Genetic and Environmental Factors in Conjunctival UV Autofluorescence

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**IMPORTANCE** Conjunctival UV autofluorescence (CUVAF) photography was developed to detect and characterize preclinical sunlight-induced ocular damage. Ocular sun exposure has been related to cases of pterygia and was recently negatively correlated with myopia. Hence, CUVAF has excellent potential as an objective biomarker of sun exposure. However, much variation in CUVAF has been observed, and the relative contributions of genes and environment to this variation have not yet been identified.

**OBJECTIVE** To investigate sources of variation in CUVAF in relation to its potential clinical relevance.

**DESIGN, SETTING, AND PARTICIPANTS** We performed a cross-sectional analysis of 3 population-based cohort studies in the general community, including the Twins Eye Study in Tasmania, the Brisbane Adolescent Twin Study, and the Western Australian Pregnancy Cohort (Raine) Study. The twin studies were conducted between 2001 and 2009, and the 20-year follow-up of the Raine Study was completed between March 2010 and February 2012. We included genotypic and phenotypic data from 295 Australian families in the Tasmanian and Brisbane twin studies and from 661 participants in the 20-year follow-up of the Raine Study. We compared CUVAF levels in the 3 cohorts and performed a classic twin study to partition variation in CUVAF. We also conducted a genome-wide association analysis to identify specific genetic variants associated with CUVAF.

**MAIN OUTCOMES AND MEASURES** The total area of CUVAF, heritability of CUVAF, and single-nucleotide polymorphisms (SNPs) associated with CUVAF from the genome-wide association study.

**RESULTS** Within twin cohorts, individuals living closer to the equator (latitude, 27.47° S) had higher levels of CUVAF compared with individuals from southern regions (latitude, 42.88° S) (median [interquartile range], 45.4 [26.8-68.5] vs 28.7 [15.0-42.3] mm²; \( P < .001 \)). The variation in CUVAF explained by the additive genetic component was 0.37 (95% CI, 0.22-0.56), whereas the variation due to the common environment was 0.50 (95% CI; 0.29-0.71). The SNP rs1060043, located approximately 800 base pairs away from the SLC1A5 gene, a member of the solute carrier family 1, had a genome-wide significant association with a \( P \) value of \( 3.2 \times 10^{-8} \). Gene-based analysis did not improve our power to detect association with other genes.

**CONCLUSIONS AND RELEVANCE** Our findings confirm that, although a large environmental component to CUVAF (equivalent of sun exposure) exists, genes also play a significant role. We identified a SNP (rs1060043) as being significantly associated with CUVAF; replication of this finding in future studies is warranted.
Excessive sun exposure, in particular UV light, increases the risk for many ocular diseases, including pterygia,7 corneal cataract,8 ocular surface squamous neoplasia,9 climatic droplet keratopathy,10 and malignant neoplasms of the eyelid.9 Despite early work suggesting that sun exposure has a role in the pathogenesis of age-related macular degeneration11 and ocular melanoma,7 these associations remain inconclusive. In recent years, a considerable number of epidemiological studies have reported that increased time spent outdoors is associated with lower rates of myopia in children, suggesting that sunlight brightness or UV light may have a beneficial effect.4 These conflicting reports on the effects of sun exposure require a better understanding of the mechanisms underlying ocular sun damage and related eye diseases.

A challenge of studying ophthalmohelioses9 (sun-related ocular diseases) is the difficulty of assessing sun exposure. The usual method of determining an individual’s sun exposure is by self-reported questionnaire, which is subject to recall error. Questions often are designed to assess whole-body sun exposure rather than ocular sun exposure; thus, the accuracy of these measures in ocular diseases is arbitrary. Conjunctival UV autofluorescence (CUVAF) photography was developed to detect precursors of ocular sun damage using a technique similar to UV fluorescence in the detection of UV exposure–related dermatologic diseases.10 Previous studies have reported an association of CUVAF with the presence of pterygia11 and shown that increasing total area of CUVAF is associated with increasing prevalence of pterygia.12 Time spent outdoors correlates highly with the area of CUVAF,8 which suggests that CUVAF could be regarded as an objective measure of sun damage corresponding to the amount of time spent outdoors and could help to characterize local sun exposure.

