

Conjunctival ultraviolet autofluorescence as a measure of past sun exposure in children

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Abbreviation list: CUVAF: conjunctival ultraviolet autofluorescence; AOR: adjusted odds ratio; CI: confidence interval; SES: socio-economic status; UVR: UV radiation

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Abstract

Background: Conjunctival ultraviolet autofluorescence (CUVAF) area detected from UVAF photographs is a recently developed potential marker for past sun exposure but its relationship with sun-related factors have not been fully investigated.

Methods: The study included 339 ethnically diverse and healthy children aged 5-15 years in Melbourne, Australia. Data were collected by questionnaire and examination at school. CUVAF area was measured using a computer programme and analysed as a continuous and dichotomous outcome (any/none).

Results: The mean age (SD) of the study population was 9.6 (2.7) years with 45.4% males. 53 children (15.6%) had detectable CUVAF and the youngest age at which a child showed sun damage was 8 years. Compared with silicone skin cast score, there was good inter-grader agreement on grading of a random selection of UVAF photographs, with Cohen's kappa 0.85 [95% confidence interval (CI), 0.65-1.00] for total CUVAF area using both eye photographs. Perfect intra-grader agreement was achieved. Fairer pigmentation including medium/fair skin colour [adjusted odds ratio (AOR), 3.42; 95% CI, 1.02-11.48 versus dark/olive] and blue/grey eye colour (AOR, 4.07; 1.73-9.55 versus brown) were associated with increased odds of CUVAF. Increasing lifetime sunburn number (e.g., AOR, 2.89; 1.14-7.35 and 4.29; 1.04-17.76 for sunburns 2-4 and ≥ 5 times, respectively, versus no sunburns, trend $P=0.004$) and freckling by the end of last summer were associated with increased odds of CUVAF.

Conclusions and impact: CUVAF area can be an a priori objective measure of past sun exposure in paediatric populations for future research.

Introduction

Recently there has been considerable research interest in the role of sun exposure in relation to various health conditions. Several innovative methods, such as skin damage score graded using silicone rubber casts on the back of the hand, have been developed to measure personal past sun exposure objectively and quantitatively (1-4). Conjunctival ultraviolet autofluorescence (CUVAF) area detected from UVAF photography is a recently developed potential marker for past sun exposure (4, 5).

UVAF photography has been developed primarily to detect and characterise early pre-clinical sunlight-induced conjunctival damage. A previous study in 71 children reported that CUVAF could be used as a marker of sun-related exposure in early life occurring many years before clinical manifestations of sun exposure related eye diseases, such as pinguecula and pterygium (5). Kerkenezov was the first to observe that patients with pterygium more frequently had hyperkeratosis and/or rodent ulcers on sun-exposed area. Furthermore, pterygia developed about a decade before the skin conditions (6). A likely explanation is that sunlight-damage associated pathologic processes are hastened as a result of exposure to sunlight focused by at least an order of magnitude by the anterior eye (7). Therefore, the sun-related damage detected on UVAF photography may be an early indicator of increased UV insolation not just in the eye but elsewhere in the body (e.g., skin). A recent study showed good intra- and inter-grader reliability for quantitative measurement of CUVAF area in an adult population (predominantly aged over 50 years) (4). The effects of cumulative sun exposures and other sun-related risk factors on the development of ocular sun damage, however, have not yet been investigated, either in adults or in children.

In this study, we aimed to evaluate the association of skin pigmentary traits, recent and lifecourse sun exposures, sun protection behaviour, and skin reactivity to sun with CUVAF area in 339 school-aged children participating as healthy controls in the Early Environment and Type 1 Diabetes Prevention Project (EET1DPP). We also examined the associations of these sun-related factors with silicone skin cast score, which has been used as an objective measure of cumulative past sun exposure in teenagers (8) and adults (3), in a subgroup of children in our study. We determined the reproducibility of CUVAF area and compared that with silicone skin cast score.

Materials and Methods

Study population

The study sample and methods of recruitment have been described previously (9). 357 children from primary and secondary schools of Melbourne, Australia who returned the signed parental consent form participated as controls in the EET1DPP during 2009-2011. 341 children had eye photographs taken and 339 who had gradable photographs for two regions (nasal and temporal) of both eyes were included in this analysis. Ethical approval was obtained from the Human Research Ethics Committee of the Royal Children's Hospital Melbourne (HREC 27111).

Sun-related exposures, behaviour and other variables

Demographic characteristics, sun-related behaviours and other early life environment history were recorded in the questionnaire by parents or guardians (10). Socio-economic status (SES) was measured using the Australian census-based Socio-Economic Indexes for Areas disadvantage score (11).

The objective assessment of natural skin colour (4 categories), hair colour (5 categories), and eye colour (4 categories) has been described in detail elsewhere (9). During the clinical examination at school, the research nurses recorded each child's ethnicity (analysed as Caucasian and non-Caucasian [Asian, Middle Eastern, and Other]) (3, 9). They also counted nevi and observed the presence of freckling (yes/no) on face, back, and left arm using standardised protocols (12). Freckling by end of last summer was self-reported. Skin pigmentation was measured at skin reflectance on sun-exposed sites (e.g., dorsum of hand) and non-exposed sites (e.g., buttock) using a hand-held spectrophotometer (Minolta 2500d, Tokyo, Japan) at wavelengths ranging from 360 to 740 nm (1).

Data were obtained for the monthly average of daily total UV radiation (UVR) dose in standard erythemal doses for Melbourne, latitude 37.8°S, from the Australian Radiation Protection and Nuclear Safety Agency (13). The ambient UVR for the month of interview was the monthly average of daily total UVR assessed at the month and year of interview. UVR was similarly determined for 1 and 2 months prior to interview.

