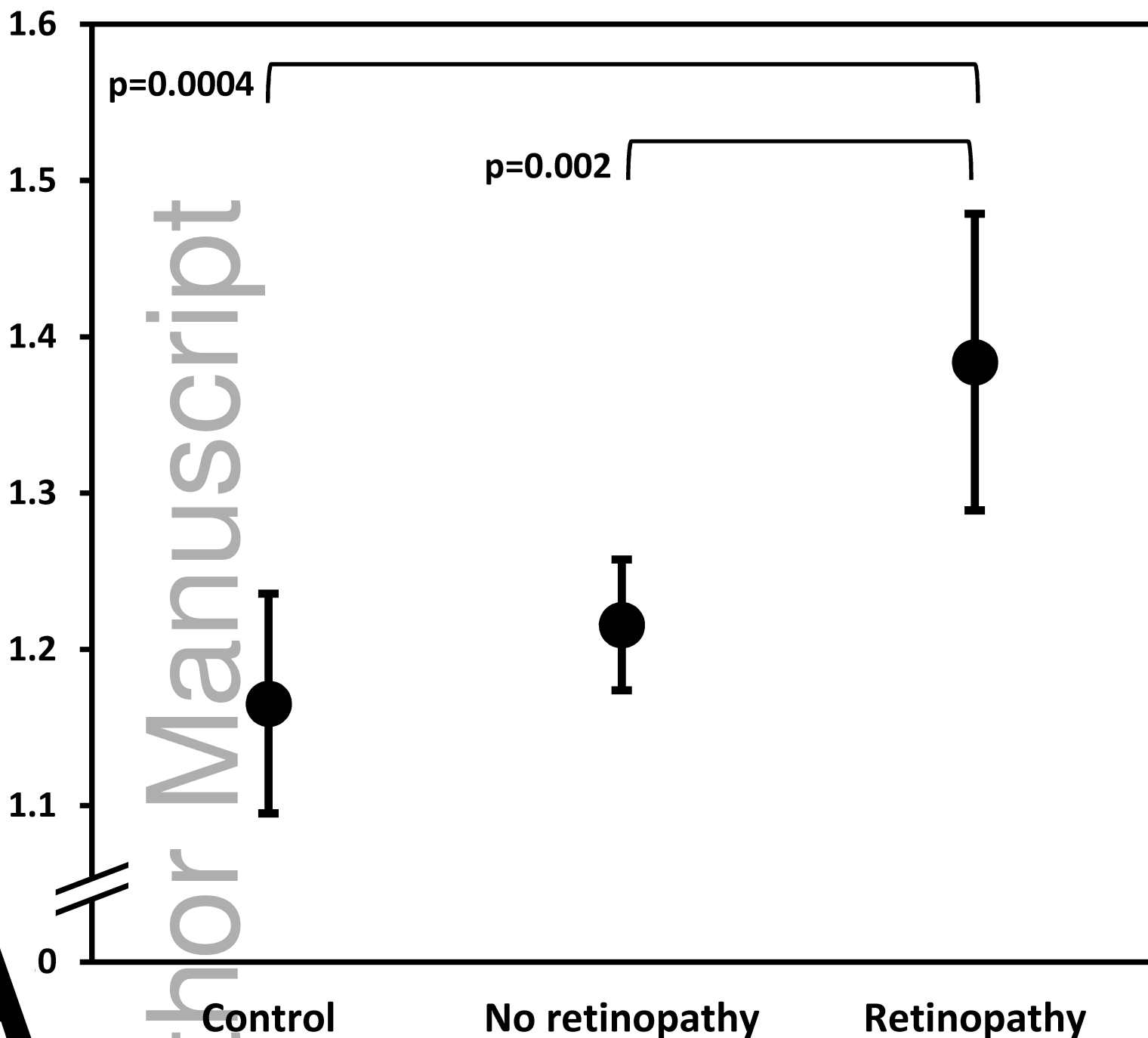
Models	Odds ratio (95% CI)	P	R ²
Unadjusted skin autofluorescence (AU*10)	1.32 (1.11–1.57)	0.002	0.13
Skin autofluorescence adjusted for age	1.27 (1.05–1.53)	0.01	0.15
Skin autofluorescence adjusted for duration	1.23 (1.02–1.49)	0.03	0.19
Skin autofluorescence adjusted for eGFR	1.35 (1.13–1.62)	0.001	0.17
Skin autofluorescence adjusted for HbA _{1c}	1.30 (0.94–1.81)	0.01	0.16
Skin autofluorescence adjusted for mean HbA _{1c} over 2.5 years	1.24 (1.03–1.49)	0.03	0.19
Skin autofluorescence adjusted for mean HbA _{1c} over 5 years	1.25 (1.04–1.51)	0.02	0.18

