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1.a. Full Title: Thyroid antibody status in subclinical hypothyroidism and the risk of cardiovascular disease.

b. Abbreviated Title (Length 26 characters):
Anti-tpo status in SHypo and the risk of CHD

2. Writing Group [please provide a middle name if available; EX: Adam Lee Williams]:
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ___OH__ **[please confirm with your initials electronically or in writing]**

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3. Timeline:

May 2023 – July 2023: Systematic review, contact new cohorts identified, contact existing TSC cohorts, data collection.
July 2023 – September 2023: Data analysis.
October 2023- December 2023: Manuscript preparation and submission to co-authors for review.

4. Rationale:

Subclinical hypothyroidism (SHypo) is defined as the combination of an elevated TSH in combination with a free thyroxine (free T4) within the reference range.[1] SHypo is common with a higher prevalence in women, ranges from 4-20% and increases with age[1,2] and has been associated with coronary heart disease (CHD) events and mortality, with a stronger association for those with TSH of 10.0 mIU/L or greater.[3] Among the multiple etiologies of SHypo (e.g. medication, iodine deficiency), autoimmunity is the most common cause, which is defined as positive anti-thyroid peroxidase antibody (anti-TPO).[4,5] Anti-TPO is the most sensitive marker for thyroid autoimmunity and can be found in 35-65% of patients with SHypo.[6-8] Because in patients who have circulating anti-TPO, there is a greater risk of progression from subclinical to overt hypothyroidism which is known to be associated with increased incidence of cardiovascular (CVD) events[9-15], one may infer that SHypo with positive thyroid antibodies might be also associated with increased risks of CVD mortality or events. Only a few previous studies have reported on SHypo with positive thyroid antibodies and clinical cardiovascular outcomes, with conflicting results.[16-18] These studies had limited power with a relatively low number of events and did not provide subgroup analyses (eg, by TSH levels or age). Therefore, in 2014 we conducted an IPD analysis on SHypo and CHD risk and we did not find a significant difference in risk according to Anti-TPO status.[7] However, this analysis was limited by 2 studies using old antibody assays,[16,19] fewer participants at the time (38,274 vs. 51,292 now with 3 additional cohorts with anti-TPO status, longer follow-up and more CV events) and did not include stroke outcomes. In another IPD analysis, we showed that stroke risk was increased in patients <65 years and in those with higher TSH but did not consider anti-TPO status.[20] Due to lack or the conflicting nature of results obtained from previous studies in the field, whether or not anti-TPO positive SHypo leads to more frequent CVD outcomes remains unclear.[21-23] Recommendations in the current guidelines differ about measuring thyroid antibodies to better identify patients who should receive levothyroxine replacement to target treatment in patients with subclinical hypothyroidism.[8,24] Therefore, analyzing individual participant data (IPD), now with a larger number of multiple longitudinal cohorts currently available in the Thyroid Studies Collaboration (TSC) including also other cohorts identified by systematic research, we can reduce heterogeneity between studies and help elucidate whether the presence of thyroid antibodies in SHypo predicts cardiovascular outcomes, such as CHD and stroke mortality or events.

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5. Main Hypothesis/Study Questions:

Aim: To conduct an individual participant data (IPD) analysis of relevant studies to compare the CVD risk of SHypo with and without anti-TPO. **Hypothesis:** Individuals with positive anti-TPO and SHypo have an increased risk of CVD outcomes.

6. Design and analysis

a) **study design**
b) **inclusion/exclusion**
c) **outcome and other variables of interest with specific reference to the time of their collection**
d) **summary of data analysis**
e) **Any anticipated methodologic limitations or challenges if present**

Systematic search

We will perform a systematic literature search in MEDLINE/EMBASE (1946-2021). Two reviewers will independently screen and select prospective cohorts with baseline and follow-up data available, pertaining to thyroid status, autoimmunity (Hashimoto, anti-TPO) and CVD outcomes (including CHD and stroke). A request will be made to authors for permission to use their IPD. If in agreement, these cohorts will supplement our existing TSC database. The Newcastle-Ottawa Quality Assessment Scale will be used to assess methodological quality of the included studies. We plan to report our study in line with the PRISMA-IPD (Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data) statement.

Criteria for study cohorts:

- Available thyroid function at baseline. serum TSH, free T4 and anti-TPO
- Prospective follow-up data on CHD events, stroke, CHD mortality, and stroke mortality. As previously defined in our studies, CHD mortality will be limited to death from CHD or sudden death. A CHD event will be defined as a nonfatal myocardial infarction or CHD death (equivalent to hard events in the Framingham risk score) and hospitalization for angina or coronary revascularization (coronary artery bypass grafting or angioplasty). Stroke will be defined according to World Health Organization criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin, including ischemic or hemorrhagic strokes.

Criteria for participants:

- Inclusion criteria: age >18 years old.

Exclusion criteria: Overt hypo- or hyperthyroidism, subclinical hyperthyroidism, participants without anti-TPO measurements, pregnancy.

Outcome and other variables of interest

All ARIC participants with thyroid function testing at Visit 2 (1990-1992) and Visit 5 (2011-2013).