Cumulative sun exposure

Cumulative UVR dose over the lifecourse was determined by number of hours the child spent in the sun per day during summer and winter weekdays, weekends and holidays, at ages 0-2, 3-5, 6-10 and 11-15 years, multiplied by the average UVR dose during summer and winter. We derived information using a specific question with demonstrated validity (14): "During last summer/winter, how much time would your child have spent in the sun? (< 1 h a day [none, some but less than half an hour, half an hour to 1 h]; 1-2 h per day, 2-3 h per day; 3-4 h per day, ≥ 4 h per day)." The time in sun categories were converted into time in minutes: None - 0 min; some, but < half an hour - 15 min; half an hour to 1 h - 45 min; <1 h - 30 min (if parents failed to indicate whether under 1 h corresponded to none; some, but < half an hour; or half an hour to 1 h); 1 to 2 h per day - 90 min; 2-3 h per day - 150 min; 3-4 h per day

- 210 min and ≥ 4 h - 270 min. Median UVR for mid-summer (January) and mid-winter (July) for the years 1996-2011 (year of birth) was multiplied by time in the sun. Time in the sun by UVR in each category was then multiplied by the number of years in the specific age category, and then added for each age group to estimate the total time spent in the sun per day.

Because the biological effect of sun exposure depends not only on time in sun but also the amount of skin exposed and whether exposed skin has a sunscreen barrier, we generated a composite sun exposure index based partly on the total sun exposure score from a previous study, which has been estimated to account for 38% of variance in serum 25 hydroxyvitamin D for adults at a latitude of 40°N (15). A composite sun exposure index last summer was created (information on sun protection behaviour was not collected for last winter). Children with greater time in the sun, less clothing and sunscreen use were given a higher score (16).

CUVAF measurement

Digital photographs were obtained using both reflected visible light (control) and UV-induced fluorescence with the aid of two portable photographic systems. The UVAF photography was taken using a specially adapted electronic flash system fitted with UV-transmission filters (transmittance range 300 to 400 nm, peak 365 nm) and thus only fluorescence was recorded by a Nikon D100 (Nikon, Melville, New York, USA) digital camera (5). We took photos for each eye separately for the nasal and temporal region. The best quality images were graded. **Figure 1** shows examples of UVAF and control photographs of the same eye from a participant.

A grader who was blind to participants' identity used Adobe Photoshop CS4 Extend (Adobe Systems Inc., San Jose, California, USA) to assess the fluorescence area in the photographs using a standardised protocol. CUVAF area representing the sum of fluorescence detected in

four photographs (right nasal and temporal, and left nasal and temporal) of each child was the main outcome of interest (expressed in square millimetres [mm²]).

For the intra-observer reliability, the grader assessed the CUVAF area twice in 3 months apart for 180 eye photographs (randomly selected 45 children). For the inter-observer reliability, two graders graded 120 eye photographs (randomly selected 30 children).

Silicone skin cast score

Silicone rubber casts on the back of the hand were photographed. A grader who was blind to any other data rated the digital images into six possible skin damage scores (1-6) based on pattern of lines on the skin casts using a standardised protocol. The lower the score, the less skin damage. Left-hand casts were graded, except where these were unavailable or were of insufficient quality to be gradable. 157 of the 160 children with silicone cast scores also had CUVAF measures.

Statistical analysis

Continuous variables were described using means and standard deviations or medians and interquartile ranges if skewed in distribution, and categorical variables using percentages. CUVAF area was mainly analyzed as a dichotomous variable (any/none). Using multivariable logistic regression models, we examined the associations between a range of sun-related risk factors and CUVAF area. We also investigated such associations among 53 children with detectable CUVAF, using CUVAF area as a continuous variable. It was natural log transformed before analysis due to its skewed distribution. We also performed Pearson correlation analysis.

We used Stata 14 software (StataCorp, College Station, TX) for all analyses.

Results

The mean age (SD) of the study population was 9.6 (2.7) years; 45.4% were males and 69.3% were Caucasian. CUVAF area had a skewed distribution with a median of 0 (ranged 0-37.6 mm²). 53 (15.6%) children had detectable CUVAF (sun-induced eye damage) and the youngest age at which a child showed such damage in this sample was 8 years (**Figure 2**). 48 children had sun damage on the nasal aspect of the conjunctiva with a median of 2.4 mm² and 21 had damage on the temporal aspect with a median of 1.4 mm² (interquartile range 2.1 to 3.7 and 0.9 to 2.2 mm², respectively). There was perfect intra- and good inter-grader agreement [Cohen's kappa 0.85; 95% confidence interval (CI), 0.65-1.00] on repeat scoring of a random selection of eye photographs for CUVAF area.

Compared to participants with no CUVAF, those with CUVAF were older, more likely to be male and Caucasian (**Table 1**). Increased odds of CUVAF was associated with increasing age, after adjusting for sex and ethnicity. Caucasians were more than twice as likely to have increased odds of CUVAF compared with children from other ethnicities, after adjusting for age and sex. Boys did not show different odds of CUVAF compared with girls.

