

# BMJ Open Retinal microvasculature: population epidemiology and concordance in Australian children aged 11–12 years and their parents

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## ABSTRACT

**Objectives** To describe distributions and concordance of retinal microvasculature measurements in a population-based sample of Australian parent–child dyads at child age 11–12 years.

**Design** Cross-sectional Child Health CheckPoint study, between waves 6 and 7 of the national population-based Longitudinal Study of Australian Children (LSAC).

**Setting** Assessment centres in seven Australian cities, February 2015–March 2016.

**Participants** Of the 1874 participating families, 1288 children (51% girls) and 1264 parents (87% mothers, mean age 43.7) were analysed. Diabetic participants and non-biological pairs were excluded from concordance analyses.

**Outcome measures** Retinal photographs were taken by non-mydratric fundus camera. Trained graders scored vascular calibre using semi-automated software, yielding estimates of central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) and arteriolar–venular ratio (AVR). Pearson's correlation coefficients and multivariable linear regression models assessed parent–child concordance. Survey weights and methods accounted for LSAC's complex sampling, stratification and clustering within postcodes.

**Results** Mean (SD) of CRAE and CRVE were larger in children (159.5 (11.8) and 231.1 (16.5)  $\mu\text{m}$ , respectively) than parents (151.5 (14.0) and 220.6 (19.0)  $\mu\text{m}$ ), yielding similar AVR (children 0.69 (0.05), parents 0.69 (0.06)). Correlation coefficients for parent–child pairs were 0.22 (95% CI 0.16 to 0.27) for CRAE, 0.23 (95% CI 0.17 to 0.28) for CRVE and 0.18 (95% CI 0.13 to 0.24) for AVR. Mother–child and father–child values were similar (0.20 and 0.32 for CRAE, 0.22 and 0.29 for CRVE, respectively). Relationships attenuated slightly on adjustment for age, sex, blood pressure, diabetes and body mass index. Percentiles and concordance are presented for the whole sample and by sex.

**Conclusions** Arteriolar and venular calibre were similar to previously documented measures in midlife adult and late childhood populations. Population parent–child concordance values align with moderate polygenic heritability reported in smaller studies.

## INTRODUCTION

The role of the microvasculature (ie, small vessels) is increasingly recognised in the

## Strengths and limitations of this study

- This is the largest Australian population-based study to investigate the concordance of microvascular structural measurements.
- Our adult sample provides novel data regarding the relatively neglected midlife phase, with most other adult population samples being elderly.
- We used gold-standard methodology and demonstrated high inter-rater and intra-rater reliability.
- Most of the participating parents were mothers, resulting in less precise descriptive and concordance estimates for fathers.

pathogenesis of cardiovascular disease.<sup>1–3</sup> Microvascular abnormalities already present in early life are thought to predispose to both cardiovascular risk factors and outcomes (eg, stroke, myocardial infarction).<sup>4–6</sup>

The retina offers a fast, non-invasive platform to study microvascular health *in vivo*, with similar pathological changes associated with abnormalities and diseases in the cerebral, coronary and renal microvasculatures.<sup>7–10</sup> The most commonly assessed retinal microvasculature measure is the vessel calibre (diameter),<sup>10</sup> summarised as the central retinal arteriolar equivalents (CRAE) and central retinal venular equivalent (CRVE) and its ratio, the arteriolar–venular ratio (AVR). Meta-analyses have demonstrated statistically significant correlations of smaller retinal arteriolar and wider retinal venular vessel calibre with subsequent cardiovascular disease, including stroke, obesity and coronary heart disease.<sup>11–14</sup> Cardiovascular risk factors (such as hypertension, diabetes mellitus and obesity) have been similarly associated with smaller retinal arterioles and larger retinal venules via both shared and unique underlying pathophysiology.<sup>6 15 16</sup> Thus, the retinal vasculature has

been suggested to be a robust biomarker of cardio-metabolic diseases and allows studying the natural course of small vessel changes over life and its relationship to cardiovascular outcomes in later years.<sup>10 15</sup>

However, an important gap in the literature is that the natural course and determinants of the retinal vascular calibre are not fully documented. There is some evidence for distinct but differing curvilinear relationships of arteriolar and venular calibres with age.<sup>10 15</sup> Thus, from disparate cross-sectional studies, Ikram's 2012 narrative review reported that both arteriolar and venular calibre increased from birth (85.5 and 130.0 µm respectively) to age 6 years (165.6 and 232.0 µm). Arteriolar calibre increased further by midlife (202.3 µm) while venular calibre remained static, with calibres of both arterioles and venules reduced from midlife to old age.<sup>10</sup> However, limitations to this literature include differing protocols for imaging and scoring and a marked under-representation of population studies, with few data from young and mid-adulthood. These limitations preclude robust conclusions regarding reference ranges and predictive cutpoints across the lifespan.