Variables:

Baseline
- Thyroid function: TSH, free T4, total T3, TPO antibodies
- Sex, age, age, date of visit 2, race, center, smoking status, height, weight, body mass index, blood pressure, lipids
- History of cardiovascular disease
- History of diabetes

Medication use at baseline and follow-up: thyroid-altering medications (including levothyroxine, anti-thyroid medication, lithium, amiodarone, glucocorticoids, iodine) and cardiovascular medications (antihypertensive and lipid lowering agents)

Primary outcomes:
- CHD, stroke

Secondary outcomes:
- CHD mortality
- Stroke mortality
- Total mortality

Planned analyses.

We will perform an individual participant data meta-analysis using a two-step method. First, we will assess the association in each individual cohort between positive anti-TPO SHypo and each outcome, using a Cox regression model. In the second step, we will pool the estimates from each cohort in a random effects meta-analysis, to calculate the overall effect on the outcome. Initial models will be age and sex-adjusted and subsequent models will be additionally adjusted for cardiovascular risk factors (body mass index (BMI), systolic blood pressure, smoking status, total cholesterol, diabetes) and cardiovascular medications (lipid-lowering and antihypertensive treatment) and TSH values. Results will be summarized using forest plots and will be presented as hazard ratio (HR) along with their relative 95% confidence interval.

Thyroid function: We will include participants with TSH levels of 0.45-19.9 mIU/L. Similar to previous studies, we will define euthyroidism as a TSH level from 0.45 to 4.49 mIU/L and subclinical hypothyroidism will be stratified according to the standard categories [4.5-6.9 mU/L (mild elevation), 7.0-9.9 mU/L (moderate elevation), and 10.0-19.9 mU/L (marked elevation)] with normal fT4 levels.

Anti-TPO status: Anti-TPO positivity will be defined according to the assay used (mostly > 35 IU/L)

We will perform several pre-specified stratified and sensitivity analyses.

Stratified analyses:

- Stratification by sex
- Stratification by age using previous categories (18-49, 50-64, 65-79, and ≥ 80 years) and the second age stratification (≥65 and <65 years)
- Stratification by TSH levels

- Stratification by levothyroxine use at baseline (yes/no)

Sensitivity analyses:

- Restrict the analyses to participants not progressing to overt hypothyroidism to clarify if the antibodies modify the effect of subclinical hypothyroidism on CVD independently of progression to overt hypothyroidism.
- Excluding participants with prior ischemic heart disease, arrhythmia, valvulopathies, congenital disease
- Excluding participants with thyroid-altering medication (anti-thyroid drugs, thyroxine, amiodarone, lithium)
- Excluding participants with immune-modulating treatments (steroids, immune-checkpoint inhibitors, IFN-alpha)
- Excluding participants with cardiac-altering medication (pioglitazone, anthracycline, trastuzumab, IFN-alpha, IL-2)
- Other exclusions of studies based on data quality.

To assess heterogeneity across studies, we will use the I^2 statistic. We will use the Egger test and age- and sex-adjusted funnel plots and selection models to assess the impact of and account for publication bias.

f) Will the author need Limited data to complete the proposed manuscript? ☐ Yes, Limited data is needed. ☒ No, De-identified data will be sufficient.

*Please note, Limited dataset access is strict and rarely provided. Limited data includes identifiable information such as dates (birthdays, visit dates, etc.). CMS, Genomic, Geocoded, and Proteomics/Somalogic data all fall under the limited data category. De-identified data does not include dates. All dates are date adjusted to "Days since Visit 1".

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ☐ Yes ☒ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ☐ Yes ☒ No
(The file ICTDER is distributed to ARIC PIs annually, 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 ☒ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the current derived consent file ICTDER05 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: https://aric.cscc.unc.edu/aric9/proposalsearch [ARIC Website→Publications→Proposal Search]

☒ 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)?

1. Prevalence and Risk Factors of Thyroid Dysfunction in Older Adults in the Community. Diab N, Daya NR, Juraschek SP, Martin SS, McEvoy JW, Schultheiß UT, Köttgen A, Selvin E. Sci Rep. 2019 Sep 11;9(1):13156. doi: 10.1038/s41598-019-49540-z. PMID: 3151158
2. Thyroid Function, Cardiovascular Risk Factors, and Incident Atherosclerotic Cardiovascular Disease: The Atherosclerosis Risk in Communities (ARIC) Study. Martin SS, Daya N, Lutsey PL, Matsushita K, Fretz A, McEvoy JW, Blumenthal RS, Coresh J, Greenland P, Kottgen A, Selvin E. J Clin Endocrinol Metab. 2017 Sep 1;102(9):3306-3315. doi: 10.1210/jc.2017- 00986.PMID: 28605456

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

11.b. If yes, is the proposal

☒ **A. primarily the result of an ancillary study (list number* 2009.24)**

☐ **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
https://aric.cscc.unc.edu/aric9/researchers/ancillary_studies/approved_ancillary_studies [ARIC Website→Ancillary Studies→Approved Ancillary Studies]

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 https://aric.cscc.unc.edu/aric9/publications/policies_forms_and_guidelines [ARIC WebsiteàPublicationsàPublication Policies, Forms, and Guidelines].
http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

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