SIREN: the journey towards accurate comprehensive stroke phenotyping in Africa

Stroke Investigative Research and Educational Network (SIREN) Investigators

Fifth H3 Africa Consortium Meeting, November 7-10, 2014, Dar es Salaam
Background

- Stroke epidemic in sub-Saharan Africa
- Need to characterize this epidemic
- Epidemiologic, phenotypic and genomic characterization
- SIREN: a response to the clarion call.
The SIREN Partners

USA

UK
Cardiovascular Topics

Understanding the rise in cardiovascular diseases in Africa: harmonising H3Africa genomic epidemiological teams and tools

Mayowa O Owolabi, George A Mensah, Paul L Kimmel, Dwomoa Adu, Michele Ramsay, Salina P Waddy, Bruce Ovbiagele, Cristina Rabadan-Diehl, Rebekah Rasooly, Sally N Akarolo-Anthony, Charles Rotimi as members of the H3Africa Consortium
To evaluate the **genomic and environmental risk factors for stroke in SSA**, while simultaneously building sustainable capacities in phenomics, biobanking, genomics, biostatistics and bioinformatics.
SIREN : Component Projects

- SIREN is comprised of three interwoven Systematic Investigation of Blacks with Stroke (SIBS) projects geared at a comprehensive investigation of stroke: SIBS-Phenomics and Community Engagement, SIBS-Genomics, SIBS-Bioinformatics and Genomic Analysis.
SIREN : Component Projects

SIREN Collaborating Center (Ibadan)

- SIREN Training, Career Development and Capacity building Core
- SIBS Phenomics and community engagement core
- SIBS Genomics Core
- SIBS Bioinformatics and Genomic Statistics Core

SIREN Project Sites (Ibadan, Abeokuta, Zaria, Kano, Kumasi, Accra) and USA (MUSC, UAB)
SIREN

3000 case – control pairs

SIBS - Phenomics

Accurate phenotyping of cases

SIBS - Genomics

Discovery Phase

GWAS
Candidate gene
Pathway /Network analysis

Replication Phase

Validate new SNPs and CNVs in REGARDS black sub-cohort

Genomic banking for future analysis

Further analysis with emerging techniques

SIBS - Bioinformatics
SIBS - Phenomics

• To generate comprehensive and accurate phenomic data on stroke patients and controls that will assure reliable phenotyping for the concurrent genomics project (SIBS-Genomics) focused on evaluating genetic risk factors for stroke (and its subtypes)
Study Design

- case-control study based on a risk-set sampling frame work
- 3000 cases and 3000 controls
### Sampling Frame

#### Cases

- All patients admitted to medical ward, neurology or neurosurgical ward, ICU, Emergency room, or reviewed in an Outpatient Clinic
- study eligibility criteria
- informed consent

#### Controls

- Primarily community–based
- Hospital-based controls may be attendants or relatives of another (non-stroke) patient, or
- Patients admitted to the hospital or visiting the hospital for conditions or procedures not related to stroke or TIA.
- Each control is matched for sex, age (+/- 5 years) and ethnicity.
- At least a control (with or without CV risks) for every case recruited.
Inclusion criteria

Cases
• Adult with first stroke within 8 days
• CT or MRI is planned within 10 days of symptom onset.

Controls
• Persons, with no clinical evidence of stroke or MI, with or without cardiovascular risk factors
Exclusion criteria

**Cases**
- Current hospitalization for coronary heart disease
- Unable to provide consent and no surrogate available
- A known previous history of stroke

**Controls**
- A known previous history of stroke, or MI
- Unable to provide consent and no surrogate available
To validate stroke–free status in our control subjects
Validating the Questionnaire for Verifying Stroke-Free Status (QVSFS) by Neurological History and Examination
William J. Jones, Linda S. Williams and James F. Meschia

Stroke. 2001;32:2232-2236
doi: 10.1161/hs1001.096191

Original Paper

Neuroepidemiology 2004;23:236–239
DOI: 10.1159/000079949

Creation of a Bilingual Spanish-English Version of the Questionnaire for Verifying Stroke-Free Status

Pablo R. Castillo a  Thomas G. Brott b  Salvador Alvarez c  James F. Meschia
1. Have you ever been told by a doctor that you had a stroke?
   Yes  No  Don’t know