Compared to participants with no CUVAF, those with CUVAF were more likely to have medium/fair skin colour, mousey blonde hair colour (45.3% vs 31.2%), and blue/grey, hazel and green eye colours, and less likely to have dark/olive, olive/medium or fair skin colour, black or light blonde hair (11.3% or 1.9% vs 21.8% or 5.3%), and brown eye colour (**Table 2**). Participants with CUVAF were also more likely to have sunburns more than twice during lifetime and have freckling by end of last summer than those without CUVAF. Natural medium/fair skin colour, compared with dark/olive skin colour, was associated with

increased odds of CUVAF, after adjusting for age, sex, and cumulative UVR dose. There was little evidence that objectively measured skin colour using spectrophotometry on body locations (buttock) unlikely to receive sun exposure, was associated with odds of CUVAF. Fairer hair colour was associated with increased odds of CUVAF (adjusted odds ratio [AOR], 3.65; 95% CI 1.07-12.51, mousey blonde versus black). Lighter eye colour (blue/grey), compared with brown eye colour, was also associated with increased odds of CUVAF. Sunburn twice or more in the lifetime was associated with a 3-4 fold increased odds of UVAF, and there was a dose response effect with a greater number of sunburns. Any freckling by the end of last summer and freckling on upper arm (AOR 12.55; 1.87-84.44) were associated with increased odds of CUVAF. There was marginal evidence that four or more nevi on the upper arm and face (AOR 2.67; 0.91-7.88) was associated with increased odds of CUVAF compared with no nevi on the upper arm or face. There was a dose response effect on CUVAF area by higher number of nevi count on upper arm.

There was no evidence that birth weight, any siblings, gestational age at birth, maternal age at birth, or season of examination were associated with odds of CUVAF. Birth as the second child was associated with increased odds of CUVAF, compared with the first-born child (age- and sex-AOR, 2.30; 95% CI 1.02-5.17). Such an association was strengthened after further adjusting for either time spent in the sun over the lifecourse (AOR, 2.53; 1.10-5.82) or cumulative leisure-time UVR dose over the lifecourse (2.60; 1.12-6.03), but was partly accounted for by composite sun exposure time last summer (2.37; 1.00-5.63). Birth as the second child was also associated with longer time of composite sun exposure last summer (either weekends, weekdays, or holidays), compared with the first-born child, after accounting for age and sex. However, there was little evidence that birth order was associated with time spent in the sun over the lifecourse and cumulative leisure-time UVR dose.

Participants with CUVAF were also more likely to report spending ≥ 3 h per day in the sun in both last summer and winter weekdays, weekends, and holidays than those without CUVAF, and less likely to use sunscreen and sun hat last summer at all times (**Table 3**). There was little evidence that lifecourse cumulative leisure-time sun exposure was associated with increased odds of CUVAF, after adjusting for age. Cumulative UVR dose was strongly correlated with age ($r=0.57$), compared with that between lifetime sunburns and age (0.34). There was a consistent pattern of associations between longer composite sun exposure time over last summer weekends or holidays and increased odds of CUVAF, after adjusting for age, sex, buttock melanin density, and SES (**Table 3**). There was little evidence that time spent in the sun during last summer or winter weekdays, weekends or holidays, or time spent in the sun per day during summer or winter weekend and holidays at age 0-2, 3-5, 6-10, 11-15 years, was associated with odds of CUVAF (data shown for time spent in the sun last summer weekends as an example). Sun hat use $< 50\%$ of the time outside last summer or never was associated with increased odds of CUVAF, compared with sun hat use all the time outside. However, there was marginal or no evidence that sunscreen (**Table 3**) and sunglass use, amount of time clothing exposing arms, and legs worn last summer were associated with odds of CUVAF (AOR 0.95; 0.36-2.51; 0.69; 0.25-1.89 and 1.76; 0.66-4.69, respectively).

When using CUVAF area as a continuous outcome, we found some, although not all due to limited power, similar pattern of associations among 53 children with detectable CUVAF, compared with the findings in the whole sample (e.g., blue/grey compared with brown eye colour was associated with increased CUVAF (natural log transformed) β 0.71; 95% CI 0.25-1.17, $P=0.003$).

Silicone skin cast

The agreement on repeat scoring of 40 randomly selected skin cast images was low in our sample, with kappa for intra- and inter-grader agreement 0.31 (95% CI 0.13-0.46) and 0.30 (0.09-0.41), respectively. There was a weak correlation between silicone skin cast score (1-5) and CUVAF (coefficient 0.06, $P=0.42$). Among 160 children, higher skin cast score was associated with increased age (sex-AOR, 2.64; 1.47-4.76 for children aged 10-15 years, compared with those aged 5-9 years), which was consistent with our finding using CUVAF (**Figure 2**). Higher skin cast score was also associated with higher number of sunburns (age- and sex-AOR, 2.85; 1.36-5.98; 1.91; 0.91-4.07, and 4.56; 1.23-16.84 for once, 2-4, or ≥ 5 lifetime sunburn, respectively), compared with no lifetime sunburns. Lower mean skin cast score was associated with female gender (age-AOR, 0.43; 0.24-0.78). Compared with children who reported 'burn then peel' (9), those 'tan only' had lower silicone cast score (age- and sex-AOR 0.28; 0.11-0.68). There was little evidence that skin pigimentary traits, ethnicity, cumulative hours sun exposure in summer or winter, nevi count, freckles or sunscreen use last summer were associated with silicone skin cast score.

Discussion

In 339 school-aged children participating as healthy controls in the EET1DPP, we found that CUVAF area was a reproducible and reliable measure of past sun damage. CUVAF was detected in children aged 8 years and over, with odds of CUVAF increasing with age. Caucasians tended to have higher odds of CUVAF than non-Caucasians. Fairer pigmentation including medium/fair skin colour (versus dark/olive), lighter eye colour (blue/grey versus brown) and hair colour (mousey blonde versus black) were associated with increased odds of CUVAF. We also found that higher numbers of lifetime sunburns and freckling by the end of last summer were associated with increased odds of CUVAF. Less sun hat use last summer

(versus sun hat use all the time) was also associated with increased odds of CUVAF, although there was marginal evidence of effect of less sunscreen use on increased odds of CUVAF. Longer composite sun exposure time over last summer, rather than amount of time in the sun, was associated with increased odds of CUVAF.

