PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Electrophysiological Ventricular Substrate of Stroke: a Prospective Cohort Study in The Atherosclerosis Risk in Communities (ARIC) Study
AUTHORS	Johnson, John; Haq, Kazi; Lutz, Katherine; Peters, Kyle; Paternostro, Kevin; Craig, Natalie; Stencel, Nathan; Hawkinson, Lila; Khayyat-Kholghi, Maedeh; Tereshchenko, Larisa

VERSION 1 – REVIEW

REVIEWER	Joung, Boyoung Yonsei University Health System
REVIEW RETURNED	05-Mar-2021

GENERAL COMMENTS	The goal of the study was to determine an association of cardiac ventricular substrate with thrombotic stroke (TS), cardioembolic stroke (ES), and intracerebral hemorrhage (ICH). ARIC participants with analyzable ECGs and no history of stroke were included. Ventricular substrate was characterized by cardiac memory, spatial QRS-T angle (QRS-Ta), sum absolute QRST integral (SAIQRST), spatial ventricular gradient magnitude (SVGmag), premature ventricular contractions (PVCs) and tachycardia-dependent intermittent bundle branch block (TD-IBBB) on 12-lead ECG at visits 1-5. The author concluded that PVC burden (reflected by cardiac memory) is associated with ischemic stroke. Transient cardiac memory (likely through TD-IBBB) precedes ICH. 1. The concern of this study is the mechanism between EKG finding and stroke and ICH is not evaluated. 2. Moreover, the relationship with known risk factors for stroke and ICH is not evaluated. 3. Finally, EKG findings cannot be directly correlated with ventricular substrate (eg. scar).

REVIEWER	Wu, Zhiyuan Capital Medical University, Public Health
REVIEW RETURNED	09-Jun-2021

GENERAL COMMENTS	There are potential residual risks associated with stroke, apart from traditional cardiovascular and metabolic risk factors. The authors investigated the association of cardiac ventricular substrate, such as PVCs and SVGmag, with the incidence of different types of stroke. This is an interesting study with clinical significance for stroke prevention. However, there are some points to be addressed. Comments

	Title: 1.The current title ‘Cardiac Ventricular Substrate of Stroke’ seems not to fully express the core elements of the article. Please modify the title. Abstract: 2.It is not clear enough how the participants in this current study were enrolled and followed. Whether the participants with ECG data at the first visit are enrolled in this study? And what is the number of participants when time-updated or time-dependent ventricular substrates analyzed. 3.Abbreviations should be defined when first appear, such as ECG. 4.Strengths and Limitations of this study: Strengths and limitations should be stated separately. Introduction: 5.This section is well organized. It is worth noting that we can only reveal the association in observational studies, and it is unreasonable to say ‘uncovering novel mechanisms of stroke’. The use of term ‘mechanism’ should be cautious. Method: 6.The design and flow chart (Figure 1) is not clear enough to understand. Please clarify the condition of data missing and lost to follow up in this cohort study. How the missing data was handled in your analyses? 7.The data are well analyzed in this study, and the time-independent effect of ventricular substrate with multiple measurements is considered in model 5. Please clarify how the death from other diseases except stroke, as a competing event, was considered in your analyses. Results: 8.The interaction between demographic variables (such as age, sex) and ventricular substrates should be presented in Subgroup analyses. 9.I suggest that integrating the figures presenting the association of ventricular substrates with TS, ES and ICH. Discussion: 10.How the monitoring of cardiac memory on ECG could be related with therapy adjustment? The manuscript should follow the format requirements and it needs to be reviewed and edited for proper grammar.

