PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Carotid artery intima-media thickness, distensibility, and elasticity: Population epidemiology and concordance in Australian 11-12 year old children and their parents
AUTHORS	Liu, Richard; Dunn, Sophie; Grobler, Anneke; Lange, Katherine; Becker, Denise; Goldsmith, Greta; Carlin, John; Juonala, Markus; Wake, Melissa; Burgner, David

VERSION 1 - REVIEW

REVIEWER	Shinichi Wada Department of Cerebrovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan
REVIEW RETURNED	28-Dec-2017

GENERAL COMMENTS	Dr Richard S Liu and colleagues reported Australian values for carotid vascular measures, and report a modest mother-child concordance. The topic is important and the manuscript was well written. However, there are several problems which need to be addressed by the authors. Major points #1. As described in limitation, more number of father should be included since the father group had more atherosclerotic risk factors. #2. Please provide the information of smoking and dyslipidemia to Tabel.1. #3. Is there any relationship between child IMT and second hands smoking? #4. As for diabetes, heart condition, pre-exiting hypertension and pacemaker, the definition is unclear. Please verify this in the method section.

REVIEWER	Jasjit Suri
	Global Biomedical Technologies, Inc., Roseville, CA, USA.
REVIEW RETURNED	24-Jan-2018

| GENERAL COMMENTS | The author describes a well-established marker of cardiovascular risk, carotid intima media thickness (IMT), and related measures (artery distensibility and elasticity) in 11-12-year-old children and mid-life adults, and examine associations within parent-child dyads. Data base consist of a large cross-sectional study consisting of 1874 families, 1489 children (50.0% girls) and 1476 parents (86.8% mothers) with approximately one in 10 parents reported a cardiovascular related health condition. Carotid IMT (mean and max) was computed approximately 10 mm (millimetres) from the carotid bulb using Carotid Analyzer semi-automatic edge detection software program. For reliability, images were reassessed twice each by two raters. For statistical analysis, Pearson's correlation coefficients and Linear regression was used. Result shows small, positive correlations in parent-child and mother-child analyses for all measures.

Major comments:

1. Bulb as a Reference Point: The authors mention: "Intima-media thickness was measured – at the vessel region of highest quality, approximately 10mm (millimetres) from the carotid bulb". It is not clear if the bulb and the CCA was taken together as a part of the acquisition protocol. How do they compute the edge of the bulb to tell that they are 10 mm away from the bulb edge? If they do not do this, then how would they know that they are 10 mm from the bulb?

2. Semi-automated Reading to be Benchmarked: There is no presence of the image in the paper. It is hard to tell if the their measurements are accurate and validated against any standard tool by GE or by AtheroEdge? GE has it awesome measurement tool and the authors should benchmark their readings against them. Even AtheroEdge is very famous and they have set standard for the carotid imaging measurement market.

3. Semi-automated Work Flow: The authors do not discuss how the semi-automated system worked? How do they handle the noisy points between the bulb and proximal to the bulb? Do the authors actually move the curves of lumen-intima interface and media-adventitia interfaces in case the curves are bumpy? This can bring an error in measurements and over-estimation of the IMT readings. This is the reason why a benchmarking reading is very necessary from the well established GE software of AtheroEdge Software. The claim of association needs to be well established by the co-software which can also demonstrate that their readings are accurate and standardized.

4. Medical Statistical Tests are missing: On page 10, section "Statistical analysis", the concordance between parents and children was assessed by 2 measures (a) Pearson's correlation coefficients and (b) Linear regression. The author can support their study by performing important tests like: z-test, Mann-Whitney test, KS test, ANNOVA test, Chi-Squared test, Friedman test, Wilcoxon test, etc. They are very standard in healthcare industry. Since the |

data pool is reasonable, they can perform lot of these tests to validate their hypothesis and claims.

5. IMT Variability: On page 15, section "Parent-child concordance", the author states that mother-child correlations were 0.12 and 0.10 for far wall mean and maximum IMT respectively, and 0.19 and 0.11 for carotid artery distensibility and elasticity. How do we validate this correlation? How can we say that they are 12% related? They must do a wall variability analysis which is so crucial in estimating variations in the interfaces. These variations signify with age. Since mother's age is 3 times the child's age, the variations should be accounted for. More important is the IMT Variability, well known and well established phenotype or biomarker for cardiovascular risk. The authors must study something like this which is more important than plain IMT.

6. Benchmarking Survey: On page 18, section "Meaning and implications for clinicians and policy makers", the author states that there results almost exactly approximate those reported by Ryder et al. [29]. Please provide a more detailed benchmarking study with a survey table.