Strengths of this study include the multiethnic population, a high survey response rate, and detailed information on both constitutive phenotype (e.g., natural skin colour) and cumulative past sun exposures. We also measured skin pigmentation using a spectrophotometer. Our study was partly limited by self-reported data (e.g., freckling by the end of last summer) given parents or guardians were asked for information up to 15 years after their child's birth. Nevertheless, a dose response effect on CUVAF with increasing number of nevi count on upper arm, assessed by the research nurse in our study, reassured the validity of such information (freckling) collected from questionnaire. A potential limitation of the CUVAF grading is the subjectivity involved in evaluating the images. Although we have demonstrated a good reliability of CUVAF grading, the study lacks the reliability and the tracking of CUVAF over periods of time. CUVAF area could mainly be analysed as a dichotomous outcome in our study as their occurrence is largely a feature of an older age group than studied here (prevalence of detectable CUVAF area 21.8%, 30.3%, 38.0% for age group 8-15, 10-15, and 12-15 years, respectively, compared with 15.6% overall). We only had information on sun protection behaviour in last summer not during the lifecourse, which might contribute to the lack of an association between CUVAF and cumulative sun exposure.

To our knowledge, this is the first study to evaluate associations of sun-related risk factors with CUVAF in a paediatric population. Increasing age was associated with increased odds of CUVAF, which is consistent with the finding from a previous study among children aged 3-

15 years (5). Such finding is also in line with that reported in an adult study (17) and concurs with findings from studies using different measures of sun-related exposures (e.g., skin damage) (2, 3). We also found being a first-born child was associated with decreased odds of CUVAF (versus being second-born). Interestingly a previous report using four cohorts showed that in large studies, being first-born child increased the risk of myopia (18), which was associated with decreased CUVAF area (19) and lower vitamin D concentration (20). Our finding on the association between birth order and CUVAF concurs with these. It is possible that parents were more careful with the sun protection of their first child. Such an association was accounted for by composite sun exposure time last summer in our study, indicating that birth order may not be an independent risk factor for CUVAF and may actually be related to composite time spent in the sun.

Consistent with a previous study using silicone skin cast score in an adult population (3), we found that fairer skin colour, lighter eye and hair colour were associated with increased odds of sunlight-induced CUVAF. A previous study reported that race correlates well with physician-diagnosed skin phototype and objectively measured skin pigmentation (21). Therefore, we did not adjust for race in **Table 2**, where the central exposures included pigmentary traits. We also found that less sun hat use last summer was associated with increased odds of CUVAF while sunglass use was not. Nevertheless, the hypothesis that Coroneo raised with regard to the UV light leading to pterygium (and presumably CUVAF) was due to scattered lateral light mainly albedo (reflected) UV (22) coming from the side, therefore standard glasses, such as sunglasses, would not greatly reduce it. Wraparound sunglasses and, theoretically, contact lenses should completely block the albedo UV reflecting on the medial limbus and thus reduce CUVAF medially. However, a previous adult study found the use of corrective lenses did not explain the lower CUVAF observed in

myopic participants (23). We did not collect information on wraparound sunglasses and corrective lenses. However, the frequency of wraparound sunglasses and contact lens wear in our young cohort would be very low as only 2.4% reported using sunglasses all times and 14.5% using $\geq 50\%$ time out last summer (as was the frequency of refractive error requiring contact lenses).

We found the association between cumulative leisure-time sun exposure UVR dose and CUVAF disappeared after adjusting for age, indicating age as a confounder might not only be expected to account for any direct effect of advancing years on sun-related exposure but also to act as a surrogate for cumulative sun exposure. However, among children aged 9 to 11 years, higher cumulative leisure-time sun exposure UVR dose remained associated with increased CUVAF (sex-AOR, 1.02; 1.00-1.05, $P=0.04$), indicating sun damage of the eye may reflect cumulative sun exposure of UVR rather than age alone. In support of this, we also found higher numbers of lifetime sunburns, a marker that combined UVR dose and host sun reaction vulnerability, was strongly associated with increased odds of CUVAF. This marker of past sun exposure was not strongly correlated with age. We adjusted for buttock melanin density, an indicator of race, as previous work indicates that race and ethnicity correlates well with objectively measured skin pigmentation ($r=0.39$, $P<0.01$) (21). In our study, buttock melanin density was also strongly correlated with ethnicity (Caucasian, Asian, Middle Eastern, and Other) ($r=0.41$, $P<0.001$).

Our finding of an association (marginal evidence) between higher numbers of nevi count and increased odds of CUVAF concurs with other work that found higher numbers of nevi are associated with increased sun damage to the skin such as lifetime sunburns and melanoma (24, 25). Meanwhile, silicone skin cast score, a valid marker of sun damage in teenagers (8)