REVIEWER	Stellos, Konstantinos Newcastle University, Biosciences Institute
REVIEW RETURNED	19-Jun-2021

GENERAL COMMENTS	In the current manuscript entitled ‘Cardiac Ventricular Substrate of Stroke: The Atherosclerosis Risk in Communities (ARIC) Prospective Cohort Study’ Johnson et al. examine the prognostic value of global electric heterogeneity (GEH) for thrombotic stroke, cardioembolic stroke and intracranial hemorrhage. For this purpose, the authors examined 14,479 individuals from the Atherosclerosis Risk in Communities (ARIC) study, who had not previously experienced any type of stroke. Study participants were followed-up from 1987 to 2018. Over a median follow-up of 24.5 years, there were 899 thrombotic stroke events (incidence 2.87; 95% CI 2.69-3.07 per 1000 person-years), 400 embolic stroke events (incidence 1.26; 95% CI 1.14-1.39 per 1000 person-years), and 120 intracranial hemorrhage-only strokes (incidence 0.38; 95% CI 0.32-0.45 per 1000 person-years). Both types of ischemic stroke (thrombotic and embolic) were diagnosed in 62

	participants, and another 16 participants endured both ICH and either thrombotic or embolic stroke. The authors used built Cox proportional hazard risk models. All continuous ECG exposure variables were expressed as their z score to standardize comparisons. To adjust for confounders, they constructed 5 models for incremental adjustment. Briefly, the models were built as follows: Model 1: demographic characteristics (age, sex, and race-study center group). Model 2: Model 1 + baseline CV risk factors and presence of CVD. Model 3: Model 2 + atrial substrate (abnormal P axis, PR interval, PTFV1, heart rate, use of antiarrhythmic drugs, anticoagulants, and aspirin, presence of PACs on 10-second 12-lead ECG at any visit, S or V median beat at any visit, and presence of AF at baseline or at any time during follow-up). Model 4: Model 3 + characteristics of the ventricular substrate. Time-updated model 5: all model 4 covariates, as well as ECG variables that were updated at the date of ECG recording in visits 1-5. The authors found that different metrics were associated with embolic or thrombotic stroke after multivariable adjustment, while SVG magnitude and SAIQRST emerged as common independent predictors of both embolic and thrombotic stroke (model 5). The manuscript is a very interesting, well-written and clinically relevant research study which extends previous work of the senior author (Dr Tereshchenko), where she examined the association between GEH alterations and ventricular arrythmias/ sudden cardiac death in the same cohort (Circulation. 2016;133:2222–2234). I only have few minor comments that the authors may consider to address: 1) Does the addition of ventricular substrate parameters in existing established algorithms (e.g. CHA2DS2-VASc) offer additive prognostic value? The authors could use the number of abnormal GEH parameters (0-5) or continuous variables that retained their association with both embolic and thrombotic stroke after multivariable adjustment (e.g. SVG magnitude, SAIQRST). Suggested statistical approach: Net reclassification index, discrimination index. 2) Does the co-existence of multiple ventricular substrate pathologies increase the risk of stroke? 3) Please add Ethics Approval Number in the respective Methods section. 4) Figure 2: please provide details of each model in the Figure legend

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

The goal of the study was to determine an association of cardiac ventricular substrate with thrombotic stroke (TS), cardioembolic stroke (ES), and intracerebral hemorrhage (ICH). ARIC participants with analyzable ECGs and no history of stroke were included. Ventricular substrate was characterized by cardiac memory, spatial QRS-T angle (QRS-Ta), sum absolute QRST integral (SAIQRST), spatial ventricular gradient magnitude (SVGmag), premature ventricular contractions (PVCs) and tachycardia-dependent intermittent bundle branch block

(TD-IBBB) on 12-lead ECG at visits 1-5. The author concluded that PVC burden (reflected by cardiac memory) is associated with ischemic stroke. Transient cardiac memory (likely through TD-IBBB) precedes ICH.

1. The concern of this study is the mechanism between EKG finding and stroke and ICH is not evaluated.

Thank you for your suggestion. To clarify this for a reader, we replaced the word "mechanisms" with the phrase "risk factors".

The study's goal was to determine an association of ventricular substrate (quantified by global electrical heterogeneity (GEH) and traditional ECG metrics) with thrombotic, cardioembolic, or hemorrhagic stroke subtypes. This is a typical epidemiologic observational study, aiming to answer whether an exposure (GEH and traditional ECG metrics reflecting ventricular substrate) is associated with outcome (incident stroke). We agree that we cannot claim cause-effect relationships between exposure and outcome because of the observational nature of the study. However, the strengths of the ARIC study are in a detailed characterization of confounders. We adjusted our models for 35 confounders in a gradual, step-by-step approach. Such strong adjustment for confounders permits us to conclude about an independent association between the exposure and outcome, implying underlying mechanisms. At the same time, we agree that any onservational study might include unmeasured confounders. To address your concern, we deleted the word "mechanisms".

