ARIC Manuscript Proposal #3790

1.   a. Full Title: Dental caries a risk factor for incident ICH and specific ischemic stroke subtype

   b. Abbreviated Title (Length 26 characters): Dental Caries, ICH and ischemic stroke subtype

2.   Writing Group:

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

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3.   Timeline:

Data analysis 03/2021
Abstract presentation 06/2021
Manuscript submission 12/2021

4. **Rationale**:

Stroke is one of the leading causes of morbidity and mortality in the United States [1, 2]. Stroke is classified into ischemic or hemorrhagic, and intracerebral hemorrhage (ICH) is the most common cause of hemorrhagic stroke [3]. ICH patients have the highest morbidity and mortality among major stroke patients [4]. Age, anticoagulant use, and heavy alcohol use are known risk factors for ICH [4, 5], but one third of cases have yet to be attributed to any particular risk factor [6].

Dental caries and periodontal disease are known promoters of atherosclerotic cardiovascular disease through systemic inflammation, molecular mimicry, and bacteremia (Figure 1) [7]. Poor oral health is a known risk factor for cerebrovascular disease (Figure 2) [8], especially ischemic stroke [9, 10], but little is known about association of dental caries with ischemic stroke and ICH. We would first like to confirm the association between dental caries and ischemic stroke. As a step further we would like to further test the association of dental caries with specific ischemic stroke subtype (cardioembolic, large artery atherosclerosis and small vessel occlusive disease). This would help to further determine the pathophysiological mechanism by which dental caries may result in ischemic stroke.

Dental caries are formed by acid-fermenting oral bacteria that break down dietary carbohydrates to form acidic byproducts that subsequently destroy dental hard tissue [11]. Bacterial infection, especially of oral origin, is known to be associated with cardiovascular diseases [12]. Streptococcus mutans is a known bacterial cause of dental caries that contains a collagen-binding protein, Cnm, and demonstrates inhibition of platelet aggregation and matrix metalloproteinase-9 activation. This strain has been linked to aggravation of ICH in mice and thus may be a risk factor for ICH [13]

This will be the first clinical-epidemiological study to evaluate the association between dental caries and ICH risk. This proposal has important clinical implications and may help us better assess whether treatment of dental caries can reduce the rate of incident vascular events in patients with ICH.

5. **Main Hypothesis/Study Questions**:
    1. Is dental caries independently associated with specific ischemic stroke subtype?
    2. Is dental caries an independent risk factor for incident ICH?

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).**
    - **Study design:** Presence of dental caries was assessed in subjects from the Dental Atherosclerosis in Communities Study (DARIC) without prior stroke or ICH. This cohort was followed for a period of incident ICH, subsequently verified by chart abstraction.
    - **Inclusion:** Participants in the ARIC study who completed a fourth clinic examination (1996 to 1998) were assessed for dental caries. All participants at visit 4 will be included.