and adults (3), was available in our study. However, compared with CUVAf, silicone skin cast scores showed poor reliability in our childhood sample. This may be due to this childhood sun-induced cast being too subtle to detect visually with the human eye. In our study, a sun-induced ocular surface measure using a computer programme seems to be a more reliable marker than the silicone skin cast score and thus may be more suitable to be applied in a paediatric population. Further prospective cohort studies that measure CUVAf at baseline and follow up the children for a certain period would be required to fully validate CUVAf as a marker of past sun exposure. Although previous studies have identified the contribution of fair skin to nevi development and higher silicone skin cast scores (3, 26), this report extends past work by using a potential objective measure of past sun-induced eye damage in a paediatric population. Previous studies using CUVAf in a predominantly adult population also support the notion that larger CUVAf area is associated with increased outdoor activity (4). Intriguingly, in our study, a longer time of composite sun exposure last summer, which has taken into account the amount of skin exposed and whether exposed skin has a sunscreen barrier, was associated with increased odds of CUVAf. This indicates that longer UVR exposure in early life, or higher vitamin D stores, may play a role in the development of sun-related damage on the ocular surface. It has been proposed that UV-mediated limbal damage acts as a trigger for pterygium pathogenesis, and that focusing on the anterior eye results in light being focused with 20 times the intensity of scattered incident light onto the nasal limbus (27). Pterygium histology reveals elastotic degeneration as seen in photo exposed and aged human skin (28). The extracellular matrix changes include accumulation of elastin, glycosaminoglycans, and altered collagen turnover. These changes may contribute to the autofluorescence detected (29).

In conclusion, CUVAf area detected in UVAf photographs could be developed as a reliable measure of past sun exposure in paediatric populations. It is associated with a range of sun-

related risk factors and therefore has potential as an objective marker and an adjunct to other measures of past sun exposure in paediatric epidemiologic studies.

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Table 1. Sun damage detected by conjunctival ultraviolet autofluorescence in relation to demographic characteristics of study group

	All participants (N=339)	With CUVAF (N=53)	Without CUVAF (N=286)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Age, years	9.6 (2.7)	12.2 (1.5)	9.1 (2.6)	1.74 (1.48-2.06)	1.74 (1.47-2.05)
Sex					
Male	154 (45.4)	52.8 (28)	44.1 (126)	1.00 (reference)	1.00 (reference)
Female	185 (54.6)	47.2 (25)	55.9 (160)	0.70 (0.39-1.27)	0.71 (0.36-1.39)
Caucasians					
No	94 (27.7)	13.2 (7)	31.5 (87/276)	1.00 (reference)	1.00 (reference)
Yes	235 (69.3)	86.8 (46)	68.5 (189/276)	3.02 (1.31-6.97)	2.77 (1.13-6.84)

Data show crude mean \pm standard deviation or proportions (n/N if different to the total numbers). Total numbers may vary due to different missing values.

CUVAF: Conjunctival ultraviolet autofluorescence; OR: Odds Ratio; CI: Confidence Interval.

*Adjusted for sex and/or ethnicity and/or age.

Table 2. Relationship of pigmentary traits and sun sensitivity with sun damage detected by conjunctival ultraviolet autofluorescence

	% (n)	With CUVAF (N=46)	Without CUVAF (N=234)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Natural skin colour					
Dark/Olive	16.9 (47)	8.7 (4)	18.5 (43/232)	1.00 (reference)	1.00 (reference)
Olive/Medium	31.7 (88)	23.9 (11)	33.2 (77/232)	1.54 (0.46-5.12)	1.89 (0.51-7.01)
Medium/Fair	42.1 (117)	60.8 (28)	38.4 (89/232)	3.38 (1.12-10.25)	3.42 (1.02-11.48)
Fair	9.4 (26)	6.5 (3)	9.9 (23/232)	1.40 (0.28-6.81)	1.15 (0.21-6.29)
				$P_{\text{trend}}=0.07$	$P_{\text{trend}}=0.23$
Eye colour					
Brown	55.4 (155)	30.4 (14)	60.3 (141)	1.00 (reference)	1.00 (reference)
Hazel	12.1 (34)	15.2 (7)	11.5 (27)	2.61 (0.96-7.07)	1.31 (0.41-4.26)
Green	7.5 (21)	8.7 (4)	7.3 (17)	2.37 (0.70-8.03)	2.11 (0.53-8.33)
Blue/Grey	25.0 (70)	45.7 (21)	20.9 (49)	4.32 (2.04-9.14)	4.07 (1.73-9.55)
				$P_{\text{trend}}<0.001$	$P_{\text{trend}}=0.001$
Buttock melanin density					
1st quartile (0-2.69)	25.3 (62)	36.6 (14/36)	23.4 (48/209)	1.00 (reference)	1.00 (reference)
2nd quartile (2.70-3.86)	24.9 (61)	26.8 (11/36)	24.6 (50/209)	0.75 (0.31-1.82)	1.10 (0.39-3.07)
3rd quartile (3.87-4.63)	24.9 (61)	22.0 (6/36)	25.4 (55/209)	0.37 (0.13-1.05)	0.68 (0.21-2.18)
4th quartile (4.64-5.55)	24.9 (61)	14.6 (5/36)	26.6 (56/209)	0.31 (0.10-0.91)	0.49 (0.14-1.72)
				$P_{\text{trend}}=0.01$	$P_{\text{trend}}=0.21$
Lifetime sunburns					
Never	43.0 (120)	19.6 (9)	47.6 (111/233)	1.00 (reference)	1.00 (reference)
Once	25.8 (72)	13.0 (6)	28.3 (66/233)	1.12 (0.38-3.29)	0.74 (0.23-2.36)
2-4 times	26.5 (74)	54.4 (25)	21.0 (49/233)	6.29 (2.74-14.47)	2.89 (1.14-7.35)
≥5 times	4.7 (13)	13.0 (6)	3.0 (7/233)	10.57 (2.93-38.20)	4.29 (1.04-17.76)
				$P_{\text{trend}}<0.001$	$P_{\text{trend}}=0.004$
Freckling by end of last summer					
No	51.6 (144)	28.3 (13)	56.2 (131/233)	1.00 (reference)	1.00 (reference)
Yes	48.4 (135)	71.7 (33)	43.8 (102/233)	3.26 (1.63-6.51)	3.12 (1.44-6.76)
				$P_{\text{trend}}=0.001$	$P_{\text{trend}}=0.004$
Nevi count on upper arm					
0	32.0 (89)	17.8 (8/45)	34.8 (81/233)	1.00 (reference)	1.00 (reference)
1	14.4 (40)	6.7 (3/45)	15.9 (37/233)	0.82 (0.21-3.27)	0.89 (0.20-3.95)
2	16.9 (47)	8.9 (4/45)	18.5 (43/233)	0.94 (0.27-3.31)	1.26 (0.30-5.27)
3	13.3(37)	17.8 (8/45)	12.5 (29/233)	2.33 (0.72-7.53)	2.19 (0.67-7.15)
≥ 4	23.4(65)	48.9 (22/45)	18.5 (43/233)	2.77 (1.05-7.36)	2.58 (0.97-6.92)
				$P_{\text{trend}}<0.001$	$P_{\text{trend}}=0.03$