2. Moreover, the relationship with known risk factors for stroke and ICH is not evaluated.

In this study, we adjusted for 35 variables that are known risk factors of stroke (as described on pages 12-13):

- *Age*
- *Gender*
- *Race*
- *hypertension (history of diagnosed hypertension, levels of systolic and diastolic blood pressure, use of blood pressure-lowering drugs),*
- *smoking,*
- *diabetes,*
- *level of physical activity,*
- *obesity (body mass index, waist-to-hip ratio),*
- *high blood cholesterol (total cholesterol, HDL, triglyceride level, use of lipid-lowering medications)*
- *carotid artery disease (presence of carotid artery plaque detected by ultrasound as described on page 10)*
- *Kidney function (eGFR)*
- *cardiovascular disease (CVD), defined as the presence of prevalent coronary heart disease (CHD), peripheral artery disease (PAD), or heart failure (HF) as defined on page 10*
- *alcohol use,*
- *Geographic location (study center) that might reflect socioeconomic factors.*
- *atrial fibrillation as described on page 9*
- *ectopic or premature atrial complexes (PACs).*
- *use of anticoagulant and aspirin-containing medications*
- *Measures of atrial substrate (described on page 9). The atrial substrate was characterized by the following P-wave indices: frontal P axis, P-terminal force in lead V1 (PTFV1), and PR interval duration.*
- *The use of antiarrhythmic medications (described on page 10). Antiarrhythmic medications included class I, II (beta-blockers), III, IV (phenylalkylamines, and benzothiazepines calcium channel blockers), or V (digoxin) antiarrhythmic agents*
- *Measures of ventricular substrate (QRS duration, QTc interval, presence of electrocardiographic left ventricular hypertrophy (ECG-LVH), bundle branch block (BBB) or interventricular conduction delay (IVCD).*
- *Premature or ectopic ventricular complexes (PVCs)*

Importantly, many known risk factors of stroke were included as time-updated risk factors (in model 5), as described on page 13.

3. Finally, EKG findings cannot be directly correlated with ventricular substrate (eg. scar).

Electrocardiogram (ECG) reflects electrophysiological ventricular substrate. Heart generates electricity with every heart beat. Electrocardiographic method is based on the cornerstone assumption that three-dimensional heart vector represents electrical generator, which moves during cardiac cycle. The movement of the heart vector is described by vectorcardiographic (VCG) loops (P, QRS, T). Projection of the VCG loops on each ECG lead over time produces ECG waveform. By the nature of the collected by ECG method information, ECG reflects electrophysiological substrate. Electrophysiological substrate goes beyond cardiac structural substrate, because it reflects "cardiac memory" of intermittent changes in ventricular activation, such as premature ventricular complexes, intermittent ventricular conduction abnormalities, and so on. Development of scar also changes ventricular activation path and, thus, it is also causes "cardiac memory", which is usually called "electrical remodeling", because scar is permanent (unlike PVCs). To clarify that we focus on electrophysiological ventricular substrate, we added the word "electrophysiological" before each phrase "ventricular substrate".

Reviewer #2

There are potential residual risks associated with stroke, apart from traditional cardiovascular and metabolic risk factors. The authors investigated the association of cardiac ventricular substrate, such as PVCs and SVGmag, with the incidence of different types of stroke. This is an interesting study with clinical significance for stroke prevention. However, there are some points to be addressed.
Comments
Title:
1.The current title 'Cardiac Ventricular Substrate of Stroke' seems not to fully express the core elements of the article. Please modify the title.

Thank you for commending our work, and for wise suggestions. In response to your request, and also considering the request of reviewer#1, we changed the title per the journal stype: "Electrophysiological Ventricular Substrate of Stroke: a Prospective Cohort Study in The Atherosclerosis Risk in Communities (ARIC) Study".

