RESEARCH ARTICLE

The Collaborative African Genomics Network (CAfGEN): Applying Genomic technologies to probe host factors important to the progression of HIV and HIV-tuberculosis infection in sub-Saharan Africa [version 1; peer review: 2 approved]

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v1 **First published:** 18 Apr 2018, **1**:3 (https://doi.org/10.12688/aasopenres.12832.1)
Latest published: 21 Jun 2018, **1**:3 (https://doi.org/10.12688/aasopenres.12832.2)

Abstract

Background: The Human Heredity and Health in Africa consortium (H3Africa) was conceived to facilitate the application of genomics technologies to improve health across Africa. Here, we describe how the Collaborative African Genomics Network (*CAfGEN)* of the H3Africa consortium is using genomics to probe host genetic factors important to the progression of HIV and

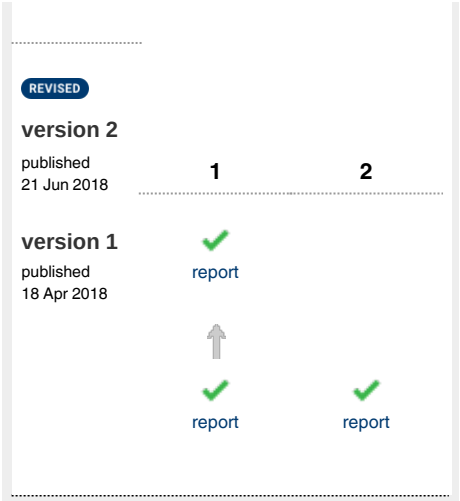
Open Peer Review

Reviewer Status ✓ ✓

Invited Reviewers

HIV-tuberculosis (TB) coinfection in sub-Saharan Africa.
Methods: *CAfGEN* is an H3Africa collaborative centre comprising expertise from the University of Botswana; Makerere University; Baylor College of Medicine Children's Clinical Centers of Excellence (COEs) in Botswana, Uganda, and Swaziland; as well as Baylor College of Medicine, Texas. The COEs provide clinical expertise for community engagement, participant recruitment and sample collection while the three University settings facilitate processing and management of genomic samples and provide infrastructure and training opportunities to sustain genomics research.
Results: The project has focused on utilizing whole-exome sequencing to identify genetic variants contributing to extreme HIV disease progression phenotypes in children, as well as RNA sequencing and integrated genomics to identify host genetic factors associated with TB disease progression among HIV-positive children. These cohorts, developed using the COEs' electronic medical records, are exceptionally well-phenotyped and present an unprecedented opportunity to assess genetic factors in individuals whose HIV was acquired by a different route than their adult counterparts in the context of a unique clinical course and disease pathophysiology.
Conclusions: Our approach offers the prospect of developing a critical mass of well-trained, highly-skilled, continent-based African genomic scientists. To ensure long term genomics research sustainability in Africa, *CAfGEN* contributes to a wide range of genomics capacity and infrastructure development on the continent, has laid a foundation for genomics graduate programs at its institutions, and continues to actively promote genomics research through innovative forms of community engagement brokered by partnerships with governments and academia to support genomics policy formulation.

Keywords
Bioinformatics, Genetics, Genomics, HIV/AIDS, Pediatrics, Tuberculosis, Education, Development



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Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: Outside the submitted work, Misaki Wayengera works as a chief scientific officer at Restrizymes Biotherapeutics (U) LTD and Graeme Mardon is a 50% owner and President of GenetiVision Corporation. There are no competing financial interests. The other authors declare no conflict of interest.

Grant information: CAfGEN is funded by NIH grant 1U54AI110398. Gerald Mboowa is also supported through the DELTAS Africa Initiative grant #DEL-15-011 to THRiVE-2 (the Training Health Researchers into Vocational Excellence in East Africa). The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust (WT) grant #107742/Z/15/Z and the UK government. Genome Adventure Comic Books were sponsored through the CAfGEN NIH grant and WT grant # 105057/z/14/z. The views expressed in this publication are those of the author(s) and not necessarily those of AAS, NEPAD Agency, or Wellcome Trust.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Mboowa G, Mwesigwa S, Katagirya E *et al.* **The Collaborative African Genomics Network (CAfGEN): Applying Genomic technologies to probe host factors important to the progression of HIV and HIV-tuberculosis infection in sub-Saharan Africa [version 1; peer review: 2 approved]** AAS Open Research 2018, **1**:3 (https://doi.org/10.12688/aasopenres.12832.1)

First published: 18 Apr 2018, **1**:3 (https://doi.org/10.12688/aasopenres.12832.1)