7. Logistic Regression (A Must): Current analysis needs "logistic regression". Also, please clearly mention, which parameter (IMT/distensibility/elasticity) is strongest between child and the parent.

8. Overlap between this work and their recent paper by the same authors using the data set of (LSAC): "Socioeconomic Position Is Associated With Carotid Intima-Media Thickness in Mid-Childhood: The Longitudinal Study of Australian Children. J Am Heart Assoc. 2017 Aug 9;6(8). pii: e005925. doi: 10.1161/JAHA.117.005925". This reviewer feels that there is an overlap between the current study and previously published study. They should clearly tell the overlapping contents.

9. Validation: How the author validates their study? This is one of the biggest weaknesses in their manuscript. There are so many variations such as: bulb location validation, no benchmarking with standard tool, no variability during the measurements, statistical tests, no logistic regression. The paper does not have technical and intellectual merits.

Cosmetic comments:
1. Please include the word "hypothesis" in the article.
2. Please include a sample image to visual the segments.
3. Please specify which artery (ICA/CCA) is analyzed in the study.
4. On Page #3 and 8, please leave a gap between the values and the unit.
(Ex. 10mm must be 10 mm)

The paper needs major revisions and cannot be published or accepted for publication in the current form.

Reviewer 1 Comments		Authors' response italics indicates original text; underline is modified text.	Reference
1.1	#1. As described in limitation, more number of father should be included since the father group had more atherosclerotic risk factors.	Unfortunately, there are no additional father data available. That said, a sample of over 150 fathers is not trivial, given that most population studies of children report only on mothers if they report on parents at all. The estimates are very similar to those for mothers, although as a consequence of the smaller sample size the confidence intervals are wider. We believe this is one of few studies to provide estimate for both mother-child and father-child concordance (Ryder et al. 2017 being the only other study to our knowledge). Pg 19, line 32, we have now added: "this suggests a degree of consistency between father and mother concordance."	Pg 19, line 32
1.2	#2. Please provide the information of smoking and dyslipidemia to Tabel.1.	Please find amended Table 1.	Pg 13-14
1.3	#3. Is there any relationship between child IMT and second hands smoking?	While a very relevant question, this is outside the scope of the aims and hypotheses of this study. We agree this is an interesting question for a separate paper examining risk factors for cardiovascular health.	NA
1.4	#4. As for diabetes, heart condition, pre-exiting hypertension and pacemaker, the definition is unclear. Please verify this in the method section.	Pg 10, line 21-3, we have now added: "Parents self-reported diabetes requiring medical treatment, high cholesterol requiring medical treatment, heart conditions, pre-existing hypertension and the presence of a pacemaker in a questionnaire at the assessment centre." We also add definitions for smoking ever and smoking current categories: Pg 10, line 23-9, we have now added: "Parental and home smoking behaviour was asked at each LSAC wave. Parents reported children's exposure to second-hand smoke as follows: "Including yourself, how many people who live with you smoke inside the house?" If parents' ever answered more than one person, children were considered exposed. Parents were classified as ever smokers if they ever answered yes to the question "Have you ever smoked?" or "Are you currently smoking?" Parents were classified as current smoker if yes was the most recent answer to "Are you currently smoking?"…"	Pg 10, line 21-3

Pg 10, line 23-9 |
| Reviewer 2 Comments | | Authors' response | Reference |

| 2.1 | Bulb as a Reference Point: The authors mention: "Intima-media thickness was measured – at the vessel region of highest quality, approximately 10mm (millimetres) from the carotid bulb". It is not clear if the bulb and the CCA was taken together as a part of the acquisition protocol. How do they compute the edge of the bulb to tell that they are 10 mm away from the bulb edge? If they do not do this, then how would they know that they are 10 mm from the bulb? | Pg 7, line 20-8: "Image acquisition occurred in two distinct phases. First, to confirm imaging location, technicians visualised a cross-section of arterial lumens both above and below the carotid bifurcation. Subsequent rotation of the probe, in the second phase of acquisition, allowed technicians to acquire a longitudinal image of the common carotid artery and proximal section of the carotid bulb. The carotid bulb was identifiable by its characteristic anatomical structure, close to the bifurcation (Figure 2)."
Pg 24, line 1-5: "Figure 2. Sample single frame of ultrasound obtained in CheckPoint, with Carotid Analyzer analysis overlayed. Yellow lines indicate the lumen-intima interface, pink lines indicate the media-adventitia interface. The distance between yellow and pink lines in the lower pair of lines (far wall) is the carotid intima-media thickness. The carotid bulb characteristics are demonstrated in the left edge of the image."
Moreover, in children and adolescents, recent evidence suggests that the results obtained from any distance 0-20 mm from the bulb are comparable.[1] | Pg 7, line 20-8