Multiple biological mechanisms have been proposed to explain the cause of detected CUVAF in other tissues. These mechanisms include alterations of collagen cross-linking or changes in cell metabolites, such as reduced levels of nicotinamide adenine dinucleotide, or derivatives of amino acids, such as tryptophan.13

 Conjunctival UV autofluorescence can be an ideal biomarker of ophthalmohelioses once its characteristics are defined better. In the present study, we sought to determine whether a genetic predisposition to variation in CUVAF area exists in 3 Australian cohorts. However, given that sun exposure depends on geographic location, we investigated the effect of latitudinal differences on CUVAF distribution. After this analysis, we explored the contribution of genes to CUVAF area variation through a classic twin study and a genome-wide association study (GWAS).

Methods

Ethics Approval

This study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all adult participants and from the parents of minors. Approval for this study was obtained from the human research ethics committees of the University of Tasmania, Tasmania, Australia; Royal Victorian Eye and Ear Hospital, Melbourne, Australia; QIMR Berghofer Medical Research Institute, Brisbane, Australia; Princess Margaret Hospital, Perth, Australia; and University of Western Australia, Perth, Australia.

Participants

This study included 2 twin cohorts and 1 singleton cohort, each of northern European ancestry and all from Australia. The twin pairs were identified from 2 existing cohorts starting in 2001, the Twins Eye Study in Tasmania (TEST)14 and the Brisbane Adolescent Twin Study (BATS).15 Methods of these studies were described in detail previously.14,15 In brief, a total of 487 twin pairs (200 monozygotic [MZ] and 287 dizygotic [DZ] twin pairs) were recruited in the TEST through several overlapping methods, including use of the national twin registry and existing statewide studies. A total of 2443 individuals who were enrolled in the BATS were invited to participate in the twin eye study. Among the 1199 individuals who agreed to participate were 185 MZ and 278 DZ twin pairs. The Western Australian Pregnancy Cohort (Raine) Study16,17 is an ongoing longitudinal birth cohort of 2868 individuals whose mothers were recruited initially to evaluate prenatal ultrasonography. Their offspring subsequently underwent assessment in detail during childhood (ages 1, 2, 3, 5, 8, and 10 years) and adolescence (ages 14 and 17 years). At the 20-year cohort follow-up, 1344 participants underwent an ocular examination from March 2010 through February 2012.18 Comparison between the individuals who did and did not participate in the 20-year follow-up has been presented previously.19

Quantitative Analysis of CUVAF

A camera system developed by Ooi and colleagues11,20 was used to obtain CUVAF images for each participant. The camera system included a height-adjustable table equipped with a headrest, camera-positioning assembly, digital single-lens reflex camera (D100; Nikon), 105-mm f/2.8 lens (Micro-Nikkor; Nikon), and filtered electronic flash. The nasal and temporal regions of both eyes were photographed at a magnification of ×0.94 in total darkness. All images were saved in RGB format at the D100 settings of JPEG fine (compression, 1:4) and large resolution (3000 × 2000 pixels). The area of fluorescence in square millimeters for each photograph was determined using graphics editing software (Adobe Photoshop CS4 Extend; Adobe Systems, Inc). Reliability of CUVAF as a biomarker of sunlight exposure has been validated previously.21

Questionnaire

As part of the Raine Study 20-year examination, participants were asked to complete questionnaires regarding their socioeconomic status, medical history, and sun exposure. In relation to sun exposure, participants were asked to estimate time spent outdoors, with 4 possible responses to the question, “In the summer, when not working at your job or at school, what part of the day do you spend outside?” Responses included none, less than one-quarter of the day, approximately half of the day, and more than three-quarters of the day. Responses of none and less than one-quarter of the day were combined into a single group owing to the low numbers who responded none. Only socioeconomic status and medical history questionnaires were available for the TEST and BATS cohorts.
The study analysis was divided into 3 main components: (1) a comparison of CUVAF areas between the TEST and BATS cohorts to identify the effect of latitude; (2) a classic twin study using the TEST and BATS cohorts to estimate heritability of CUVAF; and (3) a meta-GWAS analysis of CUVAF to identify common variants associated with this measurement by pooling data from all 3 cohorts.

