DBS Collection for DNA Methylation Profiling in the Malawi Longitudinal Studies of Family and Health (MLSFH)

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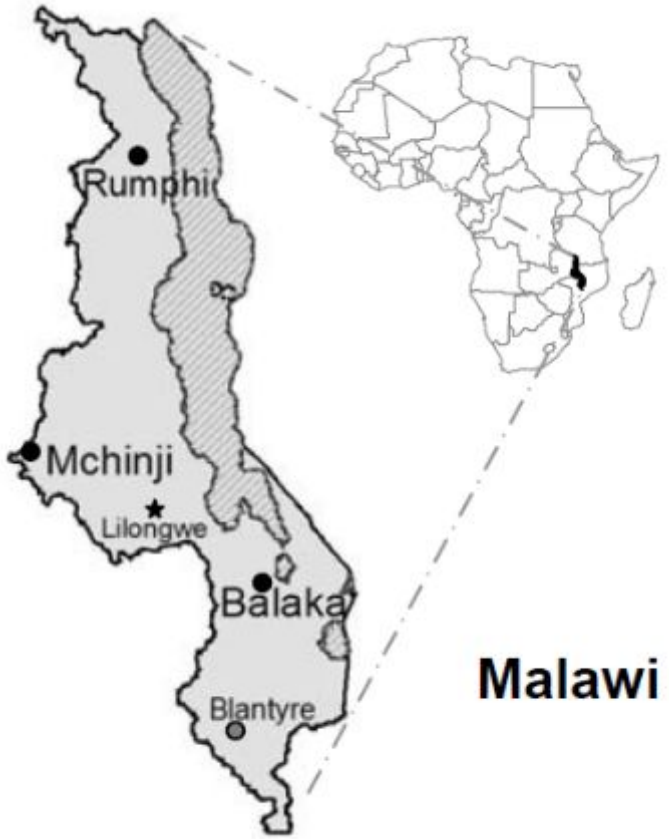
Malawi Longitudinal Study of Families and Health (**MLSFH**)

- Longitudinal household survey established in 1998
- Population-based sample broadly representing rural Malawi (~85% of the population)
- Located in three regions: Rumphi, Mchinji, and Balaka, including migration follow-up
- Rural, low-income context
- Family-focused, intergenerational, multidisciplinary



MLSFH Study Population

- **N>6,000** broken down as follows:
  - **Mature adults 45+ (N≈3,500, focus of biosocial aging studies)**
  - Adolescents and young adults (N≈2,000)
  - Adults below age 45 (N≈1,500)
- Currently 12 rounds of data collection (1998-2022)
  - Face-to-face with phone surveys in between rounds
  - Extensive longitudinal data on socioeconomic context, physical and mental health, cognitive function, intergenerational relations, NCD knowledge for 45+

Funding to continue collection in 2024 and 2027 (R01 AG079527)

- Mature adult sample (45+, n ≈ 3,500 including 1,000 siblings)
- Genomic and epigenomic data collection from DBS
  - Epigenetics: DNAm from EPIC V2 array (n ≈ 3,500)
  - Genetics: WGS at 30x (n ≈300) and 6-7x (n ≈ 3,500)
- Biosocial focus in an LMIC context
  - Accelerated aging with earlier onset of age-related diseases
  - Distinctive lifecourse trajectories that differ from HICs
- Key outcomes: accelerated aging measures, HCAP-comparable assessment of cognition and ADRD (including informant interviews and diagnostic conference)
- Summer 2024: DBS and survey data collection

DBS Dry Run (November 2023)

- Test DBS collection protocol and inform any required protocol modifications before scaling up to full collection this summer
- DBS protocol developed using NIA Biomarker Network guidelines with context-specific adjustments for a rural, LIC setting
  - Huge thanks to experts in the room today who provided additional guidance!
- Dry run was conducted in a comparable village in Balaka that is not part of the MLSFH study sample (n=50)
- Also utilizing 10 donor samples from the same individual to assess impact of field conditions on quality and accuracy of DNAm data collection from DBS

Interview training

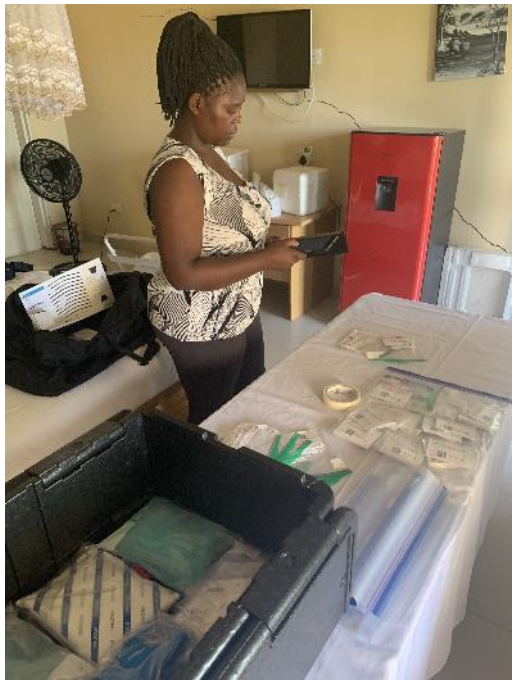
- Interview training took place at the College of Medicine in Malawi
- Further refined protocol with input from our Malawian collaborators at the College of Medicine and Compelling Works

Overview of DBS dry run

- Collected two Whatman 903 cards for each participant (5 circles/card)
- Individual drying boxes were used to protect samples from dust and debris in the field while improving air flow for drying
- Temperature, humidity, and dry time were monitored throughout the entire collection process
  - Used temperature and humidity sensors attached to interviewer backpacks
  - Also monitored temp and humidity during transportation from the field to the car, back to the base, during storage in the refrigerator, and during shipping

Overview of DBS dry run (cont.)

- Back at the base samples were put into a 4°C refrigerator after drying for at least 4 hours
  - Minimize time-to-refrigerator by organizing samples into AM and PM collections
  - Collection time, dry time, and time in the refrigerator were all recorded
- Successfully collected two DBS cards for all 49 individuals that consented (1 refusal)
- Samples were shipped on ice after proper packaging and arrived in good condition at the Kobor Lab



Early takeaways

- Kobor Lab was able to extract enough DNA for both genetic sequencing and DNAm profiling using 1.5 circles
- All 49 samples passed QC indicates collecting good quality DNAm data in a rural field setting from DBS is feasible

Next steps

- Analysis currently underway to test how variations in temperature, humidity, and storage affect DNAm profiles
  - Currently seeing that collection procedures do generate some variation in WBC counts
  - Preliminary evidence suggests that minimizing dry time and exposure to heat and humidity in the field may reduce some of this variation
- Ship extracted gDNA from Kobor Lab to sequencing lab and test genetic sequencing and downstream bioinformatics (Li San Wang's Lab)
- Epigenetic clock prediction of health and aging phenotypes in this pilot sample (Kobor Lab)