2. Have you ever been told by a doctor that you had a mild stroke or almost had a stroke (TIA)?
   Yes  No  Don’t know

3. Have you ever had sudden painless weakness on one side of your body?
   Yes  No  Don’t know

4. Have you ever had sudden numbness or a dead feeling on one side of your body?
   Yes  No  Don’t know

5. Have you ever had sudden painless loss of vision in one or both eyes?
   Yes  No  Don’t know

6. Have you ever suddenly lost one half of your vision?
   Yes  No  Don’t know

7. Have you ever suddenly lost the ability to understand what people are saying?
   Yes  No  Don’t know

8. Have you ever suddenly lost the ability to express yourself verbally or in writing?
   Yes  No  Don’t know
QVSFS SIREN

- 4 sites: Kumasi, Abeokuta, Ibadan, Zaria/Kano
- Approx. 100 patients each from each site- 50 from general clinic and 50 from neurology/stroke clinic
- Questionnaire were administered by residents in training.
- Confirmation of stroke by a neurologist or specialist at each site AND by a CT scan in a few patients (at least 32).
Performance of the SIREN – adapted 8-Item QVSFS (prelim results, n = 171)

- Sensitivity = 0.94
- Specificity = 0.84
- NPV = 0.94
- PPV = 0.83
**SIREN PROTOCOL**

1. Enter all Stroke Cases into the Stroke Register/Log
2. Use the Stroke Register to screen ALL patients admitted with probable stroke

Matching Controls:
- Unrelated sex, age (± 5 years) and ethnicity matched
- Recruit for each case within 60 days or sooner

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Eligibility Assessment

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Sign Consent Form and Obtain contact Information

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CASE/CONTROL QUESTIONNAIRE
1. Conduct Interview
2. Physical Measurements
3. Specimen Collection
4. Diagnostic work-up

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Data entry into the Neuroimaging Archive/Red Cap

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Quality Control report/action on Data

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Obtain Follow up Data at 7 days, 28 days, 3 months, 6 months, & 1 year

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Quality Control report/action on Data
ALGORITHM FOR INVESTIGATING STROKE PATIENTS

Clinically suspected Stroke

Cranial CT/MRI *confirmed stroke

Yes \(^1\)SAH \(\uparrow\) No

Note and record diagnosis & exclude

Case Report Form (CRF)/Questionnaire,
Community Engagement Survey Questionnaire;
Blood Sample Collection within 10 days of stroke;
Physical Measurements (e.g., BP, WC, HC, Ht, Wt, etc.);
Risk factor assessment, Labs- Fasting lipids, HbA1C, FBG, ECG,
Severity, functional outcomes assessed at Day 0, 7 days, 28 days, 3 mths, 6 mths, 1yr

Stroke Type

Haemorrhagic Stroke

Subtype, Hematoma volume; Location

Ischaemic Stroke

Radiologic sub-types

Echo, carotid Doppler, ± 24 hr ECG, ± TEE, TCD, + MRA/CTA

Etiologic sub-types

OCSP:- TACI, PACI, Lacunar.

Clinical sub-types

Cardio-embolic, large vessel, small vessel dx-intracranial, ASCO, TOAST

Vascular territory -ACA, MCA, PCA
Location - cortical vs subcortical


Blood samples collected from Cases/Controls must be processed WITHIN TWO HOURS. Alert the lab before taking samples. If delayed, store in a refrigerator for a maximum of one hour. * MRI preferred for suspected lacunar or posterior circulation infarct
# Variables for SIBS Phenomics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Personal and lifestyle factors</strong></td>
<td>Ethnicity, Tobacco use, alcohol use</td>
</tr>
<tr>
<td><strong>Socio economic factors</strong></td>
<td>Education, occupation.</td>
</tr>
<tr>
<td><strong>Psychosocial factors</strong></td>
<td>Stress, social support, depression,</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td>Hypertension, diabetes mellitus, hypercholesterolemia, atrial fibrillation, CVD,</td>
</tr>
<tr>
<td><strong>Physical measurements</strong></td>
<td>Blood pressure height, weight, waist and hip circumferences</td>
</tr>
<tr>
<td><strong>Neurological assessment</strong></td>
<td>Clinical assessment, neuroimaging, location of lesion, results of diagnostic tests and confirmation of stroke sub-type.</td>
</tr>
</tbody>
</table>
# Variables for SIBS Phenomics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stroke Subtype</strong></td>
<td>Based on clinical assessment and Neuroimaging (e.g. CT of brain)- Information on Neurovascular</td>
</tr>
<tr>
<td><strong>Blood tests</strong></td>
<td>Lipid profile, fasting blood sugar,</td>
</tr>
<tr>
<td><strong>Neuroimaging and other tests</strong></td>
<td>CT or MRI of brain (for all cases), ECG (for all cases), Echocardiography (for all cases of suspected cardioembolic stroke), Carotid Doppler USS (for all cases of ischemic stroke, anterior circulation).</td>
</tr>
<tr>
<td><strong>Course in hospital and status at 30 days.</strong></td>
<td>Treatments given, outcomes in hospital, m-Rankin at 30 days</td>
</tr>
</tbody>
</table>
Classification and natural history of clinically identifiable subtypes of cerebral infarction

