Burden of sickle cell trait and disease in the Uganda Sickle Surveillance Study (US3): a cross-sectional study

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Summary

Background Sickle cell disease contributes substantially to mortality in children younger than 5 years in sub-Saharan Africa. In Uganda, 20 000 babies per year are thought to be born with sickle cell disease, but accurate data are not available. We did the cross-sectional Uganda Sickle Surveillance Study to assess the burden of disease.

Methods The primary objective of the study was to calculate prevalence of sickle cell trait and disease. We obtained punch samples from dried blood spots routinely collected from HIV-exposed infants in ten regions and 112 districts across Uganda for the national Early Infant Diagnosis programme. Haemoglobin electrophoresis by isoelectric focusing was done on all samples to identify those from babies with sickle trait or disease.

Findings Between February, 2014, and March, 2015, 99 243 dried blood spots were analysed and results were available for 97 631. The overall number of children with sickle cell trait was 12 979 (13·3%) and with disease was 716 (0·7%). Sickle cell numbers ranged from 631 (4·6%) for trait and 23 (0·2%) for disease of 13 649 in the South Western region to 1306 (19·8%) for trait and 96 (1·5%) for disease of 6581 in the East Central region. Sickle cell trait was seen in all districts. The lowest prevalence was less than 3·0% in two districts. Eight districts had prevalence greater than 20·0%, with the highest being 23·9%. Sickle cell disease was less common in children older than 12 months or who were HIV positive, which is consistent with comorbidity and early mortality.

Interpretation Prevalence of sickle cell trait and disease were high in Uganda, with notable variation between regions and districts. The data will help to inform national strategies for sickle cell disease, including neonatal screening.

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Introduction

Sickle cell disease refers to a group of inherited haemoglobin disorders characterised by a predominance of abnormal sickle haemoglobin in erythrocytes.[1] Sickle cell anaemia, which results from homozygous inheritance of sickle haemoglobin from both parents, is the most common and severe form of sickle cell disease. On deoxygenation, sickle haemoglobin undergoes a conformational change that promotes intracellular polymerisation, which leads to distortion of the normal biconcave erythrocyte disc into the distinctive and pathological crescent shape. The resulting haemolytic anaemia manifests as recurrent vaso-occlusion and organ damage that together cause substantial morbidity and early mortality.[1]

Worldwide, sickle haemoglobinopathies lead to a substantial burden of disease that is not adequately addressed.[2–4] Accurate data are lacking, but the worldwide estimate for neonates born with sickle cell disease each year is 400 000, including 300 000 with sickle cell anaemia.[5] The greatest burden is seen in sub-Saharan Africa, where more than 75% of all sickle cell disease occurs, with this proportion projected to increase by 2050.[6] In Africa, sickle cell disease contributes substantially to mortality in children younger than 5 years and, therefore, limits progress towards achieving UN Sustainable Development Goal 3, Good Health and Well-Being, which includes the reduction of childhood mortality.[7,8]

In 2006, WHO issued an important report on sickle cell disease in the African region, which described the overall prevalence and provided guidelines on care and management strategies.[9] WHO also publicised the need to improve sickle cell awareness, disease prevention, and early detection.[10] Countries in sub-Saharan Africa have been challenged by WHO to formulate national strategies for sickle cell disease that address specific aims, targets, and objectives. Despite this charge, ministries of health are hindered from creating meaningful interventions by many obstacles, including lack of accurate data about the burden and distribution of disease within their countries.[11]

Uganda was among the first countries in Africa with a documented large burden of sickle cell disease. In 1949, substantial differences in the prevalence of sickle cell trait were reported between different tribes, ranging from less than 5% for Hamites in the southwest to more than 20% for the northern Nilotices (Lango and Acholi). Some Bantu tribes had even higher rates, including 45% among Bamba living in the western region.[12] A later study, however, has suggested lower values.[13] Of note,

Research in context

Evidence before this study
Decades have passed since the initial descriptions of sickle cell trait in different regions of Uganda, but accurate data on prevalence are unavailable. We searched PubMed for articles published in English up to May, 2015, with the search terms "sickle cell", "screening", "Uganda", "Africa", and "haemoglobin electrophoresis". We identified four articles published before 1960 that described ethnic origin and distribution of sickle cell trait and disease in Uganda. We found one paper from 2010 that described the distribution of sickle cell trait in several regions of Uganda and seven reports since 2005 that provided pilot neonatal screening data in other sub-Saharan African locations, but could find no comprehensive surveillance data at the national level.