Skin autofluorescence adjusted for mean HbA _{1c} over 7.5 years	1.22 (1.00–1.49)	0.05	0.21
Skin autofluorescence adjusted for mean HbA _{1c} over 10 years	1.14 (0.90–1.46)	0.28	0.20

AU, arbitrary units; eGFR, estimated GFR.

Skin autofluorescence (AU)

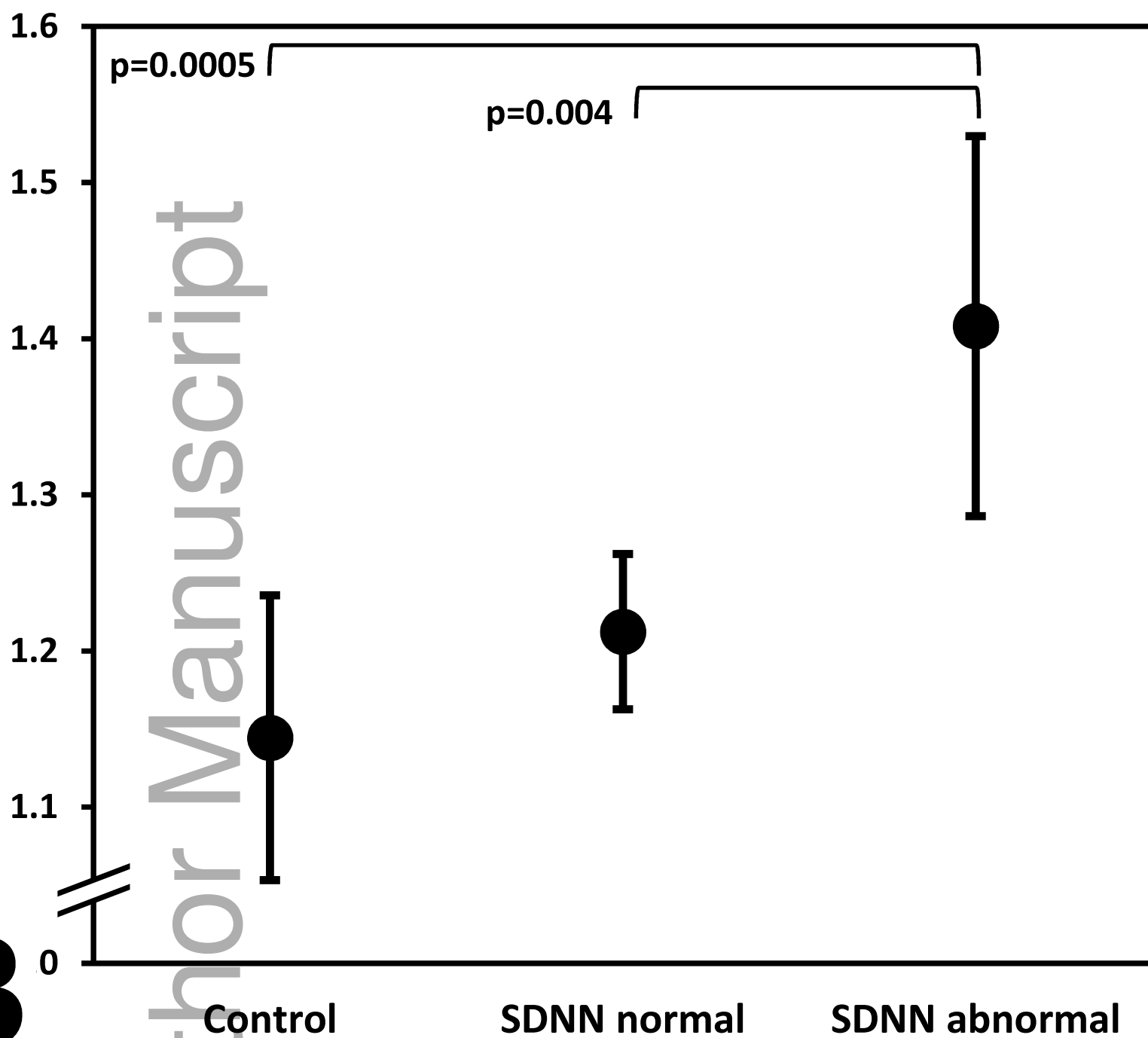
A



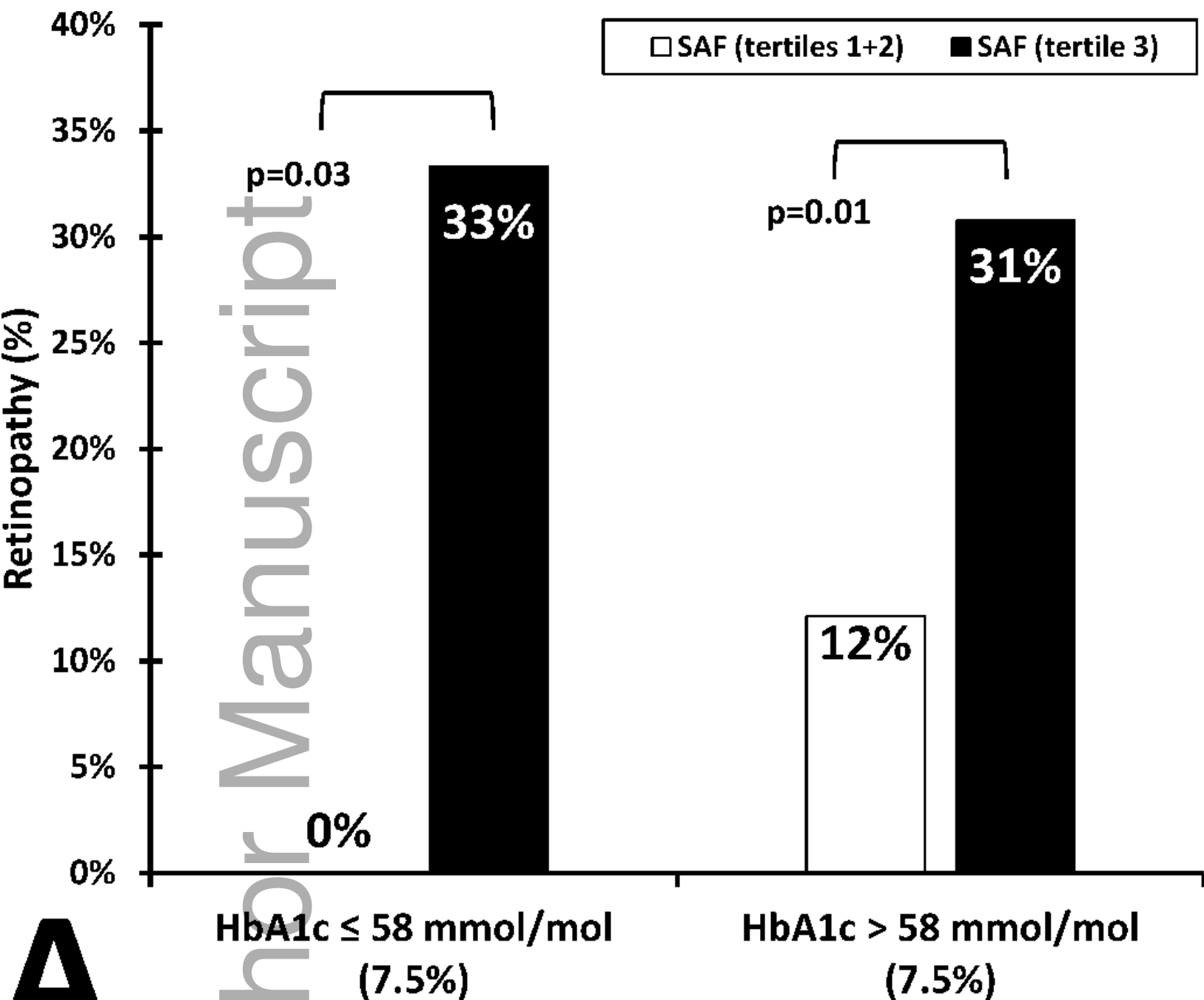
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Skin autofluorescence (AU)

