ARIC Manuscript Proposal #1630

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SC Reviewed: _________    Status: _____    Priority: _____

1. a. Full Title: Direct and Indirect Effects of Retinal Vascular Caliber, Cardiovascular Risk Factors on CVD Outcomes.

   b. Abbreviated Title (Length 26 characters): Retinal Caliber and CVD pathways

2. Writing Group:
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3. Timeline:
   Project proposal to Publication's Committee: March 2010
   Data analysis completed: March 2010
   Completed manuscript to Publication's Committee: June 2010
Retinal vessel caliber has been shown to predict systemic vascular events.\textsuperscript{1, 2} Compared to the predictive values of traditional cardiovascular disease (CVD) risk factors, however, the improvement in outcome prediction by adding retinal vessel caliber into the models containing traditional risk factors has been shown to be marginal. For example, in recent meta-analyses, it has been reported that retinal vascular caliber combined with the traditional Framingham risk factors reassigned only 1 in 5 women without diabetes at intermediate risk of CHD events to a more definitive risk category,\textsuperscript{3} and only 1 in 10 people without diabetes at intermediate risk of stroke were reclassified into different, mostly lower, risk categories with the inclusion of retinal caliber.\textsuperscript{4}

The approach of using a single risk prediction model to illustrate risk profiles of the study outcome from multiple risk factors is limited in its ability to reveal fully the disease pathways and mechanisms of complex diseases, due to the following reasons: 1) mechanisms of complex disease involve multiple factors and multiple pathways, and no single factor is sufficiently causative; 2) these multiple factors and disease pathways are not entirely independent of each other: rather they are likely interrelated, affecting on each other or may have causal-consequential relationship among the risk factors themselves, forming cascade effects in the disease mechanisms; 3) a single prediction model approach ignores the shared variances among some risk factors, their interrelationship and interactive effects, and thus may have underestimated the effect from some risk factors. It is unclear if traditional statistical modeling approaches that are widely used have substantially masked effect of some risk factors, in particular when the model contains both functional and structural co-variables, such as the case adding retinal vessel caliber variables into the Framingham CVD risk prediction model. Studying disease pathways using path analysis model to separate the direct and indirect associations of multiple risk factors with disease outcomes may have advantages over the traditional statistical modeling techniques.

We hypothesize that some CVD risk factors and retinal vascular caliber (arteriolar and venular caliber) are not independent of each other but are risk components that share CVD risk variances. Retinal vessel caliber changes may have incorporated effect of CVD risk factors on CVD outcomes, and may be intermediate variables (termed mediating variable in path analysis model) in the pathways from CVD risk factors to CVD outcomes. In addition, there are also possibilities that some common CVD risk factors (such as elevated blood pressure, white cell count and fibrinogen) share common variances with retinal vessel caliber. Therefore adjustment for all the factors in traditional statistical models may result in an over-adjustment and thus an underestimation, of the effect of some CVD risk factors.

Path analysis has been developed to provide insights into the interplay of multiple risk factors that are not entirely independent of each other on disease pathways.\textsuperscript{5} If an indirect effect is not properly considered, the relationship among the variables of interest may not be fully revealed.\textsuperscript{7} We therefore aim to assess the direct and indirect associations of CVD risk factors and retinal vessels caliber changes with CVD outcomes, using data from the Atherosclerosis Risk in Communities (ARIC) Study and path analysis techniques, to separate the direct and indirect effects by multiple factors. Cardiovascular risk factors and retinal vessel caliber were collected at the third visit (1993-95 years), and incidence of CVD outcomes collected over 10 years from the third visit. Initial analyses and writing will take place between Jan 2010 to March 2010, and final writing and manuscript submission will be in June 2010.

4. Rationale:
Cardiovascular disease (CVD) is one of the leading causes of death and disease burden globally.\textsuperscript{6} It is estimated that 17.5 million people died from CVD in the world in 2005, representing 30% of all-cause deaths.\textsuperscript{7} In Australia, the reported death rate from CVD was 34% of all-cause mortality in 2006.\textsuperscript{8} This global burden highlights the need to investigate possible early markers of CVD. Currently, many CVD risk factors have been identified, including heredity,\textsuperscript{9} male gender,\textsuperscript{10} older age,\textsuperscript{11, 12} cigarette smoking,\textsuperscript{13} high blood pressure,\textsuperscript{14} diabetes,\textsuperscript{15, 16} obesity (especially excess abdominal fat),\textsuperscript{17} lack of physical activity,\textsuperscript{18} abnormal blood cholesterol,\textsuperscript{19} and heavy alcohol consumption.\textsuperscript{20} Persons with an increasing numbers of these risk factors were found to be associated
with a greater likelihood of having CVD. However, knowledge of how these known CVD risk factors relate to each other and jointly contribute to CVD risk is still limited.