Abstract:
2.It is not clear enough how the participants in this current study were enrolled and followed. Whether the participants with ECG data at the first visit are enrolled in this study? And what is the number of participants when time-updated or time-dependent ventricular substrates analyzed.

We revised the abstract as suggested, to meet the formatting requirements. Correct, ECG was recorded during the 1st study visit. We excluded participants with missing data. If there were missing data of exposure (ECG), outcome (incident stroke), or covariates (including time-updated ECG covariates), such participants were excluded from this analysis. The exact date of ECG recording was known. Thus, we conducted a time-updated analysis using the exact date of the recorded ECG. There were no missing data in our dataset.

3.Abbreviations should be defined when first appear, such as ECG.

We added a suggested definition for ECG abbreviation in the manuscript's text.

4.Strengths and Limitations of this study: Strengths and limitations should be stated separately.

Per the Journal requirement, it is required to include an article summary with the heading "Strengths and limitations of this study", and we had to follow the Journal requirement.

Introduction:

5.This section is well organized. It is worth noting that we can only reveal the association in observational studies, and it is unreasonable to say 'uncovering novel mechanisms of stroke.' The use of term 'mechanism' should be cautious.

We agree, and we replaced the word "mechanisms" with "risk factors".

Method:

6.The design and flow chart (Figure 1) is not clear enough to understand. Please clarify the condition of data missing and lost to follow up in this cohort study. How the missing data was handled in your analyses?

Thank you for this important point. We clarified/edited Figure 1 (flowchart). We excluded participants with any missing data (missing exposure, or missing outcome, or missing covariates). There were no missing data in our analysis. Participants with lost follow-up were excluded from the analysis. To make this point clear, we edited Figure 1.

7.The data are well analyzed in this study, and the time-independent effect of ventricular substrate with multiple measurements is considered in model 5. Please clarify how the death from other diseases except stroke, as a competing event, was considered in your analyses.

Thank you for this excellent question. We added the suggested clarification, and, as suggested, expanded competing risk analyses. We agree that the risk of stroke competes with the risk of death from other causes. We employed cause-specific hazards functions, estimated using Cox proportional hazards models. For a models with incident stroke (including fatal stroke) outcomes, death from all other causes was censored at the date of death.
To further address your question, we compared the strength of association of the exposure (ECG variables reflecting ventricular substrate) between the two competing outcomes. For models with other-than-stroke death outcome, incident stroke event was censored at the date of stroke. Alive and event-free participants were censored at the date of the last follow-up. We compared the strength of association of the exposure between the two competing outcomes by testing the null hypothesis that the coefficients for a given variable (b_1 and b_2) were the same across the two competing outcomes by calculating Z-scores:

$$z = \frac{b_1 - b_2}{\sqrt{[s.e.(b_1)]^2 + [s.e.(b_2)]^2}}$$

where s.e. is a standard error, and determining their statistical significance.
We reported the association of GEH with other-than-stroke death in Supplemental Table 3

We observed several important findings (added on page 17).

There were 4,417 deaths from other-than-stroke causes (incidence 14.78; 95%CI 14.35-15.22) per 1000 person-years of follow-up. Most ECG variables had similar associations with both competing outcomes, with few notable exceptions.

Peak SVG magnitude demonstrated statistically significant discordant association with competing outcomes: larger SVG magnitude was associated with a higher risk of all types of incident stroke but lower risk of stroke-free death (Supplemental Table 3). Similarly, TD-IBBB was associated with a greater risk of ICH but not stroke-free death. Consistently, PVC's presence was stronger associated with ischemic stroke than with competing stroke-free death.

We added sub-sections reflecting comparison of two competing outcomes in the Methods, Results, and Discussion. Overal, newly added results support our previous discussion points and strengthen the evidence base.

Results:

8.The interaction between demographic variables (such as age, sex) and ventricular substrates should be presented in Subgroup analyses.

