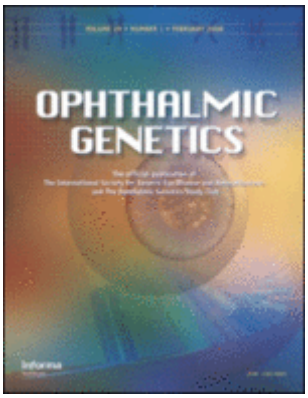

Estimation of heritability and familial correlation in myopia is not affected by past sun exposure

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Estimation of heritability and familial correlation in myopia is not affected by past sun exposure

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Abstract

Purpose:

To consider the effect of including past sun exposure in estimating heritability and familial correlation of myopia-related traits.

Methods:

We calculate the familial correlation and heritability of anterior chamber depth (ACD), axial length (AL), corneal curvature (CC) and spherical equivalent (SphE), with or without past sun exposure as a covariate, in a large number of unrelated nuclear families from the Raine Study (parents: Gen1, offspring: Gen2) residing in Perth, Australia, a city with a high amount of daily sunlight. Past sun exposure was objectively measured using conjunctival ultraviolet autofluorescence (CUVAF) photography.

Results:

When sun exposure was not included in the analysis, both familial correlation (correlation±SE; ACD: 0.308±0.065, AL: 0.374±0.061, CC: 0.436±0.063, SphE: 0.281±0.070) and heritability (ACD: 0.606±0.104, AL: 0.623±0.098, CC: 0.793±0.079, SphE: 0.591±0.106) were significant (all $P < 0.001$) for all traits. However, there was no significant change in both familial correlation and heritability estimates when sun exposure was included as an additional covariate.

Conclusions:

Past sun exposure does not affect the estimation of the additive genetic component in myopia-related traits.

Running Head

Myopia heritability not affected by sun history

Keywords

Myopia, heritability, additive genetic variance, nuclear family, sun exposure, the Raine Study

Introduction:

Myopia is a multifactorial disease arising from a mismatch of the ocular biometry parameters responsible for determining how light rays are focused on the retina (1). Light rays in a myopic eye converge in front of rather than on the retina, resulting in image defocus. The prevalence of myopia is increasing worldwide (2-4), notably in South East Asia where the disease has reached epidemic levels (4). However, much work is still required to understand the aetiology of myopia in order to develop effective treatment and prevention strategies.

It is well accepted that both environmental and genetic factors interact to underpin the overall phenotype in myopia (5, 6). Studies have shown that increased education drives the increase in the prevalence of myopia (7, 8). Conversely, it has been shown that greater outdoor light exposure is inversely related to the myopia prevalence (9-11) and myopia progression (12). However, the relationship between education and sun exposure in myopia is not straightforwardly antagonistic. It has been reported that an increase in education level results in a follow-on decrease in time spent outdoor.(7, 8, 13) Hence there may be an interactive effect between the two factors. To date, the exact mechanism(s) behind the opposing effects of education and sun exposure on myopia are unclear. Animal studies suggest dopamine pathways are involved. More specifically, bright light has been shown to increase dopamine activity which in turn inhibits myopia progression in chicken (14), mouse (15) and monkey (16) myopia models. A recent genetic study also implicated light-induced signaling pathways playing a crucial role in human myopia (17). The authors performed genome-wide association meta-analysis in 160,420 participants and replication in 95,505 participants, reported over 160 risk loci and noted light-dependent retina-to-sclera signaling cascade as a major pathway for myopia development. Taken together, the above studies provide strong evidence that light exposure history is intrinsically involved in myopia development. However, it is not clear whether light exposure history interacts with the genetic variance and protects those who are susceptible to myopia.