JOHN BAMFORD   PETER SANDERCOCK   MARTIN DENNIS   JOHN BURN   CHARLES WARLOW

We describe the incidence and natural history of four clinically identifiable subgroups of cerebral infarction in a community-based study of 675 patients with first-ever stroke. Of 543 patients with a cerebral infarct, 92 (17%) had large anterior circulation infarcts with both cortical and subcortical involvement (total anterior circulation infarcts, TACI); 185 (34%) had more restricted and predominantly cortical infarcts (partial anterior circulation infarcts, PACI); 129 (24%) had infarcts clearly associated with the vertebrobasilar arterial groups. Those in the POCI group were at greater risk of a recurrent stroke later in the first year after the index event but had the best chance of a good functional outcome. Despite the small anatomical size of the infarcts in the LACI group, many patients remained substantially handicapped. The findings have important implications for the planning of stroke treatment trials and suggest that various therapies could be directed specifically at the subgroups.

Trial of ORG 10172 in Acute Stroke Treatment, or TOAST

Epidemiology of Ischemic Stroke Subtypes According to TOAST Criteria: Incidence, Recurrence, and Long-Term Survival in Ischemic Stroke Subtypes: A Population-Based Study

Peter L. Kolominsky-Rabas, Margarete Weber, Olaf Gefeller, Bernhard Neundoerfer and Peter U. Heuschmann

Stroke. 2001;32:2735-2740
New Approach to Stroke Subtyping: The A-S-C-O (Phenotypic) Classification of Stroke

P. Amarenco\textsuperscript{a}  J. Bogousslavsky\textsuperscript{b}  L.R. Caplan\textsuperscript{c}  G.A. Donnan\textsuperscript{d}  M.G. Hennerici\textsuperscript{e}
Quality Control Strategies

• three levels of adjudication:
• first by the site PI-neurologist/neuroradiologist;
• secondly by other sites PI-neurologist/neuroradiologist for verification (peer review)
• thirdly by a *SIREN stroke phenotyping committee* comprising: all site PIs, and neuroradiologists (for ambiguous cases)
• and random checks on all classified cases.
Progress till date

✓ Start Date: 20th September, 2013.

✓ Year 1:
  - Ethical approval obtained across all sites
  - International, multidisciplinary project team of 60 professionals set up
  - Study protocols, CRFs (1000 variables over 12 months) and SOPs developed
  - Procurement of biorepository facilities across sites
  - Procurement of sample collection kits
  - Training programmes for the team
  - Site visits to inspect facilities and harmonize procedures

✓ Currently in Year 2 of the Project
<table>
<thead>
<tr>
<th>SITES</th>
<th>CAB</th>
<th>FGDs</th>
<th>FGD Subjects</th>
<th>SIREN-QVSFS</th>
<th>SIBS PHENOMICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCRA</td>
<td>6</td>
<td>4</td>
<td>27</td>
<td>Not Involved</td>
<td>50 (33 cases; 17 controls)</td>
</tr>
<tr>
<td>KUMASI</td>
<td>5</td>
<td>4</td>
<td>23</td>
<td>95</td>
<td>48</td>
</tr>
<tr>
<td>UCH</td>
<td>7</td>
<td>3</td>
<td>16</td>
<td>90</td>
<td>68 (37 cases; 31 controls)</td>
</tr>
<tr>
<td>BLOSSOM</td>
<td>6</td>
<td>3</td>
<td>17</td>
<td>Not Involved</td>
<td>3</td>
</tr>
<tr>
<td>FMC</td>
<td>6</td>
<td>3</td>
<td>17</td>
<td>90</td>
<td>21 (18 cases; 3 controls)</td>
</tr>
<tr>
<td>SACRED HEART</td>
<td>6</td>
<td>3</td>
<td>18</td>
<td>Not Involved</td>
<td>5</td>
</tr>
<tr>
<td>ZARIA</td>
<td>9</td>
<td>2</td>
<td>12</td>
<td>40</td>
<td>21</td>
</tr>
<tr>
<td>KANO</td>
<td>7</td>
<td>4</td>
<td>24</td>
<td>45</td>
<td>27 (25 cases 2 controls)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>52</td>
<td>26</td>
<td>154</td>
<td>360</td>
<td>243</td>
</tr>
</tbody>
</table>
Neuroimaging archiving with the AIM software
Centralized electronic data storage
SPECIMEN FLOW FOR SIREN SUBJECTS AT BASELINE