While heritability of retinal vascular calibres appears substantial from small community-based samples using a variety of protocols,<sup>15</sup> large studies of parent-child pairs drawn from the general population are lacking. Parent-child correlations for CRAE and CRVE respectively were 0.24 and 0.27 in 341 American adult pairs (aged 43–86 years) in the Beaver Dam Eye Study<sup>17</sup>; 0.13 and 0.20 for 174 adult pairs from the Flemish Study on Environment, Genes and Health Outcomes (aged 20+ years)<sup>18</sup> and 0.12 and 0.31 in 304 pairs from the strabismus, amblyopia and refractive error in Singaporean Children study (mean ages: 40 years for parents, 9 years for children).<sup>19</sup> In all three studies, spousal concordance was negligible for both metrics. In contrast, monozygotic twins have shown much higher concordance (CRAE 0.60 to 0.80, CRVE 0.63 to 0.88) than dizygotic pairs (CRAE 0.12 to 0.50 and CRVE 0.13 to 0.35),<sup>20–24</sup> with higher heritability estimates in twin (CRAE 0.56 to 0.70, CRVE 0.64 to 0.83) than parent-child pairs (CRAE 0.25 to 0.54, CRVE 0.28 to 0.72).<sup>17–23</sup> Genome-wide studies have identified several promising loci associated with retinal vessel calibre.<sup>23 25</sup> However, it remains unclear whether these values remain constant across the lifecourse or what opportunities these offer for preventive health.

Thus, understanding the population epidemiology of retinal calibre in larger studies of healthy midlife adults and children would inform age-specific and sex-specific reference values and facilitate secular trend analyses, international comparisons and, potentially, early risk stratification. The Child Health CheckPoint, a cross-sectional biophysical assessment nested within the Longitudinal Study of Australian Children (LSAC), provided an opportunity to address this issue/question in a national population-based sample of Australian parent-child dyads at child age 11–12 years. We aimed to (1) assess the distribution of retinal vessel calibre in both age groups

and (2) investigate parent-child concordance of these measures.

## METHODS

### Study design and participants

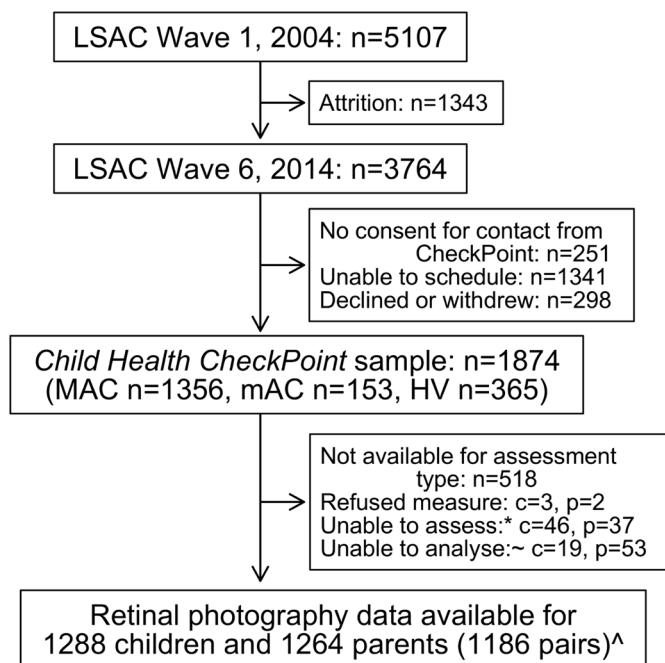
Details of the initial LSAC study design and recruitment are outlined elsewhere.<sup>26 27</sup> Briefly, LSAC recruited a nationally representative birth cohort (B cohort) of 5107 infants using a 2-stage clustered design. First, 10% of Australian postcodes (stratified by state and urban/rural locations) were randomly selected, then in-age children (born between March 2003 and February 2004) within those enrolled in the Medicare Australia database (Australia's universal healthcare system into which 98% of children are enrolled by their first birthday) were selected. Study participants were then followed up in biennial 'waves' of data collection up to 2015.<sup>27</sup> The initial recruitment rate in 2004 was 57.2%, of whom 73.7% (n=3764) were retained to LSAC wave 6 in 2014. At the wave 6 visit, all contactable and consenting families (n=3513) were invited to consent to their contact details being shared with the Child Health CheckPoint team. In 2015, families that consented to contact were sent an information pack via post and received an information and recruitment phone call. The CheckPoint's detailed cross-sectional biophysical assessment, nested between LSAC waves 6 and 7 at child age 11–12 years, took place between February 2015 and March 2016. A total of 1874 families participated. A more detailed description of the CheckPoint study design is available elsewhere.<sup>28 29</sup>