**Genotype Imputation**

The TEST and BATS cohorts were imputed against the August 4, 2010, version of the publicly released 1000 Genomes Project European genotyping using MaCH software. Likewise, the Raine Study cohort was imputed against the November 23, 2010, version of the 1000 Genome Project European genotyping using MaCH. We included SNPs with a minor allele frequency of greater than 0.01 and MaCH Rsq of greater than 0.3.

**GWAS of CUVAF**

The GWAS of the twin cohorts and the Raine Study cohort were conducted separately. Associations of 7 773 124 SNPs (439 454 genotyped) of 295 families from the TEST and BATS cohorts were performed using a multipoint engine for a rapid likelihood inference (Merlin) computer program, with the addition of the age, sex, and latitude as covariates in a linear model. For the Raine Study cohort, a linear regression model in R software with a PLINK interface was used to determine associations between 9 131 795 SNPs (561 216 genotyped) and CUVAF. In this cohort, reported time spent outdoors showed a correlation with CUVAF ($r = 0.19; P < .001$). Hence, reported time outdoors was included as a covariate with age and sex for the 661 individuals who remained in the analysis. An inverse variance–weighted meta-analysis with common SNPs imputed in both cohorts ($n = 5 003 381$) was conducted using METAL. A pathway analysis was performed by combining SNP $P$ values obtained from the Raine and TEST/BATS cohort analyses in the Versatile Gene-Based Association Study (VEGAS) analysis tool.

### Results

After quality control, 590 participants of 295 families from the TEST/BATS cohorts and a total of 661 unrelated participants from the Raine Study cohort had complete data available and were included in the present study. Characteristics of these 3 groups are given in Table 1. The age range varied between the cohorts, with the mean (range) age being 12 (5-51), 19 (13-28), and 20 (18-22) years in the TEST, BATS, and Raine Study cohorts, respectively. The TEST and BATS cohorts included more female participants (55.2% and 56.7%, respectively), whereas the Raine Study cohort included more male participants (52.1%). Sex and age were correlated with CUVAF at $r = -0.09 (P = .001)$ and $r = 0.07 (P = .01)$, respectively, in the pool of 3 cohorts.

**Effect of Latitude in the Distribution of CUVAF**

We compared areas of CUVAF in the 2 twin cohorts based on their geographic locations. Of the 590 individuals, 146 were from Tasmanian-born data from all 3 cohorts.

#### Table 1. Demographic Characteristics of CUVAF Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TEST Cohort</th>
<th>BATS Cohort</th>
<th>Raine Study Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>146</td>
<td>444</td>
<td>661</td>
</tr>
<tr>
<td>No. of families</td>
<td>73</td>
<td>222</td>
<td>661</td>
</tr>
<tr>
<td>Age, mean (range), y</td>
<td>12 (5-51)</td>
<td>19 (13-28)</td>
<td>20 (18-22)</td>
</tr>
<tr>
<td>No. of MZ/DZ twins</td>
<td>26/47</td>
<td>124/98</td>
<td>NA</td>
</tr>
<tr>
<td>Female sex, %</td>
<td>55.2</td>
<td>56.7</td>
<td>47.9</td>
</tr>
<tr>
<td>CUVAF area, median (IQR), mm²</td>
<td>28.7 (15.0-42.3)</td>
<td>45.4 (26.8-68.5)</td>
<td>44.2 (20.3-69.8)</td>
</tr>
</tbody>
</table>

Abbreviations: BATS, Brisbane Adolescent Twin Study; CUVAF, conjunctival UV autofluorescence; DZ, dizygotic; IQR, interquartile range; MZ, monozygotic; NA, not applicable; TEST, Twins Eye Study in Tasmania.