Added value of this study
The primary study objective of the Uganda Sickle Surveillance Study was to generate critical data on the prevalence of sickle cell trait and disease. Comorbidities that might be associated with early mortality in people with sickle cell disease were also explored. This national-level surveillance study in sub-Saharan

Africa documents a high burden in Uganda, with wide distribution of sickle cell trait and disease in every region and district. These national data will inform the Ministry of Health about next steps, including the launch of neonatal screening and development of a national sickle cell strategy. We also found that frequency of sickle cell disease declined in children older than 12 months or with HIV, which was consistent with comorbidity and early mortality.

Implications of all the available evidence
The Ugandan Ministry of Health needed accurate data on the prevalence and distribution of sickle cell trait and disease to help the country follow the WHO guidelines for clinical management. In view of our results, targeted neonatal screening for sickle cell disease is now warranted in the districts with the highest burden, along with efforts to improve education of health-care providers and awareness among the public and the government. Similar studies can be done in other sub-Saharan countries to help the development of national strategies for the management of sickle cell disease.

though, both studies were based on small samples and were not representative of the whole country.

The Ugandan Ministry of Health recognised the need for accurate, up-to-date data on the burden of sickle cell trait and disease to inform its efforts to pilot neonatal screening, begin early education and preventive measures, and eventually create a national strategy. We hypothesised that there is a large burden of sickle cell trait and disease across Uganda, but with substantial geographical variability. A partnership was established between Cincinnati Children's Hospital, Cincinnati, OH, USA, and Makerere University, Kampala, Uganda, to enable the Ministry of Health to do a large cross-sectional research study, the Uganda Sickle Surveillance Study (US3), to generate such data.

Methods
Programme development
The Ugandan Ministry of Health has an active programme for prevention of mother-to-child transmission of HIV that identifies and treats infected mothers and their exposed infants. Infected mothers receive antiretroviral drug therapy during pregnancy and exposed babies receive 6 weeks of treatment after birth, followed by testing with viral PCR for acquired HIV infection, typically before age 6 months. Around 100 000 HIV-exposed infants are tested each year through the Early Infant Diagnosis programme, which includes a national sample transport system.[14] Dried blood spots (DBS) are collected from exposed infants at health-care facilities across the country, carried by motorcycle to laboratory hubs at the subdistrict level, and shipped by courier to the Central Public Health Laboratories in the capital, Kampala.[14] Sample testing is completed

within 3 weeks of collection, after which DBS are stored at –20° C for about 1 year then discarded. The DBS collected between February, 2014, and March, 2015, were shared with the newly created sickle cell laboratory in the Central Public Health Laboratories within 1 week of HIV testing, to test for normal and abnormal haemoglobins.

The study protocol was approved with a waiver for informed consent by the School of Medicine Research Ethics Committee at Makerere University and the Uganda National Council for Science and Technology in Kampala. The study was also formally approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board.

Haemoglobin testing
The laboratory methods for haemoglobin testing were based on those previously described for a pilot neonatal haemoglobinopathy screening programme in Angola.[15] DBS were eluted and analysed by isoelectric focusing for the presence of normal and abnormal haemoglobins. Isoelectric focusing readily distinguishes between normal and sickle haemoglobin even in the presence of fetal haemoglobin. Staff at the Central Public Health Laboratories received on-site training by a technical team from Cincinnati Children's Hospital on the study protocol, isoelectric focusing procedures, and interpretation of results. Additional sessions were provided remotely for further training, troubleshooting, and overal technical laboratory support.

