



Exploring Phenotypic Variation in Sickle Cell Disease Leg Ulcers

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HEALTH DISPARITIES UNIT

Background

- Human disease is complex and includes influences from social, cultural, biological, and environmental factors
- In this study, sickle cell disease (SCD) is used as a model to explore variation in disease severity and health outcomes
- SCD is the most common genetically inherited blood disorder in the United States and is caused by a point mutation in the beta-globin gene
- One of the most debilitating SCD complications is a leg ulcer that commonly develops at the medial and lateral malleoli (ankle)
- Prevalence of SCD leg ulcers varies with rates of 1-2% in the US, 4-8% in Africa, and 30% in Jamaica¹⁻²
- In SCD, the etiology of leg ulcers remains unknown

Project Overview

This pilot project explores the role of the clinical phenotype, psychosocial indicators, environmental factors, and microbiome in leg ulceration in SCD.

Hypothesis

We hypothesize that leg ulceration in SCD is multifactorial and may involve contributions from the environment (physical and social), genetic modifiers, and the microbiome.

Study Design

General enrollment criteria:

- Sickle cell disease
- > 18 years of age

Microbiome specific criteria:

- No antibiotics or antifungal use 2 weeks prior to study
- No baths, showers, or moisturizers permitted 24 hours prior to sample collection
- A subset of participants will return at least 1 month after initial visit for a follow up sampling

All study participants will complete the following:

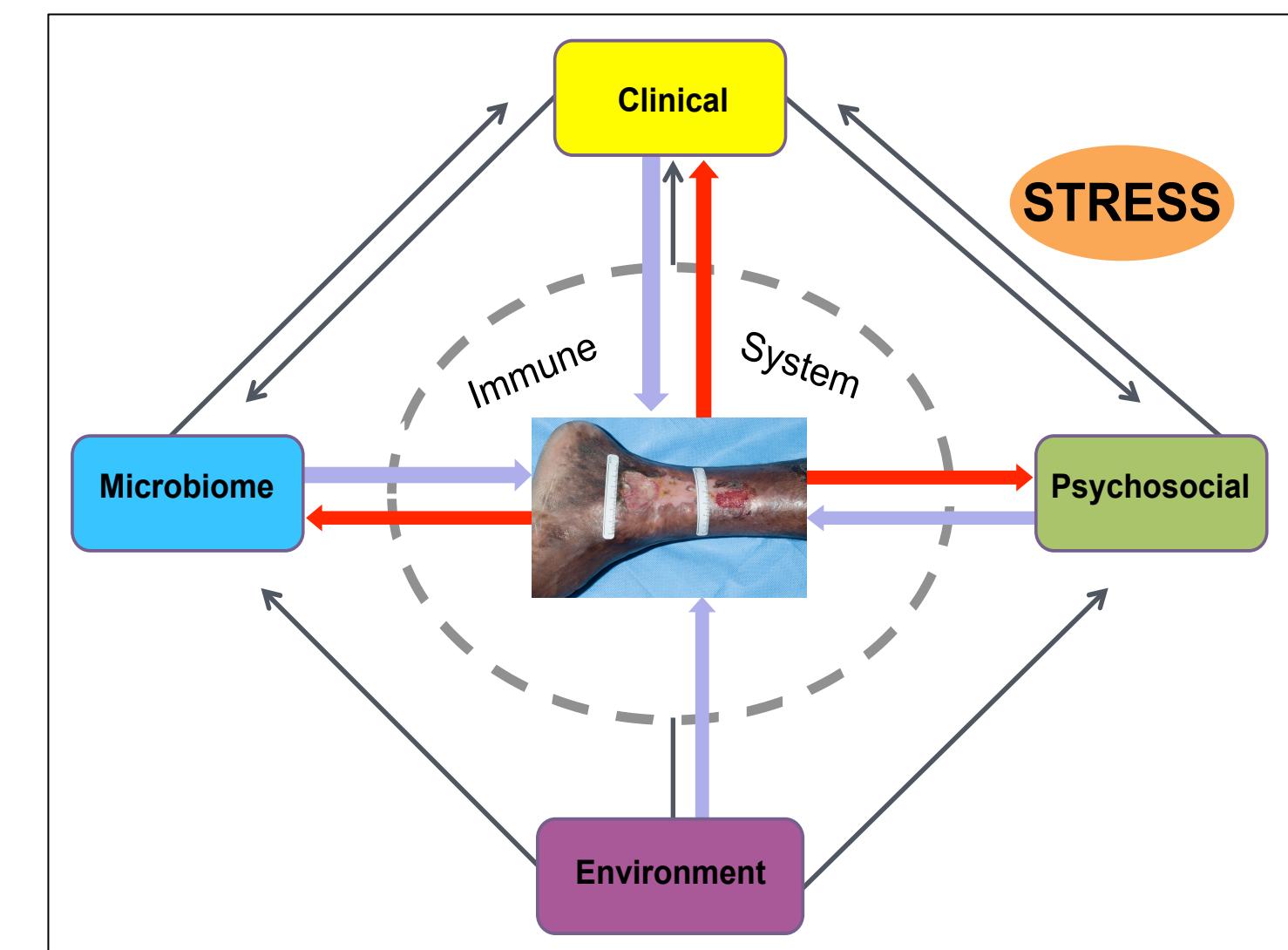
- Clinical evaluation
- Medical history
- Blood work
- Interview-administered survey
- Hair cortisol (optional)