SOP for Sample processing (Genomic phase)

Biochemical Investigations (locally executed)

2 x 5ml Plain Red top
1 x 10ml SST Gold top
1 x 4.5ml K2 EDTA Purple top

(Centrifuge)

Three (3) 1.5ml Aliquots of serum
1.8ml cryovial

Transfer cryovials & K2 EDTA Vacutainer into Zip Loc Bags

Store in -20 Freezer

Transfer periodically into transport boxes with DRY ICE/ICE PACKS for shipment to ibadan

1 x 4.5ml K3 EDTA Lavender top
1 x 4.5ml Na Citrate Blue top

(Centrifuge)

1.5ml aliquots (plasma)
0.5ml aliquot Buffy coat
1.8ml cryovial

Accucheck for Measuring sugar levels

Finger prick

Heparinized tube

lipid profiling (fasting)

HbA1c

EDTA tube

Enter results under the laboratory section of the CRFs (Cases & Controls)

ALERT LABORATORY BEFORE COLLECTING SAMPLES & PROCESS ALL SAMPLES WITHIN 2 HRS OF COLLECTION * CENTRIFUGATION SHOULD BE DONE AT 3000rpm for 20MINS
Quantity and Quality of DNA Extract

DNA conc (ng/µl)

Optical Density Ratio 260/280
Biobanking

- Procurement of biobanking facilities (-20 °C and –80°C) with solar-powered inverter back up.
- Staff trained on the use of freezerworks and bar coding for sample management.
- Daily temperature chart.
Intensive training programmes for the SIREN Team

- Regular fortnightly SIREN Team meetings by Skype
- Further specialized SIREN WG trainings and meetings (Cardiologists, Neuroradiologists, Sonologists, Laboratory)
Stroke training programmes for health workers
Community Engagement

• Established Community Advisory Boards across all SIREN performance sites
• Conducted FGDs and structured interviews to evaluate public stroke literacy and KAP of genomic research
• Developed a stroke documentary video for advocacy and educational purposes.
Challenges

• High cost of investigations
• Power supply and solution found
• Internet connectivity
• Bureaucracies of grant administration
• Political and industrial instability
• Subject retention

Victories

• Hospital CEOs providing discounts up to 50%
• Inverters including solar powered batteries
• Migrate to better networks
• Continuing engagement with institutional leaders
• Maximize stable periods and remain optimistic
• Keep in touch – mobile phone, CE activities.
“The purpose of life is to contribute in some way to making things better.”

- Robert F. Kennedy
Conclusion

- SIREN is poised to substantially enhance our understanding of factors that could be addressed to improve stroke outcomes, and possibly other vascular disease entities such as coronary artery disease and chronic kidney disease in SSA;
- discover/explore potentially modifiable genetic pathways to stroke risk that may be common to Black Africans and Black Americans

- **Making a difference:** taming the burgeoning stroke epidemic in Africa
Collaborating Institutions

- University of Ibadan, Nigeria
- Medical University of South Carolina, USA
- Department of Epidemiology and Heflin Center for Human Genetics, University of Alabama at Birmingham, USA
- Federal Medical Center, Abeokuta, Nigeria
- Sacred Heart Hospital, Abeokuta, Ibadan, Nigeria
- WFNBR-Blossom Medical Center, Ibadan, Nigeria
Collaborating Institutions

• Aminu Kano University, Kano, Nigeria
• Ahmadu Bello University, Zaria, Nigeria
• University of Ghana, Accra, Ghana
• Kwame Nkrumah University, Kumasi, Ghana
• CPGR
• Institute of Genetic Medicine, Newcastle University, UK
• University College London, UK
SIREN Investigators

- Mayowa Owolabi - Overall PI
- Bruce Ovbiagele - Overall Co-PI
- Rufus Akinyemi – PI Genomics/Site PI
- Albert Akpalu – PI Phenomics /Site PI
- Kwamena Sagoe- PI Bioinformatics
- Daniel Lackland - co – PI Phenomics
- Mulugeta Gebregziabher - Biostatistician
- Oyedunni Arulogun- Community Engagement
- Carolyn Jenkins – Community Engagement
- Fred Stephen Sarfo- Site PI
- Reginald Obiako – Site PI
- Lukman Owolabi – Site PI
SIREN Support Team

- Program Manager
- Research Coordinators
- Research Assistants
- Community Engagement Coordinators
- Neuroradiologists/Sonologists
- Cardiologists
- Resident Doctors
- Medical Laboratory Scientists
Supported by NIH U54HG007479-01
THANK YOU