The gel bands were independently compared with a standard control by two laboratory technicians and reviewed by the laboratory technologist to derive the final

	Adult haemoglobin	Sickle haemoglobin	Fetal haemoglobin	Unknown haemoglobin
Normal	Present	None	Variable	None
Trait	Present	Present	Variable	None
Sickle	None	Present	Variable	None
Variant	Present	None	Variable	Present

Table 1: Haemoglobin classifications for isoelectric focusing analysis

classification of normal, sickle cell trait, sickle cell disease, or variant (table 1). To verify the accuracy of the laboratory process, for the first 3 months of the study all samples classified as sickle cell trait or sickle cell disease were retested in a second DBS punch. Accuracy was higher than 99%. After 3 months, only samples classified as sickle cell disease were retested. If the first result was indeterminate, a second DBS punch was tested in an attempt to obtain a valid result. If the second test was indeterminate, samples were excluded at that point. Results were recorded along with the age of the infant at the time of DBS collection, location by region and district, and HIV status, which are collected in the Early Infant Diagnosis programme. Quality control for accuracy of data was periodically assessed by researchers in Cincinnati and was consistently greater than 97%. Haemoglobin results were communicated to the collection sites with the existing HIV notification system.

Statistical analysis

The primary study objective was calculation of the prevalence of sickle cell trait and disease by district and region. Secondary objectives were to calculate the effects of age and comorbidities on early mortality in children with sickle cell disease. Frequencies and percentages were calculated for ten regions and 112 districts. The relations between sickle cell disease, age, HIV status, and mortality were investigated by calculation of odds ratios and 95% CIs with the χ^2 test for binary variables. Reductions in prevalence of sickle cell disease with increasing age or positive HIV status were assumed to reflect early mortality. Correlation between prevalence of sickle cell trait and malaria (children aged 0–59 months with positive microscopy[16]) by region was tested with linear regression.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

DBS from 99 243 infants and children were analysed. 98 519 (99·3%) of samples were collected from children younger than 18 months (median age 2 months, IQR 2–9), with an equal distribution of boys and girls.

|	Median (IQR) age (months)	Sex (boys/girls/unknown [%])	Normal (%)	Sickle cell trait (%)	Sickle cell disease (%)	Variant (%)
Central 1 (n=14 756)	2 (2–8)	7127 (48·3%)/7430 (50·4%)/ 199 (1·3%)	12 757 (86·5%)	1896 (12·8%)	69 (0·5%)	34 (0·2%)
Central 2 (n=11 135)	2 (2–8)	5364 (48·2%)/5661 (50·8%)/ 110 (1·0%)	9442 (84·8%)	1566 (14·1%)	94 (0·8%)	33 (0·3%)
East Central (n=6581)	3 (2–12)	3275 (49·8%)/3243 (49·3%)/ 63 (1·0%)	5131 (78·0%)	1306 (19·8%)	96 (1·5%)	48 (0·7%)
Kampala (n=13 466)	2 (2–8)	6670 (49·5%)/6607 (49·1%)/ 189 (1·4%)	11 499 (85·4%)	1835 (13·6%)	90 (0·7%)	42 (0·3%)
Mid Eastern (n=4865)	3 (2–11)	2342 (48·1%)/2455 (50·5%)/ 68 (1·4%)	4014 (82·5%)	752 (15·5%)	60 (1·2%)	39 (0·8%)
Mid Northern (n=12 760)	3 (2–12)	6220 (48·7%)/6401 (50·2%)/ 139 (1·1%)	10 028 (78·6%)	2445 (19·2%)	160 (1·3%)	127 (1·0%)
Mid Western (n=12 945)	2 (2–10)	6271 (48·4%)/6507 (50·3%)/ 167 (1·3%)	11 418 (88·2%)	1431 (11·1%)	64 (0·5%)	32 (0·2%)
North East (n=4458)	2 (2–10)	2201 (49·4%)/2204 (49·4%)/ 53 (1·2%)	3676 (82·5%)	702 (15·7%)	46 (1·0%)	34 (0·8%)
South Western (n=13 649)	3 (2–11)	6543 (47·9%)/6896 (50·5%)/ 210 (1·5%)	12 979 (95·1%)	631 (4·6%)	23 (0·2%)	16 (0·1%)
West Nile (n=3016)	2 (2–12)	1566 (51·9%)/1406 (46·6%)/ 44 (1·5%)	2534 (84·0%)	415 (13·8%)	14 (0·5%)	53 (1·8%)
All (n=97 631)	2 (2–9)	47 579 (48·7%)/48 810 (50·0%)/1242 (1·3%)	83 478 (85·5%)	12 979 (13·3%)	716 (0·7%)	458 (0·5%)