Given the complexity associated with identifying and examining the relationship between environmental and genetic contributions in myopia, one can break this down into two components. First, instead of considering myopia as a whole, one can investigate quantifiable

ocular traits associated with the disease. The four ocular traits commonly reported in myopia are spherical equivalent (SphE; extent of myopia in the eye, as calculated by the sum of spherical refractive error and half cylindrical refractive error), anterior chamber depth (ACD; the distance between the apex of anterior lens and the corneal endothelium), axial length (AL; distance from the anterior to posterior poles of the ocular globe) and corneal curvature (CC; radius of curvature of the front surface of the eye, defined as the average of horizontal and vertical meridians, measured in dioptres of keratometric power) (1). Second, instead of quantifying the contribution of individual molecular components, one can investigate the overall genetic contribution to each of the four aforementioned traits by calculating the heritability. Heritability calculation assumes that the phenotypic variance of a quantitative trait can be decomposed into genetic and environmental components and is time- and population-specific due to variations in environmental and genetic influences (18). The genetic component can be further subdivided into additive (polygenic), dominance (interaction between alleles at the same locus) and epistatic (interaction between alleles at different loci) variance (19-21). When estimating inter-generation heritability, dominance and epistatic components are assumed to be zero; hence the ratio of the additive variance to the overall phenotype variance (i.e. heritability in the narrow sense, h^2) is typically reported (18).

Heritability studies in myopia have been conducted using twin- (22, 23), sibling- (24, 25) and population-based (26-28) cohorts. However, one common issue with twin- and sibling-based studies is that they do not appropriately account for shared environments in the modelling (29). Furthermore, previous heritability studies of traits associated with myopia usually accounted for age, sex and education in the modelling but did not consider the effect of past sun exposure; a key environmental factor which has been shown to be inversely related to myopia (10, 11). Conjunctival ultraviolet autofluorescence (CUVAF) has been shown to be an objective marker of sun exposure and will be utilized in in this study to investigate whether variation in sun exposure has a confounding effect on estimating heritability of myopic traits. It has been reported that CUVAF is influenced by both environment (50%) and genetics (37%) (30). Given the inverse relationship between light exposure and myopia development in both animal (14-16) and human (9, 10) studies, we set out to investigate whether light history will affect the estimated heritability in myopia. In this study, we examined the heritability of myopic traits in a large number of

unrelated nuclear families residing in Perth, Australia. We asked whether the heritability of myopia-related traits is modified when sunlight exposure history is included in the modelling.

Materials and Methods

Study participants

This was a cross-sectional, multi-generational, population-based study conducted as part of the Raine Study, which has been described previously (31-33). In brief, 2900 pregnant women were recruited in Perth, Australia, between May 1989 and November 1991. Since birth, the offspring have undergone serial health and socio-economic assessments, including a condensed eye examination at 27 years of age (2016 – 2018; Gen2-27 year follow-up). The same eye examination was performed on the parents around the same time (2015 – 2017; Gen1-26 year follow-up). Individuals who had ocular diseases that could affect myopia (i.e. keratoconus, glaucoma, lenticular opacification, amblyopia) or who had laser refractive or cataract surgery were excluded. The study was conducted in accordance with the tenets of the Declaration of Helsinki and the study protocol was approved by the Human Research Ethics Committee at the University of Western Australia. Informed consent was obtained from all participants.

Eye Examination

In both generations, ACD, AL and CC were measured with a noncontact partial coherence interferometry (IOL Master; Carl Zeiss Meditec AG, Jena, Germany). SphE was calculated from non-cycloplegic autorefraction (Nidek ARK-1, Nidek Co Ltd, Gamagori, Japan). Past sun history was objectively measured by conjunctival ultraviolet autofluorescence (CUVAF) photography which has been previously validated (11, 34, 35). This involves taking high-resolution digital images (Nikon D100 coupled with a 105 mm 147 f/2.8 Macro Nikkor lens; Nikon, Shinagawa, Tokyo, Japan) of the nasal and temporal conjunctiva in both eyes using ultraviolet flash illumination (Metz 36C-2, Metz, GmbH, Zirndorf, Germany fitted with Wratten glass filters). CUVAF areas were measured with a custom, semi-automated MATLAB program (36). Education attainment data, if available, were categorised into four levels for each individual (1: primary school, 2: secondary school, 3: vocational training, 4: university).