Thank you for this important question. As requested, we investigated interactions with the main demographic characteristics and ECG-ventricular substrate in fully adjusted model 5 and reported the results in Supplemental Table 4, and Supplemental Figures 3-4. We added relevant subsections in the Methods, Results, and Discussion.

Page 14: We investigated whether sex and age modify the association of electrophysiological ventricular substrate with incident stroke by adding interaction terms in the fully adjusted Cox model 5. Separate models were constructed for interaction with sex and age as a continuous variable for each outcome.

Page 18: We observed a few statistically significant interactions (Supplemental Table 4). The presence of PVCs was associated with a 2-fold higher risk of ES in women compared to men [relative hazard ratio (RHR) 2.2; 95%CI 1.0-5.0; P=0.051]. Peak SVG elevation displayed discordant association with TS in men and women. More superiorly directed SVG vector was associated with a lesser risk of TS in women but higher risk of TS in men.
ECG-LVH was associated with a 5-fold higher ICH risk in men than women (RHR 5.6; 95%CI 1.1-28.5; P=0.039). The association of age with incident ICH was modified by QRS-T angle (Supplemental Figure 3) and SVG azimuth (Supplemental Figure 4). The risk of ICH was rising steeper with age if the baseline QRS-T angle was smaller, and SVG azimuth was directed more anteriorly.

Thus, we observed important differences between men and women, warranting further investigations.

9.I suggest that integrating the figures presenting the association of ventricular substrates with TS, ES and ICH.

Thank you for this excellent idea. We merged together former figures 2 and 4 and presented them as new figure 2.

10.How the monitoring of cardiac memory on ECG could be related with therapy adjustment?

Potentially, monitoring of cardiac memory on ECG can guide short-term dual antiplatelet therapy for prevention of thrombotic stroke. Future studies are needed to test this hypothesis. We clarified this point in the Discussion.

The manuscript should follow the format requirements and it needs to be reviewed and edited for proper grammar.

The manuscript was edited as suggested. Thank you.

Reviewer #3

In the current manuscript entitled 'Cardiac Ventricular Substrate of Stroke: The Atherosclerosis Risk in Communities (ARIC) Prospective Cohort Study' Johnson et al. examine the prognostic value of global electric heterogeneity (GEH) for thrombotic stroke, cardioembolic stroke and intracranial hemorrhage.
For this purpose, the authors examined 14,479 individuals from the Atherosclerosis Risk in Communities (ARIC) study, who had not previously experienced any type of stroke. Study participants were followed-up from 1987 to 2018. Over a median follow-up of 24.5 years, there were 899 thrombotic stroke events (incidence 2.87; 95% CI 2.69-3.07 per 1000 person-years), 400 embolic stroke events (incidence 1.26; 95% CI 1.14-1.39 per 1000 person-years), and 120

intracranial hemorrhage-only strokes (incidence 0.38; 95% CI 0.32-0.45 per 1000 person-years). Both types of ischemic stroke (thrombotic and embolic) were diagnosed in 62 participants, and another 16 participants endured both ICH and either thrombotic or embolic stroke.
The authors used built Cox proportional hazard risk models. All continuous ECG exposure variables were expressed as their z score to standardize comparisons.
To adjust for confounders, they constructed 5 models for incremental adjustment. Briefly, the models were built as follows:
Model 1: demographic characteristics (age, sex, and race-study center group).
Model 2: Model 1 + baseline CV risk factors and presence of CVD.
Model 3: Model 2 + atrial substrate (abnormal P axis, PR interval, PTFV1, heart rate, use of antiarrhythmic drugs, anticoagulants, and aspirin, presence of PACs on 10-second 12-lead ECG at any visit, S or V median beat at any visit, and presence of AF at baseline or at any time during follow-up).
Model 4: Model 3 + characteristics of the ventricular substrate.
Time-updated model 5: all model 4 covariates, as well as ECG variables that were updated at the date of ECG recording in visits 1-5.
The authors found that different metrics were associated with embolic or thrombotic stroke after multivariable adjustment, while SVG magnitude and SAIQRST emerged as common independent predictors of both embolic and thrombotic stroke (model 5).