- **Exclusion:** Participants with missing periodontal disease information and those who do not meet the criteria as above will be excluded.
- **Main Exposure:** Dental caries assessed on visit 4. Criteria described by Radike was used to determine caries status. Patients were assessed for cavitations, defined as teeth with a discontinuity of the enamel surface caused by the loss of tooth substance, but must be distinguished from fractures, erosion, and abrasion. Patients with lesions but not frank cavitation were further investigated to detect pit, fissure, and smooth surface lesions as well as root and coronal caries. For each tooth, a status was recorded 1-7 denoting whether a tooth was sound, decayed or filled, missing, restored with crown, a decayed root fragment, a sound root fragment, or an implant. The main exposure is the decayed (D) component because it is the best indication that dental caries is present with possible exposure to the collagen-binding protein. However, we will also include a decayed and filled component (DF) to represent untreated caries plus caries that have been treated as a secondary analysis.
- **Outcome: 1)** <u>ICH diagnoses</u> were based on computer derived diagnosis and physician medical record review, with differences adjudicated by a second physician reviewer. Classification required evidence of sudden or rapid onset of neurological symptoms lasting >24 hours or leading to death, plus demonstration of at least one of three: (1) definite intracerebral hematoma by CT or MRI, (2) demonstration at autopsy or surgery of ICH, OR (3) evidence in the patient's clinical record that meet criteria (a), (b), (c), and (d): (a) one major or two minor neurological signs or symptoms at least 24 hours until the patient died, (b) bloody or xanthochromic spinal fluid, AND (C) avascular mass effect on CTA with no evidence of aneurysm or AVM, AND (d) no CT/MRI was performed or CT/MRI was technically inadequate.
  2) <u>Ischemic stroke</u> diagnoses based on computer derived diagnosis medical record review and imaging confirmation. Classification required evidence of sudden onset of neurological symptoms lasting ≥24 hours. Strokes were further classified according to etiology as atherothrombotic, lacunar (small vessel occlusive), and cardioembolic <u>subtypes</u>. [15]
  Covariates: Age, gender, race (categorized as European American or African-American), and smoking status at visit 4 was assessed by self-report. Hypertension defined as a systolic blood pressure of 140mmHg or higher, a diastolic blood pressure higher than 90mmHg, or use of medications to treat hypertension. Diabetes determined by a self-reported history of a physician diagnosis of diabetes, non-fasting blood glucose level of 200 mg/dL or higher, fasting blood glucose level of 126mg/dL or higher (to convert glucose to millimoles per liter, multiply by 0.0555), or use of insulin or other oral hypoglycemic medications [14]. Socioeconomic status measured by using education status as proxy. Patients reported their education status as basic (<11 years), intermediate (12-16 years), or advanced (>17 years). Regular dental care measured at the fourth visit by patient-reported responses on the dental history form and was classified into either regular or episodic use. Regular use defined as seeking routine dental care greater than or

equal to one time per year and episodic as seeking care only when in discomfort, something needed to be fixed, never, or did not receive regular dental care [15].

- **Data Analysis**: Cox regression with time-dependent covariate will be used to compute crude and adjusted hazards ratio stratified as <15 years and ≥15 years from the initial dental assessment. Univariate and multivariable analyses are planned.
- **Limitations:**
    - We are using presence of dental caries as a proxy for presence of S. mutans rather than measuring S. mutans in saliva directly. However, the link between S. mutans and dental caries is well established. in one study, S. mutans was found in the saliva of 94% of subjects. S. mutans with collagen binding activity was found in 33% of subjects. In a report by the same authors, there was also a direct relationship between Cnm-positive S. mutans and microbleeds.
    - The dental health variables measured at visit 4 may have changed over time. This study does not account for changes from baseline dental health. [3]
    - ICH stroke subtype and hemorrhage location were not assessed in these studies preventing the examination of possible associations between ICH subtypes and potential risk factors. [3]
    - Misclassification of ischemic stroke subtype: According to the algorithm, it requires the presence of a possible cardio-embolic source. Presence of a possible cardioembolic source may not necessarily mean cardioembolism as the etiology of the ischemic stroke. Also, artery-to-artery embolic stroke (e.g., dislodged carotid plaque) is classified in ARIC as "atherothrombotic". Lacunar stroke in ARIC is based on some imaging features, regardless of the presence or absence of a "lacunar stroke syndrome". The definition may miss lacunar strokes with negative scans. Also, some lacunar strokes may be cardioembolic in etiology.

Exposure variable	Dental Caries
Outcome variables	Incident ICH
Covariates	Age
	Gender
	Race
	Smoking Status
	Hypertension
	Diabetes
	Socioeconomic Status
	Regular dental care
	Periodontal disease
Analysis	Cox regression

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? _____ 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?
    _____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 ICTDER03 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)?