Statistical Analysis

Pedigrees were included in the analysis if the nuclear family requirement was met (i.e. father, mother, offspring). Only right eye data were incorporated into the analysis. Familial correlation of ACD, AL, CC and SphE were estimated using the Family Correlation (FCOR) analysis found in the S.A.G.E. (Statistical Analysis for Genetic Epidemiology; Statistical Solutions, Ltd., Cork, Ireland) package. However, as FCOR does not incorporate covariates into the analysis, *R* statistical environment (R Foundation for Statistical Computing, Vienna, Austria) was utilised to calculate the residual between each phenotype measurement and modelled prediction after adjusting for covariates (continuous covariate: age; categorical variables: sex, education attainment) (37). The residuals were then input into the FCOR analysis to estimate familial correlation for each trait. To estimate the effect of sun exposure on familial correlation, a separate set of FCOR analysis for the aforementioned myopic traits was also performed with CUVAF area inbuilt as an additional covariate in residual calculation in R.

The ASSOC procedure in S.A.G.E. (19), which utilises a maximum likelihood approach, was employed to estimate heritability. A linear regression model with sex, age and education attainment defined as covariates, was generated by the ASSOC algorithm, which partitioned the residual variance into a subject-specific and an additive polygenic component. Heritability of SphE, ACD, AL and CC were estimated by dividing the polygenic component by the total residual variance (19, 21). Age, education attainment and sex were built into the model as covariates. A separate set of ASSOC analysis was also conducted with CUVAF area built in as an additional covariate to estimate the effect of sun exposure on heritability estimates.

Results

Study demographic

A total of 992 individuals from 328 pedigrees were analysed (Table 1). The average family size was 3.02, with 13 families with 2 sibling members only, 21 families with 4 members (i.e. parents and 2 offspring) and the majority (294 families) with 3 members (i.e. parents and 1 offspring) in a nuclear family. Mean age was 57.0 years (SD 5.6 years) in the parent generation and 26.6 years (SD 0.4 years) in the offspring generation. Educational attainment was overall higher in the offspring generation (Table 2; chi-square test, $P < 0.05$), as was CUVAF area (mean ±SD, parents: 29.26 ± 26.79 mm^2; offspring: 44.79 ± 30.60 mm^2; t-test between two generations $P <$

0.05). Additionally, CUVAF area was smaller in myopic (SphE ≤ -0.50DS) compared to non-myopic (SphE > -0.50DS) eyes for both the parents (27.71±27.79 vs. 29.76±26.36 mm^2) and offspring (42.66±29.37 vs. 46.79±31.63 mm^2). SphE data were available from 582 individuals from the parent generation (mean: -0.10 ± 1.94 D) and 309 individuals from the offspring generation (mean: -0.78 ± 1.41 D). An unpaired t-test ($P < 0.001$) revealed significantly more negative SphE (i.e. greater degree of myopia) in the offspring compared to the parents. Similarly, ACD was significantly greater in the offspring (parents: 3.26 ± 0.32 mm; offspring: 3.61 ± 0.28 mm; $P < 0.001$). However, no statistical difference between the two generations was detected in AL (parents: 23.77 ± 1.02 mm; offspring: 23.71 ± 0.93 mm; $P = 0.35$) or CC (parents: 43.70 ± 1.50 D; offspring: 43.68 ± 1.43 D; $P = 0.83$).