Study accrual plan (200 participants total):

Microbiome	Non-microbiome
With an active ulcer	30
With previous history of ulcers	30
No previous history of ulcers	30
With and without an active ulcer	110

Conceptual Model

Figure 1. A model framework for the role of the microbiome and environment in SCD leg ulcer formation and healing.



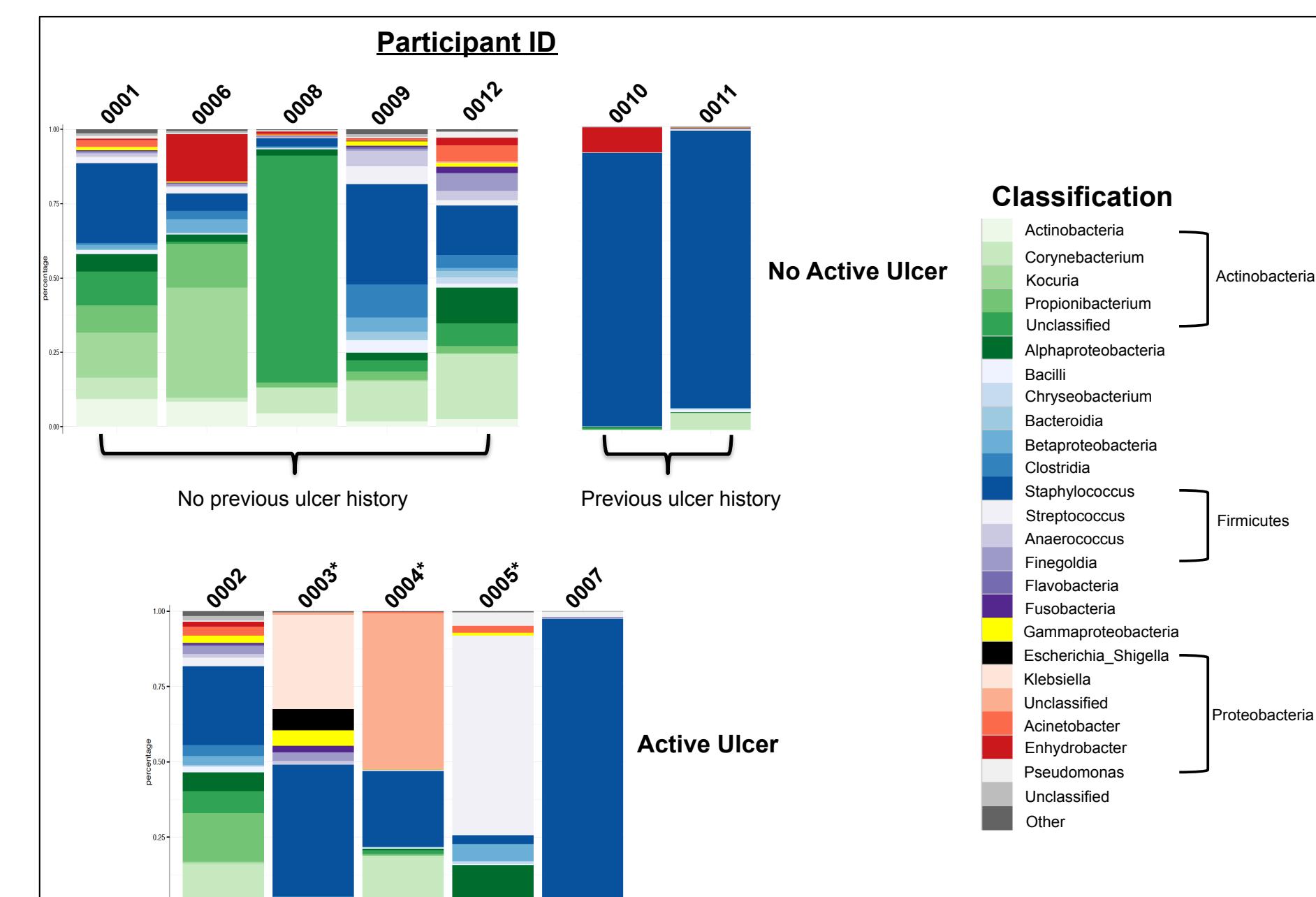