* Indicates the Western Australian Pregnancy Cohort Study. 
mania (Hobart latitude, 42.88° S) and 444 were from Queensland (Brisbane latitude, 27.47° S). The median CUVAF area was greater in individuals from Queensland (45.4 [interquartile range (IQR), 26.8-68.5] mm²) compared with that of individuals from Tasmania (28.7 [IQR, 15.0-42.3] mm²) (P < .001). To ensure that this difference did not result from a confounding effect of a difference in age and sex distribution within the 2 twin cohorts, we adjusted CUVAF for age and sex before the comparison. The difference remained, with median CUVAF area being 43.4 (IQR, 26.5-66.7) mm² in individuals from Queensland and 30.9 (IQR, 19.0-47.3) mm² in individuals from Tasmania (P < .001). Moreover, a similar difference was present when the analysis was restricted to younger twin pairs (10-20 years) (BATS cohort, 47.4 [IQR, 27.9-66.4] mm²; TEST cohort, 37.5 [IQR, 23.6-48.5] mm²; P = .006).

CUVAF Heritability

Of the 295 twin pairs included in the analysis, 150 (50.8%) were MZ twins. The pairwise correlation coefficient of CUVAF was 0.88 for MZ twins and 0.70 for DZ twins. The slightly higher correlation of MZ twins suggests a stronger common environmental contribution for the phenotype variance compared with the genetic contribution under a classic twin model. This observation was confirmed by results of univariate model fitting. The best-fit model was an ACE model (A indicates additive genetic effects; C, common environment effects; and E, unique environment effects) adjusted by age and sex. With this model, we estimated the variation explained by the additive genetic component to be 0.37 (95% CI, 0.22-0.56), whereas the common environment component explained 0.50 (95% CI, 0.29-0.71) of the variability of the trait.

Genome-wide Association

A genome-wide significant locus rs1060043 at P = 3.2 × 10⁻⁸ (SLC1A5 [HGNC 6510]) and other suggestive loci (MSANTD3 [rs1213], HDAC7 [rs7309814], and SPAG9 [rs1558253]) are shown in Figure 1 and summarized in Table 2. The effect size of the CUVAF-increasing allele was 11.34 mm² per copy. Figure 2 shows the region around the rs1060043 locus (DACT3, GNG8, CALM3, PTGIR, PPP5D1, MIR320E, STRN4, PRKD2, DACT3-AS1, FKRP, SLC1A5, SNAR-E, AP2S1, and ARHGAP25). The top 10 CUVAF-associated genes obtained from the gene-based test using VEGAS and SNP meta-analysis P value estimates are given in Table 3.

Discussion

A strong relationship between CUVAF and sun-related ocular damage has been reported previously, suggesting that CUVAF could serve as a useful biomarker of ophthalmohelioses. In this study, we investigated the genetic characteristics of CUVAF.
Given the possible confounding effect of geographic location on CUVAF, we initially explored the areas of CUVAF in 2 geographic regions defined by latitude in 2 ethnically homogeneous twin cohorts of European ancestry and identified smaller areas of CUVAF in individuals from a region of lower ambient UV radiation (Tasmania). Although previous studies report that individuals from a region of higher ambient UV radiation (Brisbane) spend less time outdoors compared with other regions of Australia, including Tasmania, the intensity of UV exposure in Tasmania is lower. The finding of greater areas of CUVAF in Brisbane is consistent with previous work by Wlodarczyk et al, who reported that Queensland had double the rate of surgery for pte-
Corneal epithelium cells (genes have been detected in human cornea, rabbit cornea, and of the genes that belong to the best VEGAS pathway result was rhesus monkeys, chimpanzees, cattle, and dogs, suggesting that zebra fish and among multiple mammalian species, including iris color. Many of the genes that belong to the SLC1 gene family and the SLC genes have been detected in human cornea, rabbit corneas, and corneal epithelium cells (SLC1A4, SLC6A14, and SLC7A5). Variants in SLC4A4 and SLC24A4 influence pigmentation traits, including iris color. The particular SNP identified in this study gives rise to a synonymous codon that is highly conserved in normal DNA repair mechanisms, such as xeroderma pigmentosum and porphyria cutanea tarda, polymorphous light eruption, and possibly Cockayne syndrome.