Familial correlation and heritability

Due to the small sample sizes, familial correlation between siblings was not calculated. Figure 1 (filled symbol) shows that, for SphE, there was a significant correlation between the parents and their offspring (205 pairs, correlation ± SE: 0.281 ± 0.070, 95% CI: 0.14 – 0.42, $P < 0.001$) after adjusting for sex, age, education attainment and CUVAF area but not between spouse pairs (249 pairs, correlation ± SE: 0.043 ± 0.064, 95% CI: -0.08 – 0.17, $P = 0.50$). Correlations between parent-offspring were found for AL (parent-offspring: 206 pairs, correlation ± SE: 0.374 ± 0.061, 95% CI: 0.25 – 0.50, $P < 0.001$; spouses: 252 pairs, correlation ± SE: 0.034 ± 0.063, 95% CI: -0.09 – 0.16, $P = 0.59$) and ACD (parent-offspring: 204 pairs, correlation ± SE: 0.308 ± 0.065, 95% CI: 0.18 – 0.43, $P < 0.001$; spouses: 261 pairs, correlation ± SE: 0.015 ± 0.063, 95% CI: -0.11 – 0.14, $P = 0.81$), but with no correlation between spouses. CC was significantly correlated between parents and offspring as well as between spouses (parent-offspring: 206 pairs, correlation ± SE: 0.436 ± 0.063, 95% CI: 0.31 – 0.56, $P < 0.001$; spouses: 252 pairs, correlation ± SE: 0.155 ± 0.062, 95% CI: 0.03 – 0.28, $P < 0.05$). Familial correlation values for all four traits were similar when estimated without including CUVAF area as a covariate (unfilled symbols).

Table 3 lists the heritability of SphE, AL, ACD and CC after adjusting for age, education attainment and sex. Heritability estimates were also calculated with or without CUVAF as an additional covariate. With CUVAF included as a covariate, heritability of SphE was moderately high at 0.583 ± 0.106 (95% CI: 0.375 – 0.791, $P < 0.001$). Heritability of ACD and AL was

similar (ACD: 0.564 ± 0.112, 95% CI 0.344 – 0.784; AL: 0.623 ± 0.098, 95% CI 0.420 – 0.804; both $P < 0.001$). CC had the highest heritability at 0.779 ± 0.082 (95% CI 0.618 – 0.940, $P < 0.001$). In all four traits, heritability calculations without including CUVAF area as a covariate returned non-significant increases.

Discussion

The heritability of myopic parameters has been estimated in different cohorts, by examining data from twins (22, 23), siblings (24, 25), or population-based pedigrees (26-28). Heritability values estimated from the current, pedigree-based study is, as expected, lower than that in twin or sibling studies. For example, in twin studies, the heritability of SphE has been estimated between 0.61 and 0.94 (22, 23, 29, 38-40). Similarly, sibling pair studies have reported SphE heritability between 0.69 to 0.90 (24, 25). The reason behind the higher heritability estimates in the twin and sibling studies could be due to the higher shared family environment arising from the lack of cross-generation or cohort effects (29) and the inclusion of dominance effects in twin and sibling heritability calculations (41). Therefore it is more rational to compare heritability values in the current study to other pedigree-based studies. In a previous pedigree-based study conducted in Melbourne, Australia (132 pedigrees, 723 individuals, family size range 2 to 36 members), the heritability of SphE, ACD, AL and CC estimated with childhood shared environment was 0.50, 0.78, 0.73 and 0.16, respectively (28). A similarly sized pedigree study in Beaver Dam, Wisconsin, USA (189 pedigrees, 715 individuals) had similar heritability estimates for SphE (0.58), ACD (0.78) and AL (0.64) but a much greater value for CC (0.95) (42). We found, without including CUVAF area as a covariate in order to be comparable to the previous studies, similar SphE heritability (0.59) but a slightly lower slightly lower heritability for ACD and AL (0.61 and 0.62, respectively). Interestingly, the heritability of CC in the current study (0.79) is closer to the US-based than the Australia-based study. The reason behind the low CC heritability in the previous Australia-based study is unclear, given that moderately high heritability values have been reported in other studies (0.64 (26), 0.57 (27)). Figure 2 compares our findings to previous works.

Familial correlation in the current study showed significant correlation between all four myopic traits examined between the parent-offspring pairs, which agree with the heritability

findings. There was no significant correlation in SphE, AL and ACD in spouse pairs, which was expected. However, a significant correlation in CC in spouse pairs was noted in the current study, which was also observed previously (28) and was speculated to be due to assortative mating.