**Do Retinal Vascular Caliber Changes Reflect Cumulative Impacts from CVD Risk Factors and Share Indirect Effect of CVD Risk Factors on CVD Outcomes?**

Some CVD risk factors may interrelate as a cascade in terms of consequential effect on CVD event. For examples, obesity and lack of exercise can adversely influence blood pressure and blood cholesterol levels, which in turn influence the blood circulation in end organs including the heart and the brain. The presence of multiple CVD risk factors over an extended period of time could lead to arteriosclerosis and arteriolosclerosis, which in turn lead to an increase in the workload and burden of the heart and reduced oxygen supply to the end organs. There are also possibilities that CVD risk factors affect each other and worsen in parallel, ultimately leading to adverse CVD outcomes.

Research in the last decade has shown a strong, consistent association between elevated blood pressure and retinal arteriolar narrowing, and that retinal microvascular structural abnormalities including retinal arteriolar caliber narrowing and venular caliber widening may be risk indicators of subsequent cerebrovascular diseases and incidence of CVD events. We hypothesize that some CVD risk factors and central retinal arteriolar equivalent (CRAE) or central retinal venular equivalent (CRVE) share CVD risk variances. We quantitate CVD risk variances shared by retinal vessel caliber changes with other CVD risk factors in terms of two major CVD outcomes: incident cerebrovascular events and cardiovascular events. If the shared risk variances are relatively large, it may indirectly support our hypothesis that retinal vascular caliber changes incorporate cumulative effects of various CVD risk factors. Therefore adjustment of these variables simultaneously in the same traditional statistical models may not be appropriate.

**5. Research Hypothesis:**

Mechanisms for incident CVD include multiple pathways with various risk components and multiple interactive cascades. It is expected that some CVD risk factors are not independent and may share CVD risk variances with CRAE and/or CRVE. CRAE/CRVE may incorporate cumulative impacts from various risk factors. Alternatively, narrower CRAE can occur before hypertension, and MABP may be a mediating variable on the pathways between CRAE and CVD events. CRVE can predict stroke and CVD events, if so, WCC and fibrinogen may act as mediating variables on the pathways between CRVE and CVD events.

**Hypothesis:**

1. Retinal vessel caliber changes may incorporate effects of various CVD risk factors and therefore may act as mediating variables on the pathways between CVD risk factors and CVD events (Please refer to Path model 1 of Appendix).

2. The effect of narrowed small arterioles on CVD events may be both direct and indirect via blood pressure (Please refer to Path model 2 of Appendix).

3. Effect of widened small venules on CVD events may be both direct and indirect via chronic inflammatory processes (Please refer to Path model 3 of Appendix).

**6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodological limitations or challenges if present).**

**6.a Methodology:** Data from the Atherosclerosis Risk in Community Study (ARIC) will be used for the proposed investigations. The ARIC is a cohort study of atherosclerosis risk factors in four U.S. communities and comprises 15792 men and women, aged between 45 to 64 years at baseline. The participants were examined every three years with the first baseline examination occurring in 1987-89, the second in 1990-92, and the third in 1993-95. Retinal vessel calibers were
measured from the retinal fundus photographs taken at the third examination. The other considered risk factors were also from the 3rd examination, and the 10 year-CVD events were taken at the 5th examination.

We will use a path analysis technique, to model the direct and indirect relationships of CVD risk factors with CVD outcome (incident cerebrovascular or cardiovascular events), either the effect of traditional CVD risk factors via CRAE and/or CRVE, or the effect of CRAE and/or CRVE via two major common CVD risk factors (high blood pressure and inflammation). Path analysis is an accepted approach used to model the direct and indirect associations of inter-related variables with the study outcomes, and has been widely used in modeling data from agricultural, social science, and medical research, including modeling for cardiovascular risk factors. No studies to date, however, have used this technique to model the interrelationship of CVD risk factors and vessel caliber with CVD outcomes.

To explain the calculation of shared variance using the path analysis technique, a simplified example with three variables (A, B and C) is used:

The correlation coefficients were $r_{AB}=0.20$, $r_{AC}=0.50$ and $r_{BC}=0.80$, respectively. Shared variance between two variables is the square of their correlation coefficients or their standardized regression coefficients. According to the correlation coefficients shown above on the diagram, the shared variances between A and C, A and B and B and C are estimated to be 4%, 25% and 64%, respectively. In this scenario, only 4% of shared variance between A and C (direct effect) can be illustrated by traditional regression modeling techniques, compared to 4% plus additional 16% (the square of the indirect effect of A on C via B, which is $(r_{AB} \times r_{BC})$, or 20%, illustrated by the path analysis techniques.