Microbiome

We will characterize microbial diversity on the ankles of participants with and without leg ulcers using high throughput sequencing.

Table 1. Demographics of the first 12 study participants.

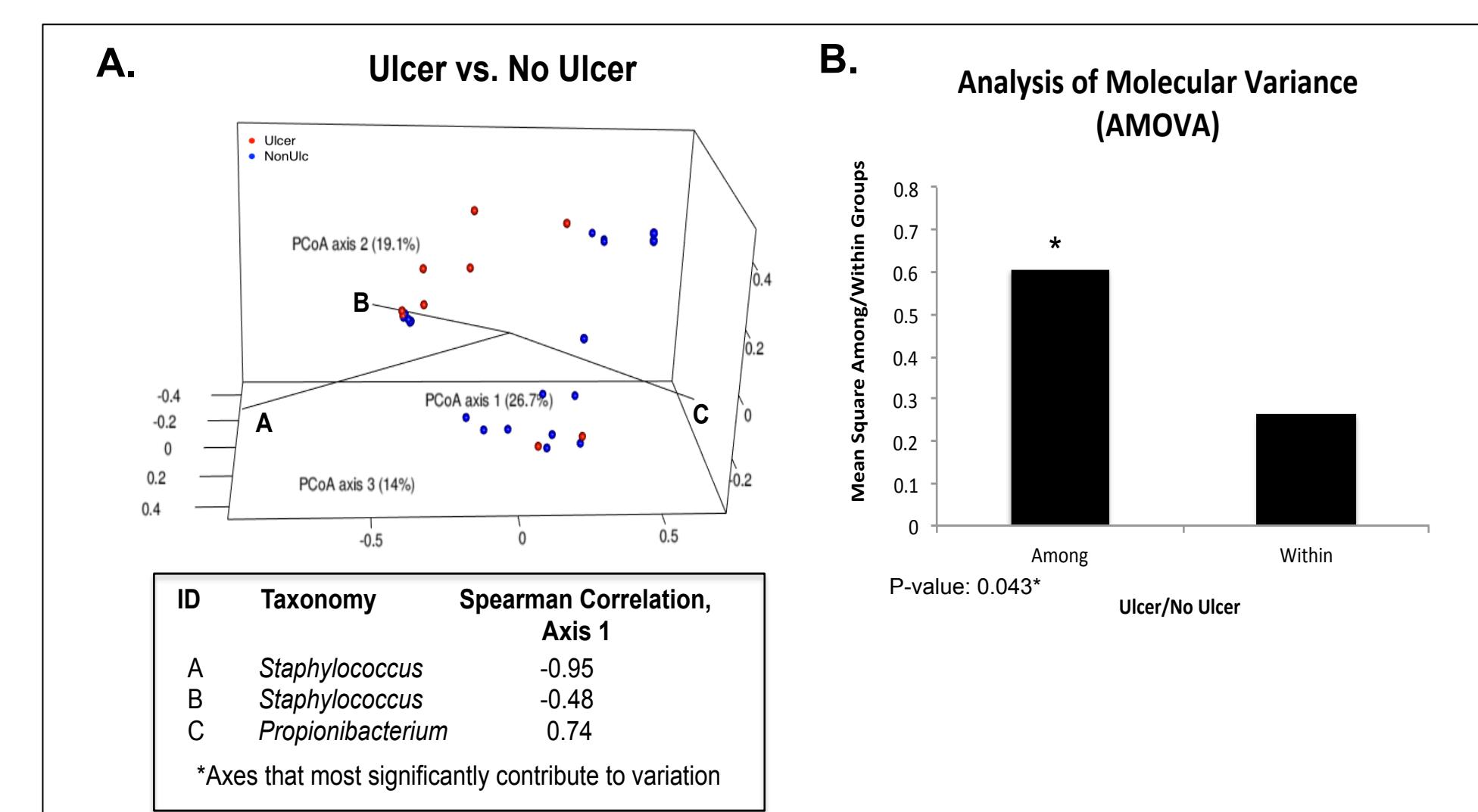
Ulcer Status	N	Age Mean (SD)	Gender (% Male)
No active ulcer	7	45.9 (15.0)	43%
Active ulcer	5	46.2 (15.5)	60%