One novel aspect of this study is the estimation of past sun exposure by measuring CUVAF area (10), which should have increased familial correlation and heritability estimates of myopia related parameters by accounting for discrepancies between family members. This was not the case. One possible explanation could be that sun exposure was too uniform within generations. However, CUVAF area, when separated into myopic and non-myopic individuals, shows a tendency of larger CUVAF area in non-myopic eyes in both generations. This inverse relationship between CUVAF area and myopia has been reported previously, in regions with high (10, 11) or low (9) annual sunlight hours. Hence we reason that past sun exposure has no true effect on the estimation of additive genetic portion of myopia related traits. Note that this suggests past sun exposure has a minimal effect on the contribution made by additive genetic variation in myopia-associated traits, not that past sun exposure has minimal influence on the final expression of the traits investigated. In support of our conclusion, although the study was conducted in Perth, a city with high annual sunlight hours (average 8.8 hr/day), heritability of myopic traits was similar to the previous two population-based studies in two cities with fewer hours of sunlight (Melbourne, Australia, 6.5 hr/day (28); Beaver Dam, Wisconsin, USA 4.3 hr/day (42)).

The strength of the current study is the enrolment of a large number of nuclear families with minimal ascertainment, which is a more representative approach to estimate heritability in the general population than estimating heritability from twin studies. A key concern in estimating heritability from twin studies is the difficulty involved in modelling shared environmental effects (43), which is circumvented by analysing data from a large number of assumed unrelated nuclear families in the current study. A potential limitation of the current study is that non-cycloplegic autorefraction was recorded in both generations. In the parent generation, given that the youngest age was 40.8 years, it is unlikely that accommodation would play a major role in determining the overall refraction in this cohort. In the offspring generation (range 25.1 – 28.1 years), although

non-cycloplegic autorefraction may be more myopic than cycloplegic autorefraction due to accommodation, it has been shown that SphE measured from cycloplegic and non-cycloplegic autorefraction were not statistically different in adults between 20 – 26 years old (44). Another limitation is that sun-exposure habits change from childhood to adulthood. However even the studies in adults are conflicting; one study reported a negative association between CUVAF area and self-reported sunglasses wear (45) while another showed no association between the two variables (46). Further research is required to tease out the effect sun-exposure habits have on CUVAF area.

In conclusion, by examining data from a large number of nuclear families in an Australian population, we have reaffirmed the previous work that showed myopia-related traits are highly heritable. Moreover, our study suggests that sun exposure has minimal effect on estimating both familial correlation and heritability in myopia-related traits.

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Competing interests

The author(s) declare no competing interests.

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Figure Legends

Figure 1: Familial correlation of myopic traits in parent-offspring and spouse pairs. Filled symbols indicate CUVAF area was included as a covariate in the analysis. Unfilled symbols indicate CUVAF area was not included as a covariate. Error bars are ± SE.

Figure 2: Heritability estimates from the current study compared to previous findings. Estimates from the current study compared to estimates from Chen and colleagues from Melbourne (28) and Klein and colleagues from Beaver Dam (42).

Table 1: Participant demographics

	n	Mean	SD
Pedigree Size	328	3.02	0.32
Parent Generation			
Age [y]	626	57.0	5.6
CUVAF in all eyes [mm^2]	568	29.16	26.80
in myopic eyes [mm^2]^	166	27.71	27.79
in non-myopic eyes [mm^2]^	402	29.76	26.36
Sex (Female)	626 (313)	-	-
Spherical equivalent [D]	582	-0.10	1.94
Anterior chamber depth [mm]	585	3.26	0.32
Axial length [mm]	586	23.77	1.02
Corneal curvature [D]	586	43.70	1.50
Education attainment*	597	3.1	0.8
Offspring Generation			
Age [y]	321	26.6	0.4
CUVAF [mm^2]	309	44.79	30.60
in myopic eyes [mm^2]^	150	42.66	29.37
in non-myopic eyes [mm^2]^	159	46.79	31.63
Sex (Female)	366 (187)	-	-
Spherical equivalent [D]	309	-0.78	1.41
Anterior chamber depth [mm]	307	3.61	0.28
Axial length [mm]	309	23.71	0.93
Corneal curvature [D]	308	43.68	1.43
Education attainment*	140	3.3	0.8