### Consent

The attending parents/caregivers provided written informed consent for themselves and their children to participate in the study.

### Procedure

Retinal photographs were taken during each participant's 3.5-hour visit to the CheckPoint assessment centres in 7 large cities (mainly state capitals) around Australia. During this visit, each child and parent rotated sequentially and separately through a number of 15 min assessment stations.<sup>28</sup> At the 'See Here' station, participants sat in front of a fundus camera (EOS 60D SLR) while the procedure was explained; the room was then darkened while the participant rested chin and forehead on the head rest of the fundus camera for several minutes. Two digital photographs (one each centring on the optic disc and macula) were taken for each eye using standard protocols from the Centre for Eye Research Australia. Because CheckPoint was unable to transport its single retinal camera to smaller regional centres or home visits, 518 CheckPoint families did not contribute to retinal vessel calibre data (figure 1).



**Figure 1** Participant flow through Child Health CheckPoint. \*Unable to collect image due to equipment failure or time constraints. ~Data excluded for images that did not meet quality criteria for 'Big 6' analysis. ^Data from 10 non-biological child-parent pairs and 29 diabetic participants excluded from concordance analyses. c, number of children; HV, home visit assessment; LSAC, Longitudinal Study of Australian Children; MAC, main assessment centre; mAC, mini assessment centre; n, number of families; p, number of attending adults

### Retinal image scoring

Right eye images were selected as first choice for scoring. Reasonable correlations ( $r > 0.71$ ) of retinal vascular diameters between right and left eyes have previously been reported,<sup>30 31</sup> and this allowed harmonisation with other vascular measures obtained in CheckPoint, such as pulse wave velocity and blood pressure, that also assessed the right-side circulation. Left eye images were used where right eye images were deemed ungradable. Issues preventing grading of images included poor focus (potentially blurring vessel edges), dark images (increasing the difficulty for graders to visually validate the vessel trace), and confounding pathology (which can obscure the vessels). Images were evaluated at the Zhongshan Ophthalmic Centre in Guangdong, China (77%) and the Centre for Eye Research Australia in Melbourne, Australia (23%), separately. In total, 2624 images were graded, including 1307 from children and 1317 from parents (87% and 92% from the right eye for children and parents, respectively). Nineteen child and 53 parent images did not meet the quality criteria for use in analyses.

Each image was graded by one of four experienced graders (two from China and two from Australia) masked to participant characteristics who measured the diameters of retinal vessels using the software programme

Integrative Vessel Analysis (IVAN, University of Wisconsin, Madison, USA). Retinal vessels were identified as arterioles or venules from a specific area (one-half to one disc diameter from the margin of the optic disc). Each grader then selected a segment of each vessel within this area for measurement. Diameters of all the selected segments were measured automatically by the IVAN software. For each participant, summary estimates of the average retinal vascular calibre were calculated according to the Big6 (revised Knudston-Parr) formula,<sup>30</sup> which combines measurements of the six largest arterioles or venules, represented as the CRAE and CRVE. The AVR was calculated as CRAE divided by the CRVE.

### Other sample characteristics

Measures of potential confounding variables were included as detailed elsewhere.<sup>28</sup> Briefly, age was calculated to the nearest week using the date of birth, either imported from Medicare Australia's database at the time of LSAC enrolment (child) or self-reported (parent), and date of assessment. Sex was self-reported.

Body mass index (BMI) was calculated as  $\text{kg}/\text{m}^2$ , using measured height and weight.<sup>28</sup> For children, age-adjusted and sex-adjusted BMI z-scores were calculated using the US Centers for Disease Control and Prevention growth reference charts.<sup>32</sup>

Brachial blood pressure was measured using the SphygmoCor XCEL (AtCor Medical Pty, West Ryde, NSW, Australia), following 7 min rest. Mean arterial blood pressure was calculated from the systolic and diastolic measures.