Table 2: Haemoglobin results, by region
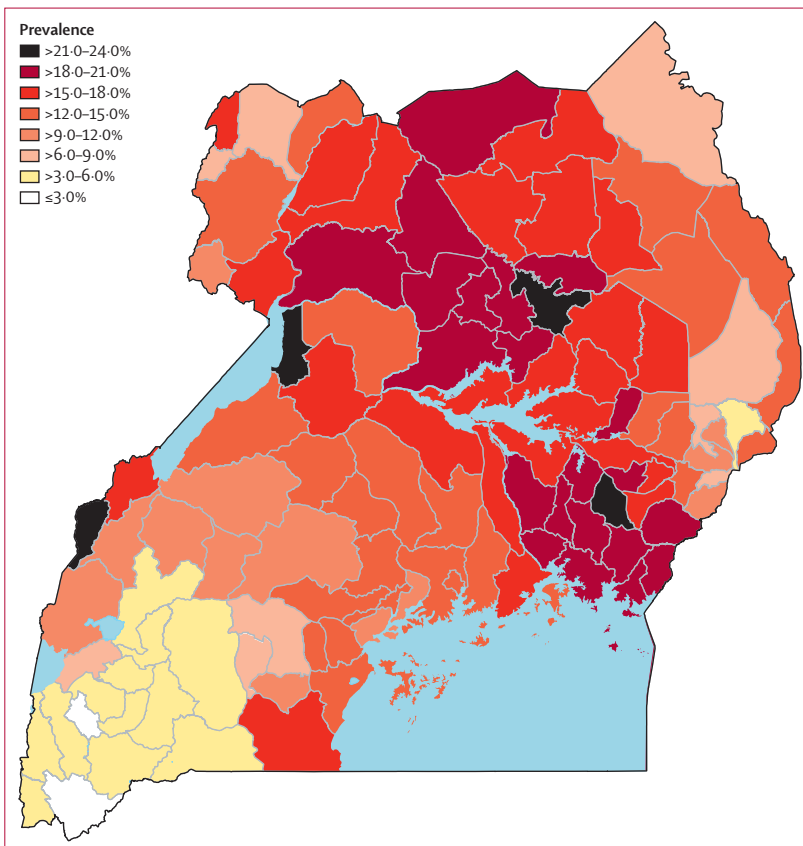
Figure 1: **Prevalence of sickle cell trait in 112 districts in Uganda**
Prevalence ranged from 2·5% to 23·9%.
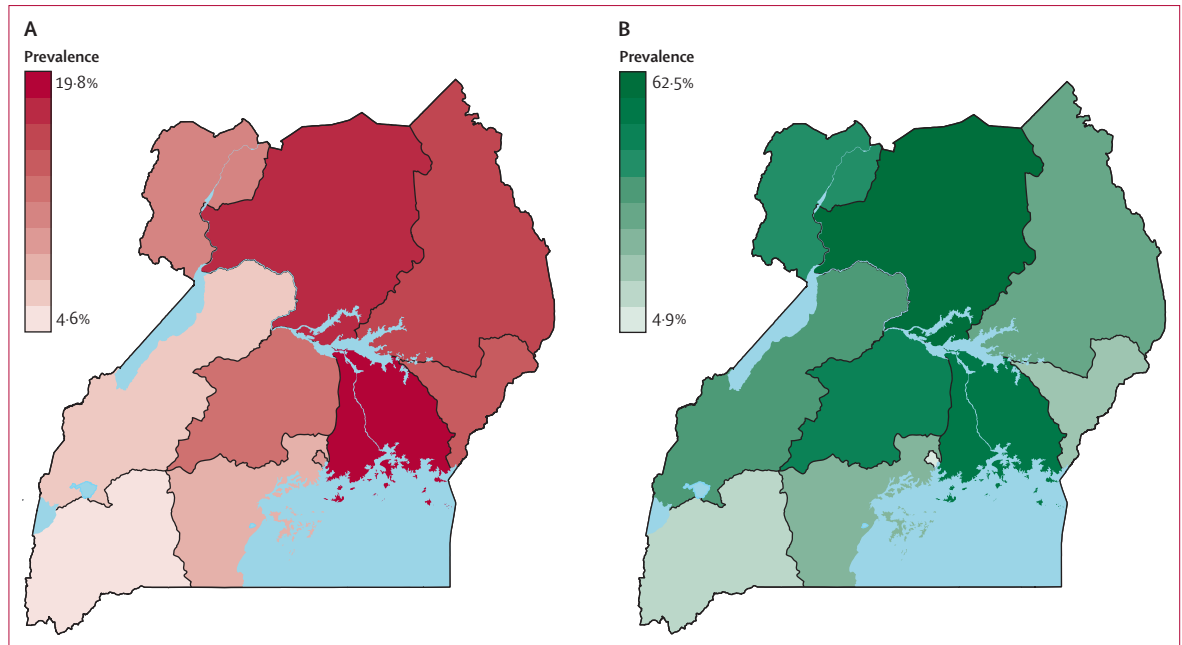
Figure 2: Prevalence of sickle cell trait and malaria in ten regions in Uganda
(A) Prevalence of sickle cell trait. (B) Prevalence of malaria, based on the Uganda Bureau of Statistics 2009 surveillance project.[16]