Figure 2. Bacterial relative abundance plots.



~1.3 million 16S rRNA bacterial sequences were taxonomically classified using the online molecular ecology tool, mothur, and the RDP classifier and training set (version 9). Relative abundance plots are shown for participants with and without an active ulcer. * denotes samples collected from the left ankle.

Figure 3. Bacterial community structure analysis.



Principal coordinate analysis (PCoA) plot using theta_c distances comparing the bacterial community structure in ulcer and non-ulcer SCD study participants. A. The red dot corresponds to participants with active ulcers while the blue dot corresponds to participants who do not have a leg ulcer. Bacterial isolates that are responsible for the observed variation are highlighted. B. An analysis of molecular variance (AMOVA) test was performed to examine differences in community structure using theta_c distances from participants with and without leg ulcers.

Clinical

We will assess the overall health status of each study participant capturing common laboratory tests and information on pain frequency and sleep in participants with and without leg ulcers.³

Table 2. Demographics of the first 56 participants.

Ulcer Status	N	Age Mean (SD)	Gender (% Male)
No active ulcer	40	44 (11.0)	35%
Active ulcer	16	42 (12.4)	50%

Table 3. Non-parametric test to compare lab test values in study participants.

STANDARD LABS	N	No active ulcer Mean (SD)	N	Active ulcer Mean (SD)	p-value
Hemoglobin (g/dL)	40	8.7 (1.5)	16	7.7 (1.1)	0.02*
Hemoglobin Fetal (%)	40	12.1 (10.4)	16	8.7 (6.5)	0.58
Hemoglobin S (%)	40	66.1 (22.1)	16	67.1 (20.3)	0.99
Hematocrit (%)	40	24.8 (4.0)	16	22.2 (3.6)	0.03*
Reticulocyte (K/uL)	40	251.7 (186.3)	15	270.5 (116.4)	0.21
White Blood Cell Count (K/uL)	40	7.6 (3.6)	16	10.1 (4.6)	0.07
INFLAMMATORY MARKERS	N	No active ulcer Mean (SD)	N	Active ulcer Mean (SD)	p-value
C-Reactive Protein (mg/L)	40	6.1 (9.1)	15	13.9 (19.8)	0.05*
Erythrocyte Sedimentation Rate (mm/hr)	40	33.2 (29.4)	16	49.2 (26.4)	0.01*

Stress

We will collect a hair sample and blood from each study participant to measure two biomarkers, cortisol and serotonin, respectively.

Figure 4. Hair cortisol collection.