In Australia, Socio-Economic Indexes for Areas provide standardised scores for socio-economic position by geographical area (postcode of family domicile) compiled from 2011 Australian census data. We used the Socio-Economic Indexes for Areas index of Relative Socio-economic Disadvantage (Disadvantage Index) which numerically summarises the social and economic conditions of Australian neighbourhoods (national mean of 1000 and SD of 100, where higher values represent less disadvantage).<sup>33</sup> Parents reported child and parent medical histories via iPad-administered questionnaires at the assessment centre.

Child and parent participants who attended the CheckPoint assessment centres also completed a visual acuity assessment (not conducted in home visits). As part of this assessment, they were asked if they 'usually wear glasses or contact lenses'. Staff members recorded their response as yes or no; the strength of prescription was not captured.

### Statistical analyses

All analyses were performed using Stata V.14.2.

Continuous descriptive variables were summarised using weighted means and SD; categorical variables were summarised by number and weighted percentage for children and adults separately and by sex. Survey weights were calculated taking into account the selection

probability of each child, and were adjusted for non-response, loss to follow-up and benchmarked to population numbers in major (post stratification) categories of the population of children born in 2004. Standard errors were calculated taking into account the complex design and survey weights.<sup>34</sup> More details on the calculation of survey weights is provided elsewhere.<sup>28 35</sup>

Concordance between parents and children was assessed by (1) Pearson's correlation coefficient (CCs) with 95% CIs; (2) linear regression with child variable as dependent variable and parent variable as independent variable adjusted for parent and child age, BMI, mean arterial blood pressure, Disadvantage Index score and sex where appropriate and (3) partial correlation coefficients adjusted for the same covariates. The Pearson's correlation and linear regression analyses were repeated using weighted multi-level survey analyses; as these yielded similar results, unweighted results are displayed. Twenty nine diabetic participants, and 10

non-biological child-parent pairs were excluded from concordance analyses.

### Reliability of retinal vessel calibre measurements

Inter-grader reliability was assessed by three of the four graders reanalysing a subset of 50 randomly chosen images. To assess intra-grader reliability, the same three graders each re-graded 25 of the 50 randomly chosen images. Hence, an assessment was made as to reproducibility of grading made by different graders and repeatability of gradings made by the same grader. Two-way mixed-effects intraclass correlation coefficients were used for the reliability analysis.

### Patient and public involvement

Because LSAC is a population-based longitudinal study, no patient groups were involved in its design or conduct. To our knowledge, the public was not involved in the study design, recruitment or conduct of the LSAC study

**Table 1** Sample characteristics and retinal vessel calibre measures, stratified by sex, of children and parents

Sample characteristics	Mean (SD)*		
	All	Male	Female
<b>Children†</b>	<b>(n=1288)</b>	<b>(n=633)</b>	<b>(n=655)</b>
Age, years	11.96 (0.4)	11.96 (0.4)	11.96 (0.4)
BMI, kg/m <sup>2</sup>	19.4 (3.6)	19.2 (3.6)	19.6 (3.6)
BMI z-score	0.37 (1.00)	0.37 (1.02)	0.38 (0.99)
Systolic blood pressure, mm Hg	108.7 (8.2)	108.3 (8.4)	109.1 (7.9)
Diastolic blood pressure, mm Hg	63.3 (5.7)	63.0 (6.1)	63.6 (5.2)
Neighbourhood Disadvantage Index	1011.2 (61.0)	1009.6 (63.2)	1012.8 (58.8)
Diabetes (%)	0.4	0.2	0.6
Eye condition or glasses/contact lenses (%)	20.9	17.9	23.8
Retinal vessel measures			
CRAE, µm	159.5 (11.8)	156.8 (11.8)	162.1 (11.2)
CRVE, µm	231.1 (16.5)	227.8 (15.6)	234.3 (16.8)
AVR	0.69 (0.05)	0.69 (0.05)	0.69 (0.05)
<b>Parents‡</b>	<b>(n=1264)</b>	<b>(n=169)</b>	<b>(n=1095)</b>
Age, years	43.7 (5.6)	46.2 (6.5)	43.4 (5.3)
BMI, kg/m <sup>2</sup>	28.4 (6.5)	28.7 (4.2)	28.3 (6.7)
Systolic blood pressure, mm Hg	120.5 (12.9)	128.4 (11.6)	119.3 (12.7)
Diastolic blood pressure, mm Hg	73.8 (8.8)	78.3 (8.4)	73.1 (8.6)
Diabetes (%)	2.9	5.3	2.6
Eye condition or glasses/contact lenses (%)	53.2	47.0	54.12
Retinal vessel measures			
CRAE, µm	151.5 (14.0)	148.4 (14.3)	152.0 (13.8)
CRVE, µm	220.6 (19.0)	217.9 (21.3)	221.0 (18.6)
AVR	0.69 (0.06)	0.68 (0.06)	0.69 (0.06)