The manuscript is a very interesting, well-written and clinically relevant research study which extends previous work of the senior author (Dr Tereshchenko), where she examined the association between GEH alterations and ventricular arrythmias/ sudden cardiac death in the same cohort (Circulation. 2016;133:2222–2234).

Thank you for commending our study.

I only have few minor comments that the authors may consider to address:
1) Does the addition of ventricular substrate parameters in existing established algorithms (e.g. CHA2DS2-VASc) offer additive prognostic value? The authors could use the number of abnormal GEH parameters (0-5) or continuous variables that retained their association with both embolic and thrombotic stroke after multivariable adjustment (e.g. SVG magnitude, SAIQRST). Suggested statistical approach: Net reclassification index, discrimination index.

Thank you for your excellent comments and wise suggestions. As requested, we conducted additional analyses.
To determine the additive predictive value in comparison with CHA2DS2-VASc risk score, which was developed to prognosticate 1-year risk of stroke in AFib patients, we studied an incident AFib cohort in our study. From our main study population (n=14,479) we excluded participants with prevalent AFib (n=26), and then those without incident AFib during follow-up (n=11,178), and those with missing data about incident AFib status in 2017 (n=264). The remaining 3,011 participants with incident AFib were included in the analysis. The CHA2DS2-VASc score was calculated at the date of incident AFib diagnosis. The exposure ECG variables were taken as measured prior (as close as possible) to incident AFib. Time at risk was calculated from the date of AFib ascertainment until the date of ischemic stroke, other-than-stroke death, or loss of follow-up, whichever occurred first. To compare predictive value of GEH for ischemic stroke, we compared the performance of CHA2DS2-VASc score alone and after addition of GEH variables. We assessed model performance by C-statistic, relative integrated discrimination improvement (IDI), and categorical net reclassification improvement (NRI) for categories of <1%, 1-2%, and >2% 1-year risk of stroke.
Results: Out of 3011 ARIC participants with incident AFib, there were 204 ischemic strokes (either ES or TS) within the first year after AFib diagnosis. The addition of all GEH variables to CHA2DS2-VASc score improved the C-statistic (95%CI) from 0.560 (0.515-0.604) to 0.605 (0.561-0.650); P=0.005. Reclassification was also improved. Relative IDI 1.31 (95%CI 0.81 – 1.79); P<0.0001. Categorical NRI 0.282 (95%CI 0.182 – 0.381); P<0.0001. Out of participants with ischemic stroke, 62% were appropriately re-classified to higher risk categories. Out of those free from stroke within 1 year, 53% were appropriately re-classified to lower-risk categories.

We added the requested analyses in the Methods and Results, but did not focus on it in the Discussion, because the Discussion section and the manuscript overall is already large. Development and validation of the risk score will require a separate study, which should include another, second cohort for validation of the risk score performance.

2) Does the co-existence of multiple ventricular substrate pathologies increase the risk of stroke?

Yes, to a small degree. Further addition to the predictive model traditional ECG metrics of ventricular substrate (ECG-LVH, QTc, QRS duration), including presence of PVCs, did not improve C-statistic above the previous model reported above (C-statistic 0.611; 95%CI 0.567-0.655) versus 0.605 (95%CI 0.561-0.650); P=0.211). However, reclassification indices indicated statistically significant improvement. Relative IDI 0.131 (P-value 0.009) and NRI 0.100 (P-value 0.048).

3) Please add Ethics Approval Number in the respective Methods section.

Added as suggested (on page 23). This study was approved by the Oregon Health & Science University Institutional Review Board (eIRB ID IRB00010346). In addition, the ARIC Publication Committee approved this manuscript (ARIC Manuscript #3333).

4) Figure 2: please provide details of each model in the Figure legend.

Added as suggested.

VERSION 2 – REVIEW

REVIEWER	Joung, Boyoung Yonsei University Health System
REVIEW RETURNED	13-Aug-2021

GENERAL COMMENTS	I have no further comment.

REVIEWER	Wu, Zhiyuan Capital Medical University, Public Health
REVIEW RETURNED	09-Aug-2021

GENERAL COMMENTS	The authors have addressed my concerns.