To further understand the genetic contribution to the development of CUVAF, we conducted a GWAS in both twin cohorts and the Raine Study cohort. The meta-analysis of the GWAS allowed the identification of a significant association of rs1060043, which is located 800 base pairs upstream of the solute carrier family 1 (neutral amino acid transporter), member 5 (SLC1A5) gene on chromosome 19q13. SLC1A5 is a peptide transporter gene expressed in retinal Müller cells and also serves as an effluxer of D-serine agonist in N-methyl-D-aspartate receptor sites. Many of the genes that belong to the SLC1 gene family and the SLC genes have been detected in human cornea, rabbit corneas, and corneal epithelium cells (SLC1A4, SLC6A14, and SLC7A5). Variants in SLC4A4 and SLC24A4 influence pigmentation traits, including iris color. The particular SNP identified in this study gives rise to a synonymous codon that is highly conserved in zebra fish and among multiple mammalian species, including rhesus monkeys, chimpanzees, cattle, and dogs, suggesting that this gene has a critical function in mammals. The only locus in the best VEGAS pathway result was C2orf58 (NCBI Entrezgene 205428). This gene, and none of the other genes identified in the gene-based analysis, had an ocular function.

The present study was designed to investigate whether genetic and environmental factors play a role in the development of CUVAF. This investigation had 3 important results. First, individuals living in areas with higher levels of UV radiation are more likely to have increased areas of CUVAF. Second, although CUVAF was caused primarily by environmental factors, genetic factors also play a role in its development. Finally, we detected a susceptibility locus related to CUVAF. Although the study successfully demonstrated these findings, certain limitations in terms of its design and sample size must be acknowledged. For example, the inclusion of older adult twin pairs may have caused a selection bias when comparing the role of environment in the presentation of CUVAF. On the other hand, when the analysis was restricted to younger twin pairs, the effect of latitude on CUVAF remained the same. Thus, this finding indicated that the effect of older individuals was minimal on the representation of the young twin pairs in the present study. Moreover, single GWASs are commonly underpowered. Our twin and singleton discovery cohorts were limited in sample size, which resulted in the detection of inconsistent signals in individual cohort analysis. This issue was overcome by performing a meta-analysis that resulted in more reliable outcomes.

Conclusions

Overall, the present findings add to a growing body of literature contributing to the understanding of CUVAF development. Further research investigating the role of genetics and the environment would assist in identifying individuals who are predisposed to ocular sun damage to recommend personalized health messages.
Study concept and design: Yazar, Cuellar-Partida, McKnight, Coroneo, Hewitt, MacGregor, Mackay. Acquisition, analysis, or interpretation of data: Yazar, Cuellar-Partida, McKnight, Quach-Thansii, Mountain, Pennell, Hewitt, MacGregor, Mackay. Drafting of the manuscript: Yazar, Cuellar-Partida, Quach-Thansii, Coroneo, MacGregor, Mackay. Critical revision of the manuscript for important intellectual content: Yazar, Cuellar-Partida, McKnight, Mountain, Pennell, Hewitt, MacGregor, Mackay. Statistical analysis: Yazar, Cuellar-Partida, Quach-Thansii, MacGregor. Obtained funding: Coroneo, Pennell, Hewitt, MacGregor, Mackay. Administrative, technical, or material support: Yazar, McKnight, Mountain, Coroneo, Pennell, Mackay. Study supervision: Hewitt, MacGregor, Mackay.

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