\*Mean (SD), unless otherwise specified. †n for each child variable ranges from 1213 to 1288.

‡n for each parent variable ranges from 1169 to 1264.

AVR, arteriolar-venular ratio; BMI, body mass index; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent.



**Table 2** Retinal vessel calibre percentiles

Retinal calibre	Child							Parent						
	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95
Central retinal arteriolar equivalent, $\mu\text{m}$														
Male	137.1	141.5	148.0	158.0	165.1	170.7	174.2	123.6	128.7	139.9	148.3	158.2	167.2	171.6
Female	143.8	147.3	153.8	162.5	169.9	175.3	179.3	128.8	134.8	143.3	152.1	162.3	169.1	174.0
All	139.8	144.0	151.3	160.2	167.6	173.1	178.5	128.1	134.1	143.0	151.4	161.7	169.0	173.7
Central retinal venular equivalent, $\mu\text{m}$														
Male	203.3	208.9	217.7	226.7	237.4	247.4	252.7	186.8	190.9	202.1	215.4	231.5	247.6	261.5
Female	208.8	213.3	222.6	234.0	245.4	256.8	262.8	192.3	197.5	208.4	220.8	233.8	244.8	253.0
All	206.1	210.8	220.4	230.4	241.4	252.1	259.3	190.9	196.8	207.2	220.2	233.7	245.7	253.0
Arteriolar–venular ratio														
Male	0.61	0.63	0.66	0.69	0.72	0.76	0.78	0.60	0.61	0.64	0.67	0.73	0.78	0.79
Female	0.61	0.62	0.66	0.69	0.73	0.76	0.78	0.59	0.61	0.65	0.69	0.73	0.77	0.78
All	0.61	0.63	0.66	0.69	0.72	0.76	0.78	0.59	0.61	0.65	0.69	0.73	0.77	0.78

P, percentile.

or its CheckPoint module. Parents received a summary health report for their child and themselves at or soon after the assessment visit. They consented to take part knowing that they would not otherwise receive individual results about themselves or their child.

## RESULTS

### Sample characteristics

A total of 2552 participants (1288 children and 1264 adults) were included in the descriptive aim 1 analyses (figure 1). This represents 95% of the 1356 pairs who attended CheckPoint assessment centres (where retinal photography was offered). Reasons for participants not having retinal photography images and data are attendance at smaller assessment centres without a retinal camera or home visit (n=518 participants), participant

refusal (n=160 participants), and image not taken (eg, the camera required repair) or the quality was too poor (n=5 participants, figure 1). A total of 1186 parent–child pairs were included in aim 2 analyses; 10 non-biologic adult–child pairs and 29 diabetic participants were excluded from the concordance assessments (figure 1).

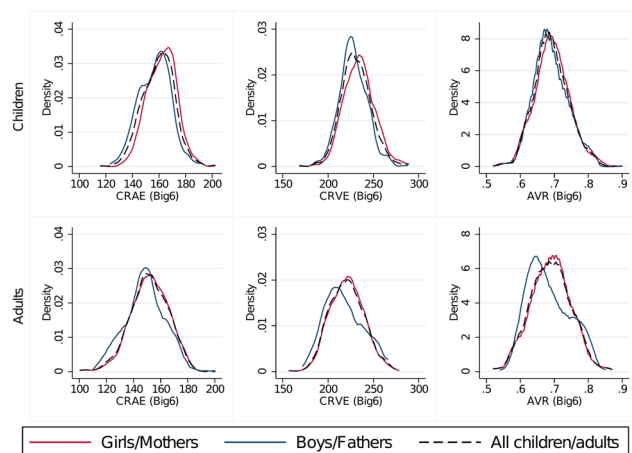
Table 1 shows sample characteristics of children and parents stratified by sex. In children, sex was evenly represented (51% girls), while mean age-specific and sex-specific BMI z-scores were higher than Center for Disease Control historical norms (0.37). The parent sample largely comprised mothers (86.6%). Participants on average were slightly more advantaged (Disadvantage Index mean score 1011, SD 61) than the national Australian population (mean 1000, SD 100).<sup>33</sup>