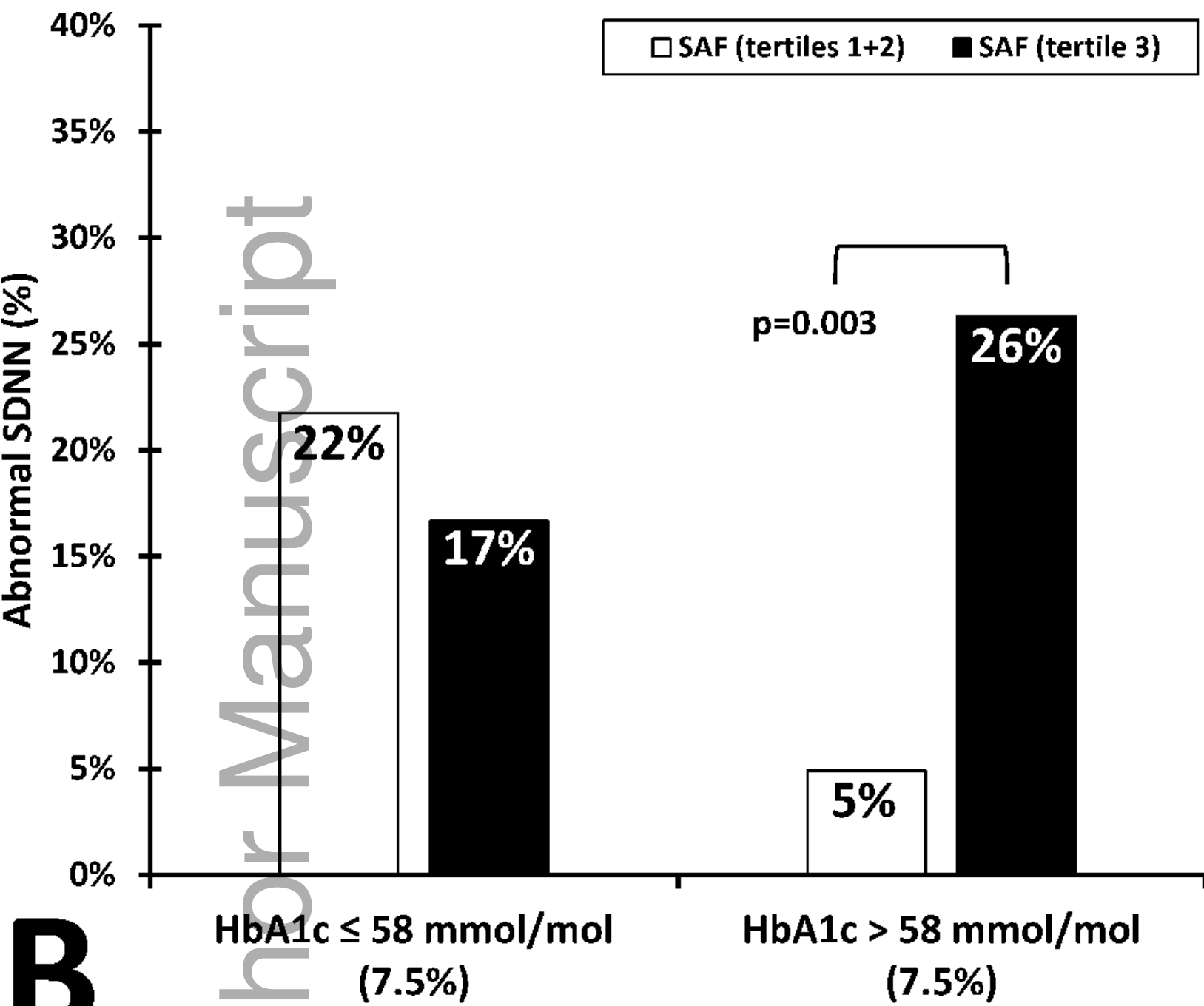
B



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