Ferket, B. S., van Kempen, B. J., Wieberdink, R. G., Steyerberg, E. W., Koudstaal, P. J., Hofman, A., Shahar, E., Gottesman, R. F., Rosamond, W., Kizer, J. R., Kronmal, R. A., Psaty, B. M., Longstreth, W. T., Jr, Mosley, T., Folsom, A. R., Hunink, M. G., & Ikram, M. A. (2014). **Separate prediction of ICH and ischemic stroke.** *Neurology*, *82*(20), 1804–1812. https://doi.org/10.1212/WNL.0000000000000427

Folsom, A. R., Yatsuya, H., Mosley, T. H., Jr, Psaty, B. M., & Longstreth, W. T., Jr (2012). **Risk of intraparenchymal hemorrhage with magnetic resonance imaging-defined leukoaraiosis and brain infarcts.** *Annals of neurology*, *71*(4), 552–559. https://doi.org/10.1002/ana.22690

Fornage, M. **Using ARIC controls for Whole Genome Sequence Analysis of ICH**

Gottesman, RF. **Cerebral microhemorrhages and cortical amyloid: The ARIC-PET study**

Mahmoodi BK, Yatsuya H, Matsushita K, et al. **Association of kidney disease measures with ischemic versus hemorrhagic strokes: pooled analyses of 4 prospective community-based cohorts**. *Stroke*. 2014;45(7):1925-1931. doi:10.1161/STROKEAHA.114.004900

Marsh, EB. **The Association Between Renal Dysfunction and Clinical and Subclinical Intracranial Hemorrhage: The ARIC Study.**

Murthy, S. **New Diagnosis of ICH and the Risk of Subsequent Ischemic Stroke and Acute Myocardial Infarction**

[2016] Sen S, Offenbacher S, Beck J, Moss, K, et al. **Association of PD levels with incident ischemic stroke as well as the etiological stroke subtypes in the Atherosclerosis Risk In Communities study.** Published Stroke, 2018. 49(2): p. 355-362**.**

Sharma, R. **Can the risk of intracranial hemorrhage be predicted in patients treated with statins, antiplatelets, and anticoagulants? The Atherosclerosis Risk in Communities Study.**

Smith, L. G., Yatsuya, H., Psaty, B. M., Longstreth, W. T., Jr, & Folsom, A. R. (2013). **Height and risk of incident intraparenchymal hemorrhage: Atherosclerosis Risk in Communities and Cardiovascular Health study cohorts**. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association*, *22*(4), 323–328. https://doi.org/10.1016/j.jstrokecerebrovasdis.2011.09.004

Sturgeon, J. D., Folsom, A. R., Longstreth, W. T., Jr, Shahar, E., Rosamond, W. D., & Cushman, M. (2008). **Hemostatic and inflammatory risk factors for ICH in a pooled cohort.** *Stroke*, *39*(8), 2268–2273. https://doi.org/10.1161/STROKEAHA.107.505800

Sturgeon, J. D., Folsom, A. R., Longstreth, W. T., Jr, Shahar, E., Rosamond, W. D., & Cushman, M. (2007). **Risk factors for ICH in a pooled prospective study.** *Stroke*, *38*(10), 2718–2725. https://doi.org/10.1161/STROKEAHA.107.487090