6. b Primary exposure Variables:
Age, gender, race, study centre, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arteriolar blood pressure (MABP), hypertension status, body mass index (BMI), waist to hip ratio (WHR), serum total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol and triglyceride levels, serum glucose levels, diabetes mellitus status, measure of activity (e.g., sport index, leisure index), current smoking and current drinking status.

6. c Secondary exposure (intermediate) variables:
Central retinal arteriolar equivalent (CRAE), Central retinal venular equivalent (CRVE), carotid artery intima-media thickness (CIMT), for comparison with CRAE and CRVE.

6. d Final Outcome Variables:
Incident stroke and cardiovascular events over 10 years since the third visits will be the study outcomes. We define incidence CVD diseases as new stroke and cardiovascular events since the third visits (1993-95) to the fifth visits (2005) among subjects who were free of the events at the beginning of this time period.

6. e Study Design
We classified incidence of stroke or cardiovascular disease in individuals who were free of these diseases at the third visit and developed stroke (ischaemic/hemorrhagic) or myocardial infarction / heart failure in the subsequent ten year’s period.

Associations between various CVD risk factors and CRAE/CRVE, and associations between CRAE/CRVE and three CVD risk factors (MABP, WCC and fibrinogen levels), will be explored.
using linear and logistic regression analyses. Additionally, the associations between CVD risk factors, CRAE/CRVE and the incidence of CVD will be evaluated first using logistic regression models. These statistical models will enable us to identify the risk factors that are directly associated with CRAE/CRVE, and also variables that are directly associated with the incidence of CVD outcomes. This information will guide us to form our hypothetical disease pathway models to investigate the inter-relationship of these factors and their contribution to CVD events.

The total effect of explanatory (independent) variable on CVD outcomes (dependent variables) can be disintegrated into direct and indirect components. We will use LISREL 8.8 (Scientific software international Lincolnwood, IL) to investigate the direct and indirect roles of major CVD risk factors and retinal vessel caliber changes, to address the question: Do CRAE/CRVE share risk variances with known CVD risk factors and thus may play intermediate roles on the pathway from CVD risk factors to CVD events?

6. f Software use for data analysis: Data analyses were conducted with SPSS 17.0 program (SPSS, Chicago, IL) and LISREL 8.8 (Scientific software international Lincolnwood, IL)

6. g Findings from initial analysis:
Initial analyses revealed that some CVD risk factors (e.g., Mean arteirilor blood pressure (MABP), total cholesterol levels, high density lipoprotein (HDL), sport index, current smoking and current drinker) are associated with CRAE and CRVE, and CRAE and CRVE were associated with incident CVD events. The role of CRAE and CRVE differs between diabetic and non-diabetic groups. In adults with diabetes, CVD risk factors were shown to have an indirect effect via CRAE and CRVE on the incidence of cerebral vascular events (6.6% and 11.0% of the total effect, the two path analysis models containing CRAE or CRVE explained 2.8% and 3.0% of the risk variations of cerebral vascular events, respectively). For the incidence of cardiovascular events, the indirect effect was 0.9% and 2.5% of the total effect, with 3.5% of risk variations explained by each of the two path analysis models. In adults without diabetes, the indirect effects via CRAE and CRVE on the incidence of cerebral vascular events were 5.7% and 0.5% of the total effect, with 2% of risk variations explained by each of the two path analysis models. For cardiovascular events, the corresponding indirect effect was 0.15% and 1.8% of the total effect, with 3.8% of risk variations explained by each of the two path analysis models.

In comparison with CIMT, in adults with diabetes, CVD risks factors were found to have indirect effect via CIMT, 5.1% and 4.0% of the total effect, and 2.9% and 3.6% of risk variances of cerebral vascular and cardiovascular events were explained by the path analysis models containing CIMT, respectively. In adults without diabetes, the indirect effect via CIMT was 8.2% and 7.0% of the total effect, with 2.0% and 4.1% of risk variations of cerebral vascular and cardiovascular events explained by the path analysis models containing CIMT, respectively.

These findings suggest that CVD risk factors affect CRAE, CRVE and CIMT, which may act as intermediate or mediating variables on the pathways to CVD events, particularly for cerebral vascular events. However, the proportions of total effect were small via the indirect effects. These preliminary findings seem justify the traditional statistical models by showing that there is no substantial over-adjustment of the risk factors, i.e. path analysis models may not needed routinely in most situations.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes  ___X__ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used?  ___ Yes  ___ No
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?  ____ Yes  __X__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”?  ____ Yes  ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php  ___ X__  Yes  ______ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  ____ Yes  __X__ No

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _________)
___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________ __________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a Manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.
REFERENCES

5. Raykob T, ed. A First Course In Structure Equatio Modelling, 2nd ed: LEA LONDON, 2006; 238.


