1a. **Full Title**: Causality between LDL-cholesterol and occurrence of coronary heart disease (CHD) in old age - an age-stratified Mendelian Randomization analysis

b. **Abbreviated Title (Length 26 characters)**: Age-stratified LDL-CHD MR

2. **Writing Group**:
   Writing group members: Roelof Smit, Stella Trompet, Ton de Craen, Wouter Jukema (and to be determined members of the CHARGE and CARDioGRAM+C4D consortia)

ARIC co-authors: Nora Franceschini, Alanna Morrison, Eric Boerwinkle

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ___RAJ___ [please confirm with your initials electronically or in writing]

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3. **Timeline**: Data analyses/interpretation: 4 months; drafting of manuscript: 2 months. Estimated analysis time for experienced analyst: 2-3 days.
4. **Rationale:** Prevention and treatment of cardiovascular disease is critically dependent on adequate control of established cardiovascular risk factors. However, most of the epidemiological and experimental evidence is based on relatively healthy subjects in middle-aged and young-old populations (< 70 years). Various studies have shown that the association of cholesterol with cardiovascular disease and mortality attenuates with increasing age [e.g. 1-3]. It is currently unknown whether the reversed associations are causal or the result of confounding and reverse causality. Using genetic epidemiology, it is possible to disentangle this question of causality.

5. **Main Hypothesis/Study Questions:** Does the association between genetically raised LDL-c and (a.) phenotypic LDL-c, and (b.) the occurrence of CHD, attenuate with age?

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 methodologic limitations or challenges if present).**

After proposing the project to collaborating studies of the CHARGE and CARDIoGRAM +C4D consortia, a standardized analysis plan was recently circulated. In short, the analyses focus on (a.) the association between a genetic risk score (GRS) for LDL-c and phenotypic levels of LDL-c, and (b.) the association between the LDL-c GRS and occurrence of CHD. Both analyses will be performed in an age-stratified manner, using 5-year age groups from 55 years onwards. While preferable, it is not necessary to have both LDL-c and clinical outcomes available, as these involve separate analyses.

**In/exclusion:** include participants of 55 years and older, of European descent, for whom genome-wide SNP data is available. Exclude prevalent CHD-disease, unless the CHD event occurred (a) no more than five years before study entry and (b) the CHD event occurred at or after the age of 55.

**Stratification:** analyses should be age-stratified, using five-year increments from 55 years onwards (e.g. 55-59, 60-64, etc.). This stratification is dependent on age at measurement (usually baseline) for analyses with LDL-c, and age at event for analyses with CHD.

**Outcomes:**
1. phenotypic LDL-c (Friedewald-estimated or directly measured)
2. CHD: (suspected or probable) fatal or non-fatal MI, fatal CHD or coronary revascularization (coronary angioplasty or coronary artery bypass graft) procedures. Self-reported events are allowed.

**Covariates:** all analyses should be adjusted for sex and study-specific parameters (e.g. familial structure, principal components, matching). Where available, additional covariates will be included in additional models: triglycerides, systolic blood pressure, BMI, smoking status (current), lipid-lowering medication (yes/no). If data is not available
on one or more of the additional covariates, please run the most complete model.
Whenever possible, covariates should be taken near to the time of the LDL measurement.

Summary of data analysis:
1. Generate a weighted GRS from 42 known LDL-c SNPs, obtained from the most recent Global Lipids Genetics Consortium paper [4]
2. Age-stratified analyses between the LDL-c GRS and phenotypic LDL-c levels, using linear regression (if available, 2 models). Participants with prevalent CHD-disease will be excluded from this analysis, and lipid- and blood pressure-lowering treatment effects adjusted for by means of sensible constants [5].
3. Assessment of instrument strength: calculate squared partial correlation and F-statistic for the LDL-c GRS (extension of analysis described under step 2)
4. Age-stratified analyses between the LDL-c GRS and occurrence of CHD (if available, 3 models). Incident cases should be analyzed using Poisson regression with Lexis expansion [6]. R- and Stata-syntax will be provided. If present, prevalent cases can be analyzed using unconditional logistic regression, using non-cases at study entry as controls.

Methodological limitations/challenges: performing both the phenotypic LDL-c and event analyses should be feasible within the ARIC study population. However, it is possible that within the very oldest age categories there are insufficient events to obtain a reliable effect estimate for the CHD-analysis.

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 ICTDER03 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? ___x__ Yes ___ No
(This file ICTDER 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? ___x__ Yes ___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”? ___x__ 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.cscn.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.cscn.unc.edu/aric/forms/

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscn.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __x__ No.

References