	All	Sickle cell trait (%)	Sickle cell disease (%)
Age (months)			
0–6·0	66670	8802 (13·2%)	521 (0·8%)
>6·0–12·0	13182	1758 (13·3%)	87 (0·7%)
>12·0–18·0	17075	2326 (13·6%)	105 (0·6%)
HIV status			
Negative	92024	12293 (13·4%)	693 (0·8%)
Positive	5080	672 (13·2%)	23 (0·5%)

Table 3: **Prevalence of sickle cell trait and disease by age and HIV status**

1612 (1·6%) samples did not yield a valid isoelectric focusing result and could not be classified and, therefore, 97631 were included in the analysis. The overall number of children with sickle cell trait was 12979 (13·3%) and ranged from 631 (4·6%) of 13649 in the South Western region to 1306 (19·8%) of 6581 in the East Central region. The overall number with sickle cell disease was 716 (0·7%) and ranged from 23 (0·2%) of 13649 in the South Western region to 96 (1·5%) of 6581 in the East Central region (table 2).

Sickle cell trait was found in all 112 districts (figure 1, appendix). Those in the South Western region had the lowest prevalence, being less than 5% in nine and less than 3% in two. Eight districts had prevalence greater than 20%, with the highest being 23·9% in Alebtong.

The national prevalence of haemoglobin variants, specifically those other than sickle haemoglobin, was 0·5%, but ranged from 0·1% in the South Western region to 1·8% in the West Nile region (table 2). The

highest frequency of DBS with variants by district was seen in Arua, with 26 (2·7%) in 973.

Comparison of the data for prevalence of sickle cell trait and malaria in ten regions showed good overall visual concordance (figure 2). The numerical data supported this finding, showing strong correlation between sickle cell trait and malaria prevalence ($r=0·69$, $p=0·026$, appendix).

Prevalence of sickle cell disease fell with increasing age (table 3). The odds ratio for mortality was 0·79 (95% CI 0·64–0·97, $p=0·027$) for the oldest age group compared with the youngest, which suggests early mortality in babies born with sickle cell disease. The odds ratio of having sickle cell disease for infants with positive versus negative HIV status was 0·60 (95% CI 0·40–0·91, $p=0·022$), which is consistent with early mortality due to HIV in babies with sickle cell disease, irrespective of age.

Discussion

We used haemoglobin testing of around 100000 infants to provide quantitative data on the prevalence and distribution of sickle cell trait and disease across Uganda. The data showed an enormous burden, with sickle cell trait being present in all 112 districts assessed. Prevalence was highest in the Mid Northern and East Central regions. Overall, the prevalence of sickle cell trait was 13·3%, but it was more than 20% in eight districts. Among babies aged 6 months or younger, the overall prevalence of sickle cell trait was 13·2% and of disease was 0·8%, which suggests that at least 15000 babies per year are born with sickle cell disease in Uganda. The prevalence of sickle cell disease approached or exceeded 1·0% in four regions,

which is higher than predicted when based on the prevalence of sickle cell trait. This finding might reflect assortative (non-random) mating, where individuals have a preference for specific genotypes and phenotypes. Additional haemoglobin variants with prevalence of 1·0% or higher in the Mid Northern and West Nile regions was unexpected, but we await molecular diagnoses to confirm their identities and relevance.