### Descriptive epidemiology of retinal vessel calibre

Summary statistics for parent and child retinal values are displayed in table 1, and broken down for reference into 5th to 95th percentiles in table 2. The corresponding distribution graphs (figure 2) demonstrate approximately normal distribution in all measures for both children and adults. Mean CRAE and CRVE were around 0.6 SD larger in children (159.5 (11.8) and 231.1 (16.5)  $\mu\text{m}$ , respectively) than parents (151.5 (14.0) and 220.6 (19.0)  $\mu\text{m}$ ), yielding similar AVR (children 0.69 (0.05), parents 0.69 (0.06)), table 1). Boys' CRAE and CRVE mean values were marginally smaller than girls' values (CRAE 156.8  $\mu\text{m}$  vs 162.1  $\mu\text{m}$ , CRVE 227.8  $\mu\text{m}$  vs 234.3  $\mu\text{m}$ ), and fathers similarly showed smaller mean values than mothers (CRAE 148.4  $\mu\text{m}$  vs 152.0  $\mu\text{m}$ , CRVE 217.9  $\mu\text{m}$  vs 221.0  $\mu\text{m}$ ). Mean AVR was strikingly similar in parents and children and between all four groups.

### Parent–child concordance

Correlations for the 1186 parent–child pairs are displayed in table 3. CCs for parent–child dyads were 0.22 for CRAE



**Figure 2** Density plots for retinal vessel calibre measures. AVR, arteriolar–venular ratio; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; BIG6, revised Knudston–Parr formula for calculating CRAE and CRVE using IVAN software.<sup>30</sup>

**Table 3** Parent–child concordance

Pearson's correlation coefficient	Parent–child			Mother–child			Father–child		
	n	CC	95% CI	n	CC	95% CI	n	CC	95% CI
CRAE, $\mu\text{m}$	1186	0.22	0.16 to 0.27	1029	0.20	0.14 to 0.26	157	0.32	0.17 to 0.45
CRVE, $\mu\text{m}$	1186	0.23	0.17 to 0.28	1029	0.22	0.16 to 0.28	157	0.29	0.14 to 0.42
AVR	1186	0.18	0.13 to 0.24	1029	0.18	0.12 to 0.24	157	0.20	0.04 to 0.35
Adjusted linear regression	n	RC	P value	n	RC	P value	n	RC	P value
CRAE, $\mu\text{m}$	998	0.18	<0.001	867	0.18	<0.001	131	0.21	0.004
CRVE, $\mu\text{m}$	998	0.20	<0.001	867	0.20	<0.001	131	0.22	0.003
AVR	998	0.16	<0.001	867	0.16	<0.001	131	0.15	0.06

Non-biological caregivers (n=10) and diabetic participants (n=29) were excluded from these analyses. Covariates in adjusted linear regression models include parent and child ages, Body mass index and Disadvantage Index, mean arterial blood pressure and parent and child sex in models including both sexes.

AVR, arteriolar–venular ratio ; CC, estimated Pearson's correlation coefficient; n, number of biological child–parent pairs with this measure; RC, estimated regression coefficient.

and 0.23 for CRVE, and (as expected for a derived ratio value) slightly smaller for AVR (0.18). Mother–child and father–child correlations are presented for reference but not compared statistically; noting slightly higher values for father–child dyads but wider CIs in keeping with the much smaller numbers. In linear regression adjusting for all covariates, estimates attenuated only marginally and all associations remained strong (estimated regression coefficients for parent–child dyads: CRAE 0.18, CRVE 0.20, AVR 0.16) with the exception of father–child AVR. Similarly, partial correlations adjusted for covariates attenuated slightly (correlation coefficient decreased by 0.01 to 0.06, data not shown).