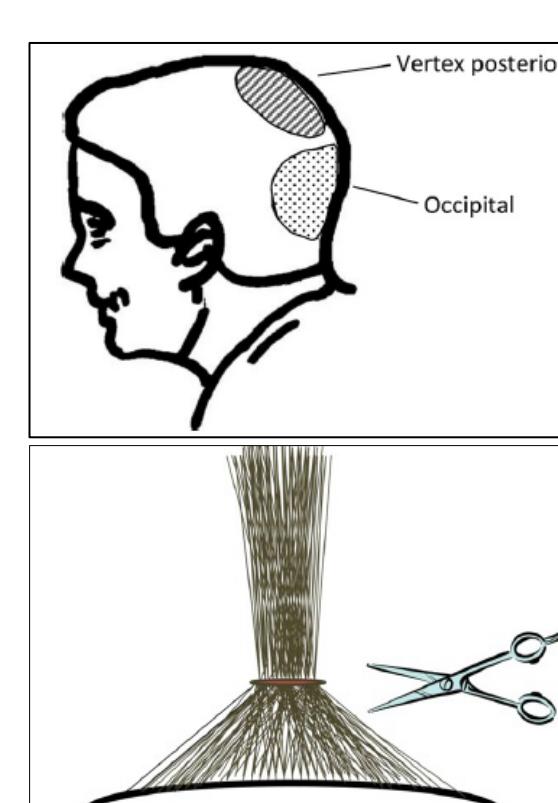


Table 4. Serotonin lab test values in study participants.

	N	No active ulcer Mean (SD)	N	Active Ulcer Mean (SD)
Serotonin (ng/mL)	38	170.4 (87.9)	16	271.1 (188.7)

Normal range is 101-283 ng/mL. Low serotonin is linked to an increase in stress, depression, and insomnia.

Psychosocial

We will collect information to assess the overall psychosocial state (i.e. depression, social support, perceived stress, discrimination and stigma) of each study participant comparing those with and without leg ulcers.

Table 5. Non-parametric test to compare social measures in study participants.

Study Measures	Scoring	N	No active ulcer Mean (SD)	N	Active ulcer Mean (SD)	p-value
Discrimination experience subscale due to SCD Stigma	5 item scale: 0-14 minimal to moderate ≥ 15 severe	39	12.94 (0.50)	15	12.89 (0.47)	0.64
Resistance subscale due to SCD Stigma	5 item scale: 0-20 with higher scores indicate more effort to resist stigma	40	3.13 (0.48)	16	2.92 (0.46)	0.10
The Cohen Perceived Stress Scale	10 item scale: < 13 low stress 13 average ≥ 20 high stress	37	19.49 (7.58)	16	19.88 (8.07)	0.98
The Beck Depression Inventory	21 item scale: 1-16 low 17-30 moderate ≥ 30 severe to extreme	39	11.49 (9.11)	16	13.75 (11.43)	0.61

Environment

We will collect information on the physical environment which will include exposures at home, work, and in the neighborhood for each study participant. Additionally, we will record health-related behaviors and demographic information such as country of origin comparing participants with and without leg ulcers.

Figure 5. Birthplace of the first 56 participants.