Data show proportions (n/N if different to the total numbers). Total numbers may vary due to different missing values.

CUVAF: Conjunctival ultraviolet autofluorescence; OR: Odds Ratio; CI: Confidence Interval.

*Adjusted for age, sex, and cumulative UVR dose.

Table 3. Relationship between leisure-time sun exposure and sun damage detected by conjunctival ultraviolet autofluorescence

	% (n)	With CUVAF (N=33)	Without CUVAF (N=194)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*	Fully adjusted OR (95% CI)†
Cumulative leisure-time sun exposure UVR dose (per 1,000 SED)‡						
19.3-47.4	25.4 (50)	9.4 (3/32)	28.5 (47/165)	1.00 (reference)	1.00 (reference)	1.00 (reference)
47.5-61.6	24.9(49)	15.6 (5/32)	26.7 (44/165)	1.78 (0.40-7.89)	0.57 (0.10-3.08)	0.34 (0.05-2.16)
61.7-82.6	24.9 (49)	28.1 (9/32)	24.2 (40/165)	3.53 (0.89-13.91)	0.81 (0.16-4.02)	0.46 (0.08-2.61)
82.7-182.2	24.9(49)	46.9 (15/32)	20.6 (34/165)	6.91 (1.85-25.76)	0.89 (0.17-4.67)	0.48 (0.08-2.87)
				$P_{\text{trend}}=0.001$	$P_{\text{trend}}=0.73$	$P_{\text{trend}}=0.83$
Time spent in the sun during last summer weekends						
< 1 h	4.1 (8)	34.1 (1/32)	5.7 (11/193)	1.00 (reference)	1.00 (reference)	1.00 (reference)
1-2 h	27.8 (54)	21.9 (7/32)	30.1 (58/193)	1.33 (0.15-11.89)	0.57 (0.05-6.10)	0.50 (0.05-5.37)
2-3 h	35.6 (69)	31.2 (10/32)	34.7 (67/193)	1.64 (0.19-14.13)	1.07 (0.10-11.05)	0.77 (0.07-8.23)
≥ 3 h	32.5 (63)	43.8 (14/32)	29.5 (57/193)	2.70 (0.32-22.71)	2.30 (0.23-22.96)	1.85 (0.19-18.35)
				$P_{\text{trend}}=0.11$	$P_{\text{trend}}=0.02$	$P_{\text{trend}}=0.05$
Sunscreen use last summer						
All times	30.9 (65)	10. (3/30)	33.3 (62/186)	1.00 (reference)	1.00 (reference)	1.00 (reference)
≥50% times out	49.1 (106)	66.7 (20/30)	46.2 (86/186)	4.81 (1.37-16.89)	4.92 (1.29-18.71)	3.84 (0.95-15.45)
<50% times out or never	20.8 (45)	23.3 (7/30)	20.4 (38/186)	3.81 (0.93-15.62)	2.59 (0.57-11.74)	2.77 (0.56-13.67)
				$P_{\text{trend}}=0.06$	$P_{\text{trend}}=0.28$	$P_{\text{trend}}=0.25$
Sun hat wear last summer						
All the time	30.4 (69)	15.2 (5)	33.0 (64)	1.00 (reference)	1.00 (reference)	1.00 (reference)
≥50% of time outside	48.5 (110)	39.4 (13)	50.0 (97)	1.72 (0.58-5.04)	1.59 (0.50-5.03)	1.29 (0.36-4.65)
<50% of time outside or never	19.4 (48)	45.5 (15)	17.0 (33)	5.82 (1.94-17.41)	4.83 (1.45-16.13)	5.45 (1.40-21.01)
				$P_{\text{trend}}=0.001$	$P_{\text{trend}}=0.007$	$P_{\text{trend}}=0.009$
Composite time in sun last summer weekday	6.0 (4.8-7.4)	6.5 (5.0-7.8)	5.8 (4.8-7.8)	1.10 (0.98-1.25)	1.14 (0.96-1.34)	1.12 (0.95-1.33)
Composite time in sun last summer weekend	7.3 (5.4-8.9)	7.5 (7.0-9.8)	7.0 (5.0-8.5)	1.26 (1.07-1.49)	1.33 (1.09-1.62)	1.30 (1.05-1.60)
Composite time in sun last summer holiday	8.3 (6.5-10.0)	9.3 (7.5-10.8)	8.0 (6.3-9.8)	1.26 (1.05-1.50)	1.36 (1.09-1.69)	1.32 (1.05-1.66)

Data show proportions (n/N if different to the total numbers) or median (interquartile range). Total numbers may vary due to different missing values.