The association between sickle cell trait and malaria was suggested many years ago, and was based on the epidemiological overlap of the two disorders in central Africa.[17,18] Piel and colleagues[19] confirmed this association at the global level, but our results confirm this association at the regional level (figure 2). The overlap in Uganda suggests continuing differential selection pressure for children with sickle cell trait to survive malaria,[20] which is supported by findings in Gabon,[21] and probably also reflects low migration of tribal populations within regions. Since malaria is frequently fatal in children with sickle cell disease,[22] improved understanding of prevalence and distribution of sickle cell trait and disease might be relevant to national malaria control strategies.

Sickle cell disease is frequently associated with early mortality for infants and young children in sub-Saharan Africa,[2,4,6–8] including in two reports by WHO on the African Region,[9,10] but no definitive data are available due to inadequate studies. Our data, although not longitudinal, support this association. Age and HIV status also seemed to be associated with early mortality in children with sickle cell disease, as we took reduced prevalence of sickle cell disease in children older than 12 months and those who were HIV positive to indicate early mortality. The low odds ratio indicates an important potential comorbidity between HIV and sickle cell disease that should be investigated prospectively. Further assessment is especially important, as the combination is poorly understood, with potential protective effects and synergism for disease complications both having been described.[23]

This study had several strengths, including the large sample size in the Early Infant Diagnosis programme, which provided national coverage, high-quality laboratory testing, and the ability to create an accurate geospatial map at the regional and district levels. The study design was efficient, as repurposing of DBS kept effort, costs, and time low. Other countries within sub-Saharan Africa that have comprehensive early infant diagnosis programmes could obtain similar accurate sickle cell prevalence data with this strategy. Finally, the study established a strong infrastructure, with laboratory staff being trained to assess sickle cell trait and disease, which met the important goal of building local laboratory and research capacity. This study reflects a highly successful partnership between government and academia in Uganda and the USA, which generated data that will inform policy.

The study also had several limitations. All DBS were collected from children exposed to HIV. However, no

evidence suggests any systematic bias between HIV exposure and the inheritance of sickle cell trait or disease. Indeed, prevalence was around 13% in HIV-positive (representing 5% of the total Early Infant Diagnosis cohort) and HIV-negative infants. Second, the cross-sectional study design did not allow collection of longitudinal data, but the Central Public Health Laboratories plan to track these affected infants over time. Finally, optimum clinical care could not be assured for all children identified as having sickle cell disease because there are no national treatment guidelines and knowledge of the disease among health-care providers is poor. The Ugandan Ministry of Health, however, is to issue national standards of care to promote appropriate clinical management in all regions and districts.

The WHO strategy for sickle cell disease in the African Region is bold and comprehensive. The guiding principles include country ownership of the strategy, partnership and team building, evidence-based interventions, and cultural sensitivity.[10] The Ugandan Ministry of Health commissioned the US3 study in response to the WHO guidelines, specifically to document the burden of sickle cell disease and to decide how and where to prioritise its limited resources. Pilot neonatal haemoglobinopathy screening efforts in sub-Saharan Africa have successfully identified a large burden of sickle cell disease,[15,24–26] and are typically coupled with immunisations to prevent fatal bacterial infections.[27] Additionally, hydroxyurea has been identified as a potentially transformative disease-modifying therapy for use in low-resource settings.[28] Accordingly, the proposed next steps for Uganda include targeted neonatal screening in the districts with the highest burden; training of health-care providers and establishment of regional and district sickle cell clinics to provide penicillin and antimalarial prophylaxis, family education, and immunisations; research of the safety, dosing, and benefits of hydroxyurea; and creation of a national sickle cell awareness strategy that will include premarital testing and counselling aimed at lowering the burden of sickle cell disease.

The UN Sustainable Development Goals emphasise non-communicable diseases as public health concerns, and sickle cell disease deserves recognition as a widespread disorder that can lead to serious morbidity, poor quality of life, and early mortality. Despite a plea from key thought leaders to document the natural history of this disease and the associated mortality in Uganda,[29] sickle cell disease remains neglected with few reliable data and little political will to improve the situation.[30] The US3 study provides important data that will help to rectify these deficits, and supports efforts by government and academia to improve the lives of children and adults with sickle cell disease who are living in Uganda.

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