### Reliability analysis

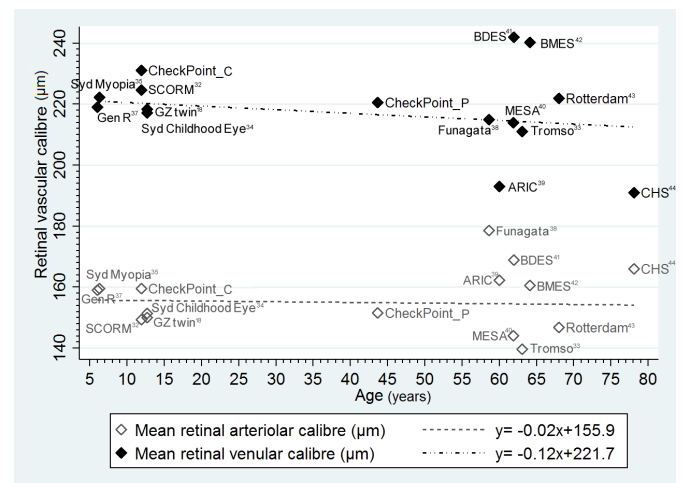
Within-grader intraclass correlations were very high for all three graders for CRAE (0.90 to 0.99, 95% CI 0.76 to 0.99) and CRVE (0.92 to 0.98, 95% CI 0.82 to 0.99). This equated to a greater within-grader range for the derived AVR variable (0.69 to 0.97, 95% CI 0.28 to 0.99). Between-grader interclass correlations were also high at 0.79 (95% CI 0.47 to 0.91), 0.92 (95% CI 0.77 to 0.96) and 0.75 (95% CI 0.58 to 0.86) for CRAE, CRVE and AVR respectively.

### Unplanned post-hoc analysis

As noted above, both CRAE and CRVE were around 0.6 SD smaller in the midlife parents than the 11–12-year olds. This contrasts with Ikram's 2012 review, from which we had expected that CRAE would be substantially larger by midlife, but that CRVE would remain static (prior to both reducing in old age).<sup>10</sup> Because these results were surprising, we therefore conducted some unplanned post-hoc analyses to determine how our findings fit within the existing literature. These should be considered as exploratory and hypothesis-generating only.

We plotted mean values for CRAE and CRVE by mean age (figure 3) for 15 community-based studies published since 2004 with participants aged from 6 years to old age.<sup>11 22 36–47</sup> We included all adult studies (summarised

in online supplementary table 1) in Sun *et al's* systematic review<sup>15</sup> that had a community or population-based sample of >1000 individuals<sup>40–47</sup>; the Norwegian Tromso Eye Study was identified through cited reference searches.<sup>37</sup> Sun's review also identified the child population in the Singapore Cohort Study of the Risk Factors for Myopia (SCORM),<sup>36</sup> and we searched the SCORM study's bibliography and other published reviews for additional children's cohorts.<sup>11 22 38 39</sup> We superimposed the



**Figure 3** Epidemiology of retinal vascular calibre by age. Mean retinal arteriolar and venular calibre by age from CheckPoint and published community-based studies with n>1000. Each symbol represents the mean value for a single study. Each study is summarised in online supplementary table 1, and the paper from which that study's data are drawn is provided in the list of references. ARIC, Atherosclerosis Risk in Communities Study; BDES, Beaver Dame Eye Study; BMES, Blue Mountains Eye Study; CHS: Cardiovascular Health Study; CheckPoint\_C, Child Health CheckPoint data of children; CheckPoint\_P, Child Health CheckPoint data of parents; MESA, Multi-Ethnic Study of Atherosclerosis; SCORM, Singapore Cohort Study of the Risk Factors for Myopia.

results from CheckPoint, separately for adults and children. As visual inspection did not suggest a curvilinear relationship, we then fitted two exploratory linear regression models, with mean age for each study (CheckPoint children and parents considered separately) as the independent variable and with calibre size (arteriolar and venular) as the two dependent variables. It can be seen that venular calibre appears to narrow slightly (though not significantly differently from 0) at a rate of approximately  $-1.2\ \mu\text{m}$  per decade from childhood through adulthood ( $\beta$  coefficient  $-0.12$ , 95% CI  $-0.37$  to  $0.14$ ,  $R^2=0.03$ ). However, there was no obvious trend for arteriolar calibre ( $\beta$  coefficient  $-0.02$ , 95% CI  $-0.21$  to  $0.17$ ,  $R^2=0.002$ ). Two striking features of the figure are the dearth of published values between childhood and late life, and the marked spread of mean values in the elderly.