Pg 24, line 1-5 |
| 2.2 | Semi-automated Reading to be Benchmarked: There is no presence of the image in the paper. It is hard to tell if the their measurements are accurate and validated against any standard tool by GE or by AtheroEdge? GE has it awesome measurement tool and the authors should benchmark their readings against them. Even AtheroEdge is very famous and they have set standard for the carotid imaging measurement market. | We have benchmarked the software and methods against manual readings and they have been used extensively in many high quality studies (see references 2 and 3 below,[2][3] also in the appropriate Methods sections as follows).
Pg 8, line 6-7: "Carotid Analyzer (Medical Imaging Applications, Coralville, IA, USA), a commercially available semi-automatic edge detection software program.[35][36]"

We question the reasoning for the reviewer recommending AtheroEdge to analyse carotid IMT images, as there are many different comparable software and measurement methods. We also note the reviewer is affiliated with the company that owns the software,[4] and is the original trademark holder of the name "AtheroEdge".

Our image raters were trained by expert ultrasonographers. Our protocols, documented in the attached Standard Operating Procedures, comply with available consensus statements from two respected organisations with an interest in measuring carotid IMT. | Pg 8, line 6-7

See supporting documents

Pg 3, line 17-9
Pg 11, line 13-8
Pg 19, line 1-6 |

		On the more general issue of accuracy, and in line with the Mannheim Consensus statement, we thoroughly investigated and reported the reliability of our analysis. This is clearly stated in the abstract, and methods. We are transparent with the reporting of our internal reliability study. In line with the principles of BMJ Open, we believe readers are able to make a judgement for themselves about the accuracy and precision of our measurements. Many other papers have not shown this information for carotid IMT, which we think an omission.	
2.3	Semi-automated Work Flow: The authors do not discuss how the semi-automated system worked? How do they handle the noisy points between the bulb and proximal to the bulb? Do the authors actually move the curves of lumen-intima interface and media-adventitia interfaces in case the curves are bumpy? This can bring an error in measurements and overestimation of the IMT readings. This is the reason why a benchmarking reading is very necessary from the well-established GE software of AtheroEdge Software. The claim of association needs to be well established by the co-software which can also demonstrate that their readings are accurate and standardized.	As above, we believe the reviewer's request to benchmark against other proprietary software is a) addressed externally by other studies we reference, b) addressed internally by our reliability study, and c) could be of some concern if the reviewer had significant competing interests. To help clarify the issue regarding "noisy points", we refer the reviewer to this line in the methods: Pg 8, line 10-12: "After algorithmic detection of the intima-media interface over the entire cine-loop, frames were manually adjusted as needed or rejected if the intima-media interface was unclear or blurred." We agree that there is potential for measurement error, as is the case when using any software. Random measurement error generally biases towards the null hypothesis (no association), differential measurement error leading to overestimation of an association is unlikely when assessors are blinded to family status or were selecting images randomly, which was the case in our protocol.	Pg 8, line 10-12
2.4	Medical Statistical Tests are missing: On page 10, section "Statistical analysis", the concordance between parents and children was assessed by 2 measures (a) Pearson's correlation coefficients and (b) Linear regression. The author can support their study by performing important tests like: z-test, Mann-Whitney test, KS test, ANNOVA test, Chi-Squared test, Friedman test, Wilcoxon test, etc. They are very standard in healthcare industry. Since the data pool is reasonable, they can perform lot of these tests to	The statistical tests used in a study depend upon the type of research question being asked. Within the scope of our research question, we believe the tests we used are the necessary and sufficient tests for our aims to report population values across two generations and examine parent-child concordance. The extensive list of statistical tests requested by the reviewer are not appropriate to answer the research question. Additional statistical testing of the same hypothesis produces little new information, may be potentially inappropriate for the research question or type of data available, and falsely inflates the consistency of the result.	NA