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

11.b. If yes, is the proposal
    _x_   **A. primarily the result of an ancillary study (list number***   **1996.01**)
    ___   **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/
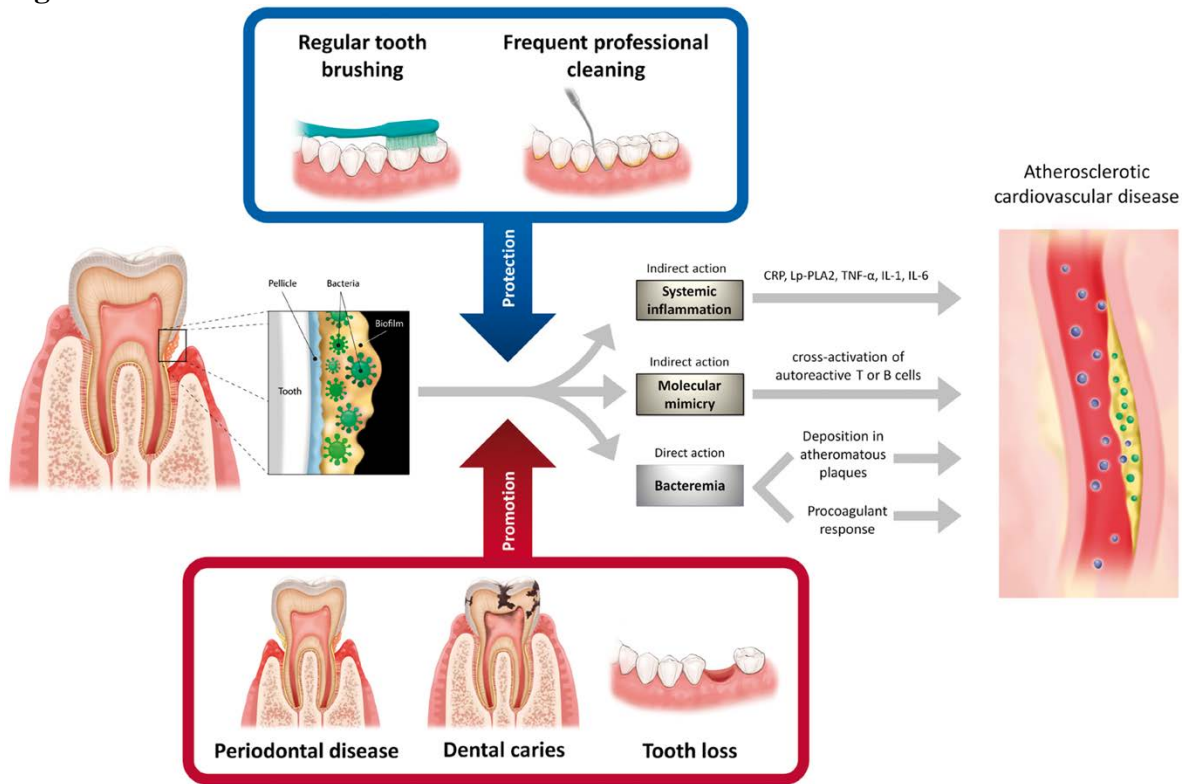
12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

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.cscc.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
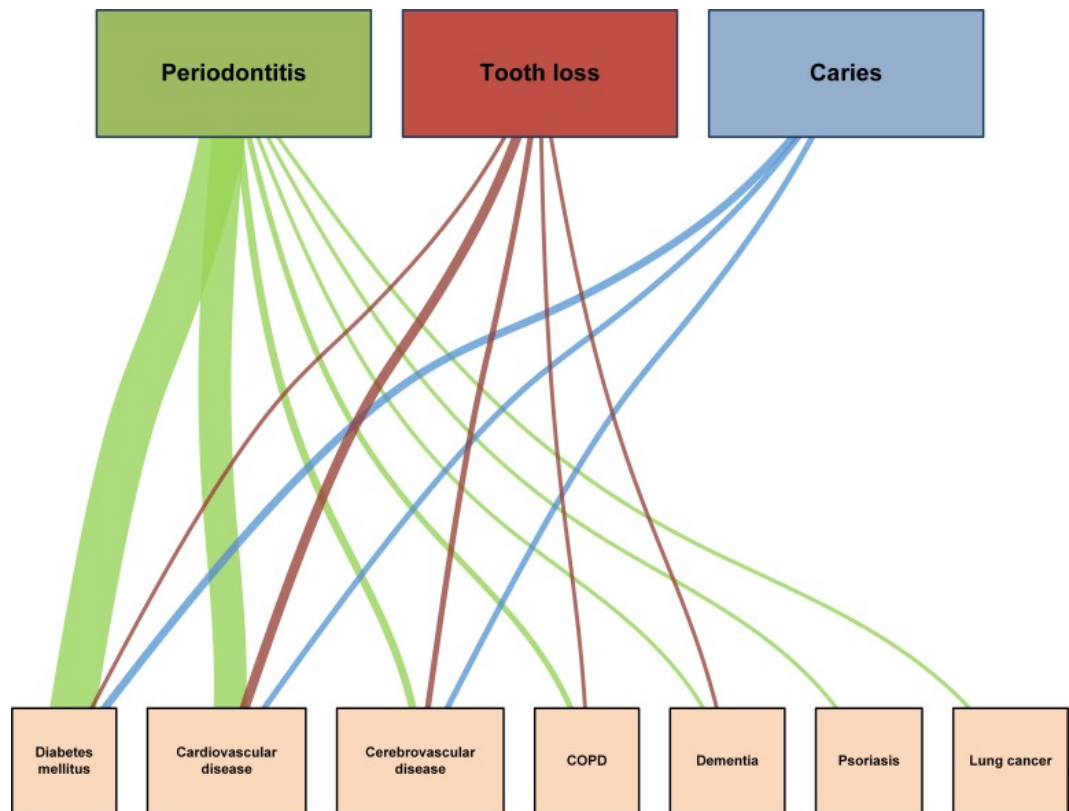
1. Feigin, V.L., et al., *Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015.* The Lancet Neurology, 2017. **16**(11): p. 877-897.