^ Myopia defined by spherical equivalent ≤-0.50 DS

* Categorical: 1 - primary school, 2 - secondary school, 3 - vocational training, 4 - university

Table 2: Educational attainment in the two generations

	Generation 1 (%)	Generation 2 (%)
1 Primary school	0.9	0.0
2 Secondary school	25.0	18.6
3 Vocational training	34.0	28.6
4 University	40.1	52.8

Table 3: Heritability of myopic traits estimated with or without CUVAF as a covariate

		h^2	SE	*P*
Spherical equivalent	With CUVAF	0.583	0.106	<0.001
	Without CUVAF	0.591	0.106	<0.001
Anterior chamber depth	With CUVAF	0.564	0.112	<0.001
	Without CUVAF	0.606	0.104	<0.001
Axial length	With CUVAF	0.612	0.098	<0.001
	Without CUVAF	0.623	0.098	<0.001
Corneal curvature	With CUVAF	0.779	0.082	<0.001
	Without CUVAF	0.793	0.079	<0.001

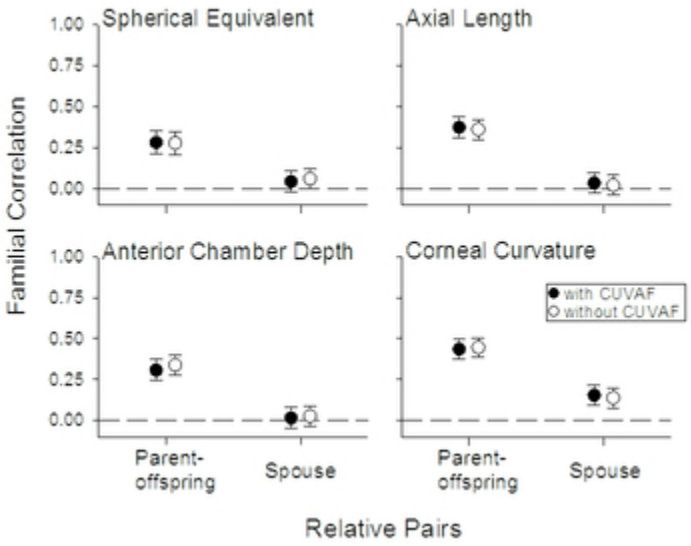

Figure 1: Familial correlation of myopic traits in parent-offspring and spouse pairs. Filled symbols indicate CUVAF area was included as a covariate in the analysis. Unfilled symbols indicate CUVAF area was not included as a covariate. Error bars are ± SE.

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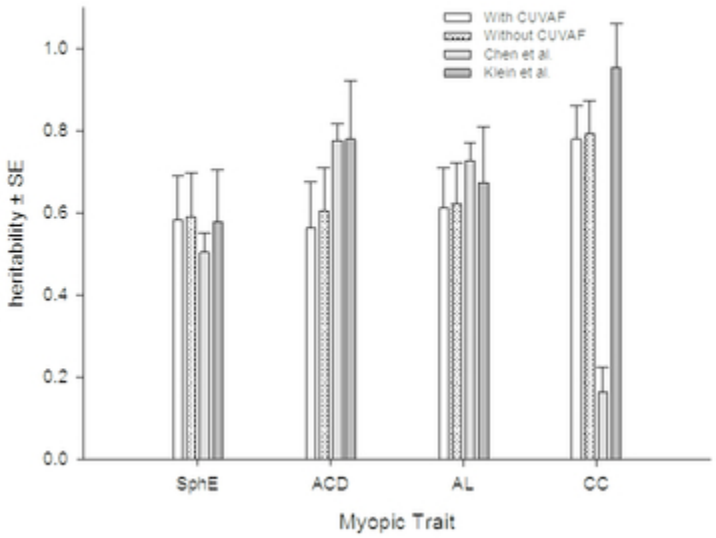

Figure 2: Heritability estimates from the current study compared to previous findings. Estimates from the current study compared to estimates from Chen and colleagues from Melbourne (27) and Klein and colleagues from Beaver Dam (41).

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