CUVAF: Conjunctival ultraviolet autofluorescence; OR: Odds Ratio; CI: Confidence Interval; SED: standard erythemal doses.

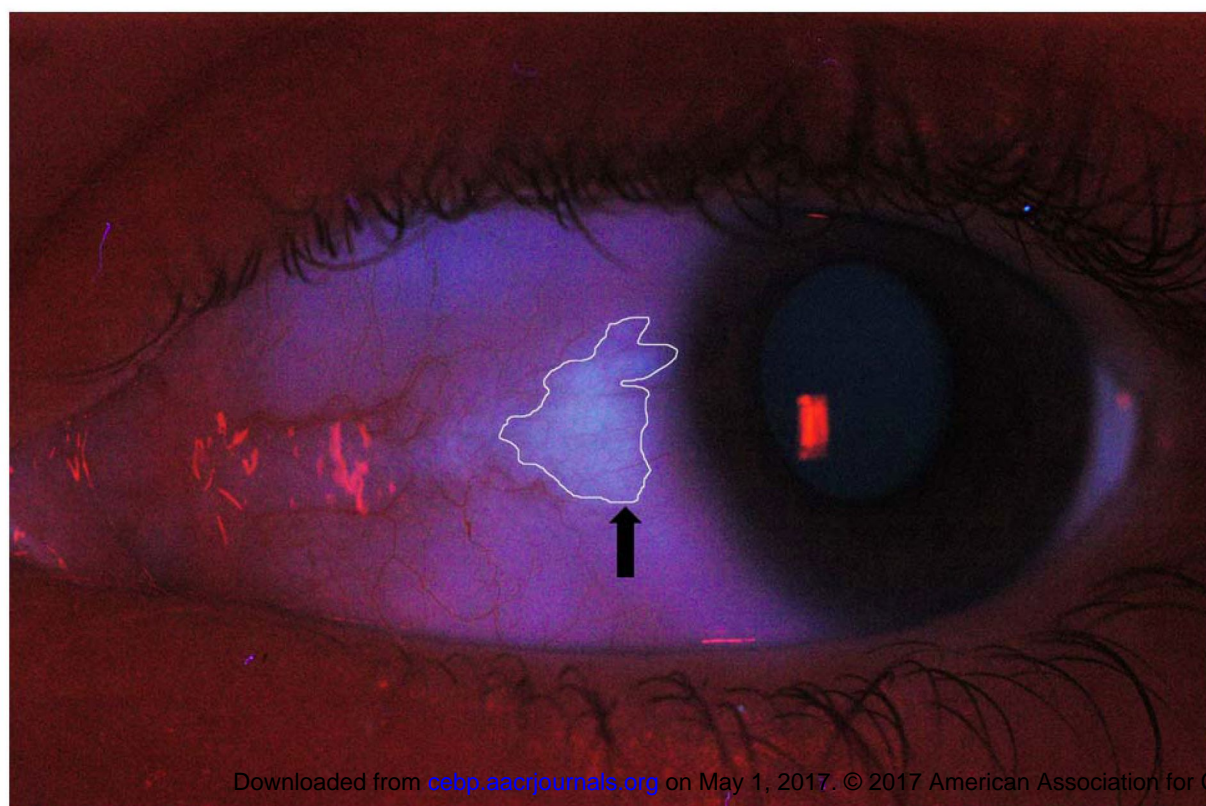
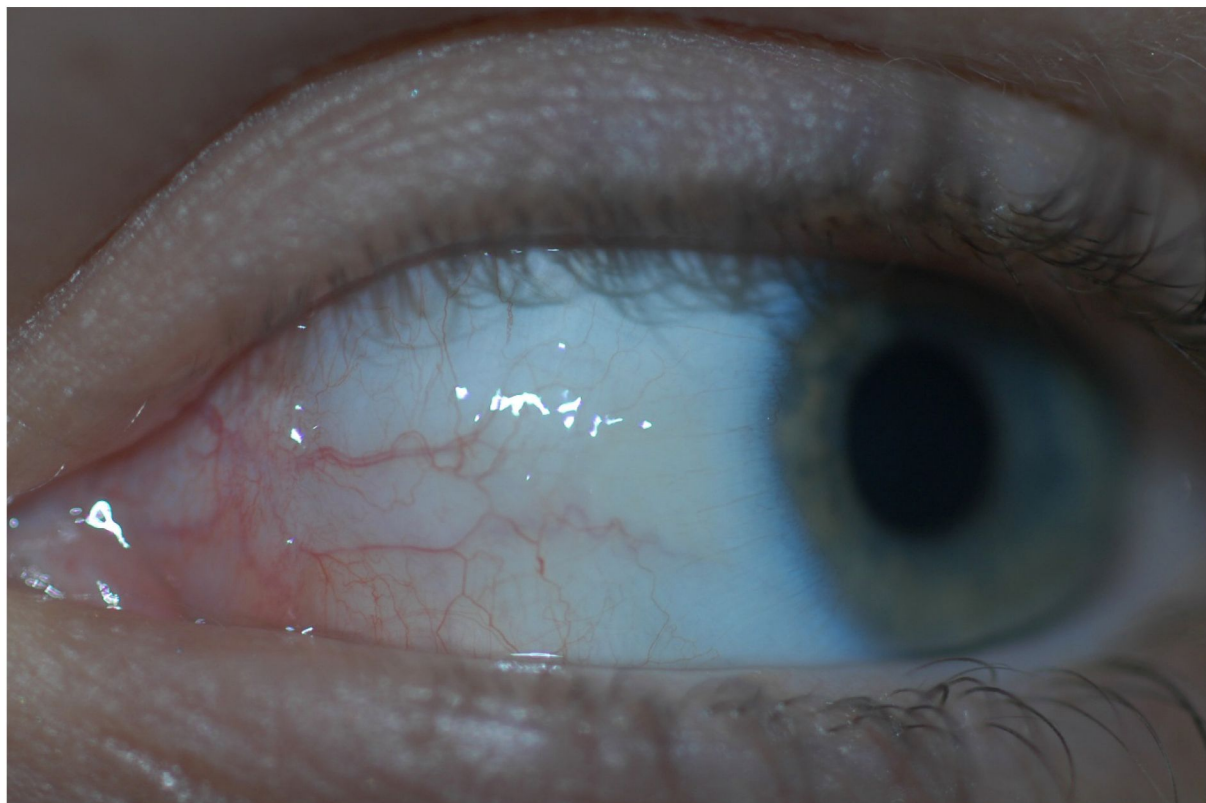
*Adjusted for age and sex.