2. Bonita, R., *Stroke prevention: a global perspective. , in Stroke Prevention*, H.V. Norris JW, Editor. 2001, Oxford University Press: New York, NY. p. 259-274.

3. Sturgeon, J.D., et al., *Hemostatic and inflammatory risk factors for intracerebral hemorrhage in a pooled cohort.* Stroke, 2008. **39**(8): p. 2268-73.

4. Sawyer, R.P., et al., *Racial/ethnic variation of APOE alleles for lobar intracerebral hemorrhage.* Neurology, 2018. **91**(5): p. e410-e420.

5. Woo, D., et al., *Genetic and environmental risk factors for intracerebral hemorrhage: preliminary results of a population-based study.* Stroke, 2002. **33**(5): p. 1190-5.

6. Nakano, K., et al., *The collagen-binding protein of Streptococcus mutans is involved in haemorrhagic stroke.* Nat Commun, 2011. **2**: p. 485.

7. Park, S.Y., et al., *Improved oral hygiene care attenuates the cardiovascular risk of oral health disease: a population-based study from Korea.* Eur Heart J, 2019. **40**(14): p. 1138-1145.

8. Seitz, M.W., et al., *Current Knowledge on Correlations Between Highly Prevalent Dental Conditions and Chronic Diseases: An Umbrella Review.* Prev Chronic Dis, 2019. **16**: p. E132.

9. Grau, A.J., et al., *Periodontal disease as a risk factor for ischemic stroke.* Stroke, 2004. **35**(2): p. 496-501.

10. Zeng, L.N., et al., *Oral health in patients with stroke: a meta-analysis of comparative studies.* Top Stroke Rehabil, 2020. **27**(1): p. 75-80.

11. Selwitz, R.H., A.I. Ismail, and N.B. Pitts, *Dental caries.* The Lancet, 2007. **369**(9555): p. 51-59.

12. Friedewald, V.E., et al., *The American Journal of Cardiology and Journal of Periodontology editors' consensus: periodontitis and atherosclerotic cardiovascular disease.* J Periodontol, 2009. **80**(7): p. 1021-32.

13. Watanabe, I., et al., *Oral Cnm-positive Streptococcus Mutans Expressing Collagen Binding Activity is a Risk Factor for Cerebral Microbleeds and Cognitive Impairment.* Sci Rep, 2016. **6**: p. 38561.

14. *The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives.* Am J Epidemiol, 1989. **129**(4): p. 687-702.

15. Sen, S., et al., *Periodontal Disease, Regular Dental Care Use, and Incident Ischemic Stroke.* Stroke, 2018. **49**(2): p. 355-362.

Figure 1

[7]

Figure 2

[8]