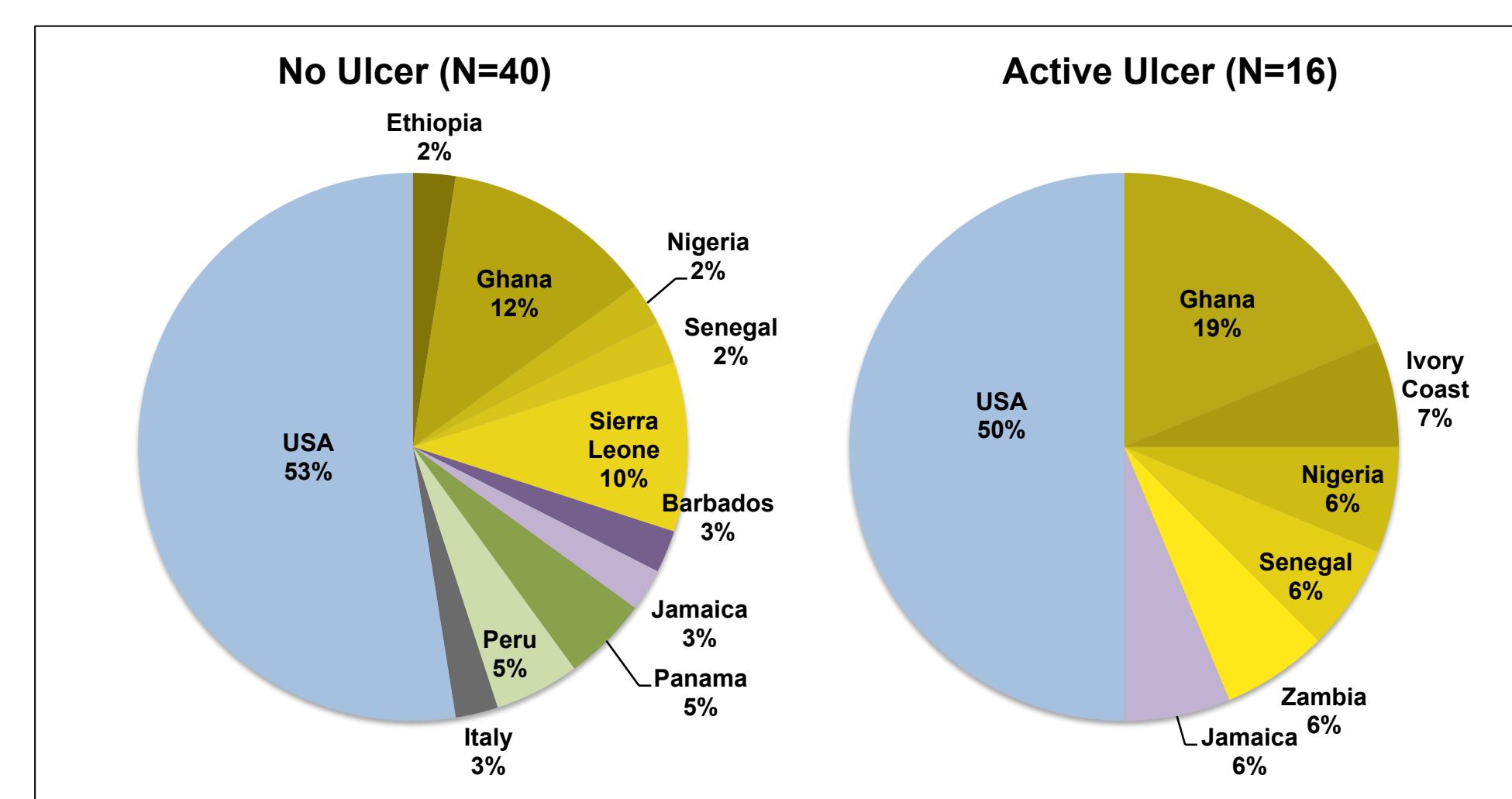


Table 6. Demographics and environmental data.

	N	No active ulcer (% Yes)	N	Active ulcer (% Yes)
Household pets	40	22.5%	16	18.8%
Neighborhood violence	40	7.5%	16	6.7%
Home break-ins	40	10%	16	14.3%
Tobacco exposure at home (at least 100 cigarettes)	40	7.5%	16	18.8%
Lifetime tobacco use (at least 100 cigarettes)	40	33%	16	14.3%
Employment status: Working	40	32.5%	16	37.5%
Disabled/Unemployed	40	32.5%	16	31.3%
Marital Status: Married	40	37.5%	16	31.3%
Never married	40	35%	16	56.3%
Household size Mean (SD)	39	2.89 (1.99)	15	2.73 (2.37)

Future Directions

- Continue efforts to increase participant recruitment and geographic diversity of study participants
- Perform processing, sequencing, and data analysis for the remaining microbiome participants
- Since *Staphylococcus* appears to be the most abundant bacterial genera in SCD leg ulcers, classification according to the species level is necessary
- Compare the microbiome and clinical data to identify microbial signatures that may play a role in leg ulcer formation and healing
- Conduct further analyses to identify if there is any correlation between the environment, social, and clinical data
- This study may lead to the identification of new and improved treatment options and interventions to better manage, if not prevent, the onset and delayed healing of SCD leg ulcers
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