## DISCUSSION

### Principal findings

We provide the first national population-based percentile values for the pattern and distributions of retinal vessel calibre in Australian 11–12 year old children and midlife adults. Surprisingly, in light of previous literature, mean scores for both CRAE and CRVE were around 0.6 SD smaller in parents than in children. Parent–child correlations were similar for all retinal vessel calibre metrics (CRAE 0.22, CRVE 0.23, AVR 0.18) to studies whose offspring probands were already adults, suggesting that the strong element of heritability in microvascular anatomical structure is largely phenotypically overt within the first decade of life.

### Strengths and weaknesses

Strengths include the national recruitment basis for this large study, providing a reference point for microvascular measurement for future Australian studies and also filling a significant international gap in population-based studies of these age groups. Results were largely consistent with previous studies and should generalise to the wider Australian child population.

Limitations include our under-representation of the most disadvantaged sector (reflecting social biases in both recruitment and retention, issues common to large longitudinal studies) and the small number of fathers in the parent sample. We adjusted only for a limited range of potential confounders. While the distributions of parent retinal vessel calibre might have changed slightly had we further adjusted for smoking status, sedentary lifestyle and diet (all previously associated with altered retinal vessel calibre<sup>48</sup>), their impact on concordance would likely be small because these factors are all strongly socially patterned. By 2019 this cohort will also be able to consider genome-wide association (GWAS) data, potentially shedding further light on the roles of genetic and shared environmental factors. We were not able to measure refractive errors in this study. Retinal vascular calibre measurements may be influenced by refractive

errors and refraction is different between children and adults.<sup>49 50</sup> However, we would expect these effects to be small, particularly as other sources of systematic bias were minimised (measured on the same day with the same equipment by the same person who was blind to dyadic membership).

### Meaning and implications for clinicians and policy makers

Findings for all outcomes are consistent with the previous literature. We showed a larger SD in the parent than the child group, indicating a greater spread of variation in retinal calibre with age. This would be in keeping with greater physiological dysregulation for some individuals with age in response to genetic and risk exposure (eg, higher blood pressure, obesity etc) over multiple decades. Direct comparisons of CRAE and CRVE across the lifecourse and between ethnicities remain difficult due to differences in equipment, software and analysis formulae for CRAE and CRVE. Such factors could well account for the differences between our own and three international studies at mean age 11.1–12.7 years: smaller mean CRAE and CRVE in Chinese twins (CRAE  $150.1\ \mu\text{m}$ , CRVE  $218.4\ \mu\text{m}$ ) and Singapore Chinese children (CRAE  $149.3\ \mu\text{m}$ , CRVE  $224.6\ \mu\text{m}$ ), but much larger values in a smaller study of German children (CRAE  $208\ \mu\text{m}$ , CRVE  $236.2\ \mu\text{m}$ ).<sup>22 36 51</sup> These discrepancies highlight the need for a uniform approach to measurement of retinal parameters in future epidemiological investigations. Our post-hoc analysis of published literature suggests both a dearth of population studies between childhood and old age and a likely fall in venular calibre from childhood through midlife. An individual participant meta-analysis would be the next step to more precisely determine how retinal calibre varies by age, incorporating any additional large-scale studies that now exist.

The moderate parent–child correlations of this Australian population are very similar to those in previous studies with much older adult offspring, suggesting that in terms of microvasculature, children do not become more phenotypically similar to their parents between childhood and midlife. Although this is the first study to report inter-generational concordance by parent sex, our marginally stronger father–child than mother–child correlations must be taken with caution due to the small sample of fathers. Like all other parent–child and twin studies, except the Beaver Dam Eye study, our CRVE correlations were marginally larger than those for CRAE.<sup>17–23</sup> Previously, Li *et al* have postulated that the retinal arteriolar phenotype may be more sensitive to environmental influences than the venular phenotype, despite having a significant, moderate degree of inheritance.<sup>19</sup> This is further supported by GWAS studies, where only CRVE showed significant associations with the identified gene loci.<sup>23 25</sup>

### Unanswered questions and future research

We provide normative values for retinal vessel calibre for Australian children aged 11–12 years and midlife adults using standardised protocols. Our findings make



explicit the need for reliable age-specific normative reference values across the life course. Ideally, this would be extended to large long-running cohort studies with access to clinical outcomes if retinal calibre is to realise the potential as a population screening and risk stratification tool for cardiovascular disease, with or without consideration of other retinal vascular features such as branching angles, tortuosity and fractal dimension, polygenic risk scores and macrovascular risk.

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