		validate their hypothesis and claims.		
2. 5	IMT Variability: On page 15, section "Parent-child concordance", the author states that mother-child correlations were 0.12 and 0.10 for far wall mean and maximum IMT respectively, and 0.19 and 0.11 for carotid artery distensibility and elasticity. How do we validate this correlation? How can we say that they are 12% related? They must do a wall variability analysis which is so crucial in estimating variations in the interfaces. These variations signify with age. Since mother's age is 3 times the child's age, the variations should be accounted for. More important is the IMT Variability, well known and well established phenotype or biomarker for cardiovascular risk. The authors must study something like this which is more important than plain IMT.	With regard to validation, please see our response to Reviewer comment 2.4. We do not claim that parents and children are 12% related. We simply report a correlation of 0.12, which is a small positive correlation. With regard to wall variability analysis, the question of carotid IMT variability is potentially interesting, but is outside the scope of this paper. Mean-mean and mean-max measurements of carotid IMT are well established measures of cardiovascular risk, and we aim to provide reference values and parent-child concordance of this measurement. We agree with the reviewer that variability is of interest; we suggest another paper to discuss this.	NA
2. 6	Benchmarking Survey: On page 18, section "Meaning and implications for clinicians and policy makers", the author states that there results almost exactly approximate those reported by Ryder et al. [29]. Please provide a more detailed benchmarking study with a survey table.	Added as supplementary table 2.	Pg 20, line 7 See supporti ng docume nts
2. 7	Logistic Regression (A Must): Current analysis needs "logistic regression". Also, please clearly mention, which parameter (IMT/distensibility/elasticity) is strongest between child and the parent.	As our outcome measure is not binary, logistic regression is inappropriate. Instead we have done linear regression, which is appropriate with continuous data. As best as we can interpret the reviewer's definition of 'strongest', we mention a higher concordance/correlation in carotid artery distensiblity in pg 20, line 28-9. "The relatively higher concordance in carotid artery distensibility (r=0.19) compared to other measures suggests differences between structural and functional vascular measures."	Pg 20, line 28-9
2. 8	Overlap between this work and their recent paper by the same authors using the data set of	While we appreciate the reviewer referencing our previous work, we believe these two papers	NA

	(LSAC): "Socioeconomic Position Is Associated With Carotid Intima-Media Thickness in Mid-Childhood: The Longitudinal Study of Australian Children. J Am Heart Assoc. 2017 Aug 9;6(8). pii: e005925. doi: 10.1161/JAHA.117.005925". This reviewer feels that there is an overlap between the current study and previously published study. They should clearly tell the overlapping contents.	address separate research questions using the same data. The referenced paper examines the association between socioeconomic position, measured through various methods, and child mean and maximum carotid IMT. We found strong associations with maximum IMT, which were not as apparent with mean IMT. We did not examine adult IMT, or child or adult distensibility and elasticity. This current paper examines the concordance between child and parent mean and maximum IMT, distensibility, and elasticity – three measures obtainable from carotid ultrasound. Further, this paper serves as an extended description of the methods for our measurement.	
2.9	Validation: How the author validates their study? This is one of the biggest weaknesses in their manuscript. There are so many variations such as: bulb location validation, no benchmarking with standard tool, no variability during the measurements, statistical tests, no logistic regression. The paper does not have technical and intellectual merits.	Please see our response to Reviewer comments 2.1-2.8 above.	NA
2.10	Please include the word "hypothesis" in the article.	The goal of our study is to describe cIMT values at a population level and to investigate the concordance between parents and children. We are not testing a specific hypothesis.	NA
2.11	Please include a sample image to visual the segments.	Included as figure 2, and current figure 2 moved to figure 3. Pg 24, line 1-5, we have added: "Figure 2. Sample single frame of ultrasound obtained in CheckPoint, with Carotid Analyzer analysis overlayed. Yellow lines indicate the lumen-intima interface, pink lines indicate the media-adventitia interface. The distance between yellow and pink lines in the lower pair of lines (far wall) is the carotid intima-media thickness. The carotid bulb characteristics are demonstrated in the left edge of the image."	Pg 24, line 1-9
2.12	Please specify which artery (ICA/CCA) is analyzed in the study.	Please see our response to Reviewer comments 2.1 above. Also, pg 7, line 1: "Procedure: Common carotid artery IMT, lumen diameter…"	Pg 7, line 1
2.13	On Page #3 and 8, please leave a gap between the values and the unit.	Fixed, with thanks.	

| (Ex. 10mm must be 10 mm) | | |

VERSION 2 – REVIEW

REVIEWER	Shinichi Wada
	National Cerebral and Cardiovascular Center
REVIEW RETURNED	30-Mar-2018

GENERAL COMMENTS	The topic is important and the manuscript was well written.

REVIEWER	Samantha R. Seals
	University of West Florida, United States of America
REVIEW RETURNED	03-Aug-2018

GENERAL COMMENTS	I am not convinced that the data follows a normal distribution given what's shown in Figure 3. However, the assumption of normality is on the residuals, not the outcome. Were the residuals considered? As long as normality was assessed properly, the methods presented are appropriate.