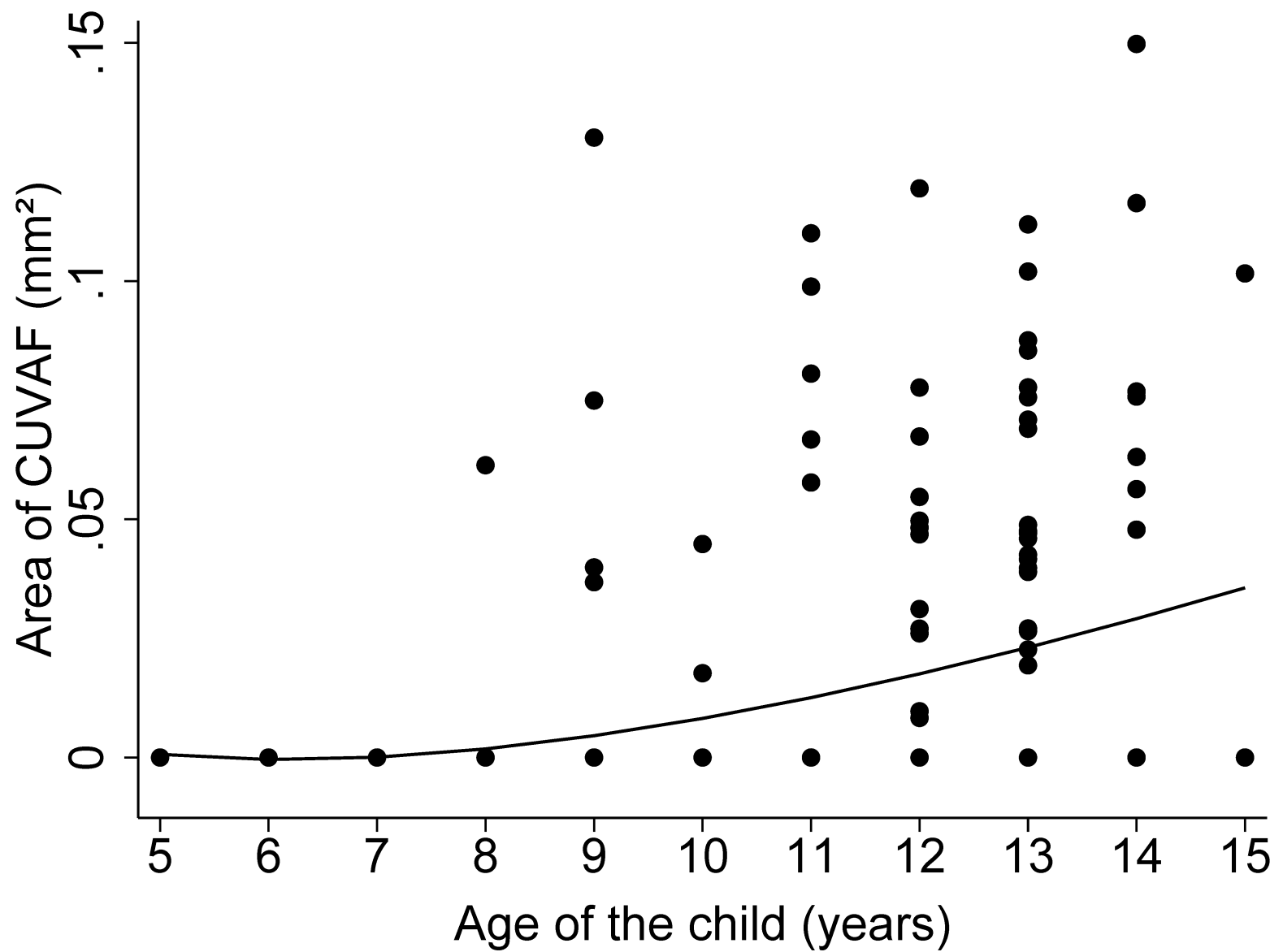
†Adjusted for age, sex, buttock melanin density, and socioeconomic status.

Figure Legends

Figure 1. (Top) Control photograph of left eye nasal region for a 13-year-old girl. (Bottom) Corresponding ultraviolet autofluorescence photograph demonstrated autofluorescence (measured area = 8.2 mm² as pointed by a black arrow).

Figure 2. Age of the child and area of conjunctival ultraviolet autofluorescence (CUVAF) in the children participating as controls of Early Environment and Type 1 Diabetes Prevention Project (excluded one child with extreme damage [CUVAF area=37.6 mm²] and there was little influence of that outlier on the plot).





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Conjunctival ultraviolet autofluorescence as a measure of past sun exposure in children

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