Introduction

In 2011, a group of scientists came together and conceived the white paper "Harnessing Genomic Technologies Toward Improving Health in Africa: Opportunities and Challenges". This white paper was used to solicit ideas from senior African scientists and scientists with extensive research experience in African populations, and ultimately led to the creation of the Human Heredity and Health in Africa (H3Africa) initiative. A major theme was to solicit suggestions for research priorities, infrastructure development, human resource training, and coalescing researchers working in Africa. The long-term goal of H3Africa was to increase the capacity to do cutting-edge genomic and biomedical research in Africa as a means of improving health[1]. To facilitate this, it became clear that there needed to be systemic improvements in the way African research was funded and how African scientists were trained. Currently, much research in Africa is funded through foreign granting mechanisms[2] and many PhD level scientists in Africa have received their training at foreign institutions[3]. Unfortunately, scientists often leave for training and do not return to Africa because of limited opportunities on the continent[4]. H3Africa was initiated, in part, to reverse this 'brain drain' and has already made substantial strides towards this goal.

The completion of the Human Genome Project in 2003 catapulted efforts to use genetics to enhance our understanding of human diseases[5]. Key to this, is identifying and utilizing variation in the human genome that is associated with disease-specific onset, complications, or outcomes. Some inherited diseases, such as Sickle cell disease (SCD), are caused by relatively straightforward variation in a single-gene, while others, such as heart disease, are the result of a complex interplay of factors that can include a multitude of both genetic and environmental influences, acting to both increase or decrease susceptibility. Added to this are circumstances where there is inter-individual variation in infection susceptibility or resistance[6,7] or severity of infection[6–8], and host genetics can also play a major role in determining the most effective treatment for diseases such as cancer or predicting adverse reactions to certain drugs[9].

Genetics and genomics have a particularly important role to play in biomedical research as DNA variation both directly and indirectly impacts disease outcomes[10,11]. Understanding this variation, therefore, has the potential to lead to a better understanding of the biology, physiology, and chemical pathways involved in disease and can help direct scientists to new areas of research that may result in better medications and treatment options. To date, this approach has yielded several pharmacogenomic biomarkers that have been translated into clinical practice, affecting the use of medications to improve quality of life for individuals on specific treatments[12]. Reflecting on the safety of antiretroviral medications; in sub-Saharan Africa, efavirenz forms the preferred first-line anti-retroviral therapy for those over the age of 3 years but adverse drug events have been reported. Additionally, some HIV-infected individuals of African origin are predisposed to developing adverse efavirenz-induced neuropsychiatric responses due to variants in *CYP2B6* that impair normal metabolism of efavirenz[13]. The *CYP2B6*6* allele occurs at a high frequency in people of African origin and is associated with high efavirenz concentrations and genotype-screening is recommended in these populations[14]. Genomic screening in populations has also identified the *HLA-B*5701* variant that predicts Abacavir hypersensitivity; assays for this variant are now routinely performed ahead of time to minimize drug associated reactions in the Western world[12,15]. Africa is classically considered to be the wellspring of modern humanity; and thus, comprises the greatest human genetic diversity among major world populations[10–12]. In addition, Africa hosts a wide variety of pathogens, climates, lifestyles, and habitats, some of which are unique to the continent. These observations underscore the immense potential for learning more about human health and our environment through systematic study of the interplay of genetics and environment in African populations[11].

Genesis of the Collaborative African Genomics Network (*CAfGEN*)

Subsequent to the H3Africa white paper, a request for application (RFA) was released and collaborative centers applied for funding in health-related areas of research (see NIH H3Africa grant page), including ;

1. The genetic/environmental contributors to non-communicable disease in Africa
2. The genetic/environmental contributors to communicable disease in Africa
3. The contribution of the human microbiome to health and disease in Africa
4. Mendelian diseases in Africa
5. Pharmacogenomics
6. AIDS and co-morbidities
7. Genetic and genomic basis of HIV/AIDS disease, abuse of licit or illicit substances (including alcohol, tobacco, cannabis, stimulants, opiates), and related co-infections or co-morbidities.

This call proved to be the genesis for the formation of the Collaborative African Genomics Network - *CAfGEN*. *CAfGEN* aims to redress the scientific imbalance of genomics research of African paediatric populations. Our network incorporates six sites – the Botswana, Uganda and Swaziland Children's Clinical Centres of Excellence (COEs); the University of Botswana; Makerere University; and Baylor College of Medicine in Houston, Texas. The *CAfGEN* research agenda includes:

1. Recruitment of prospective and retrospective cohorts of HIV and HIV-TB infected children;
2. Development of core genomics facilities within Africa for sample processing and storage;
3. Candidate gene re-sequencing, HLA allelotyping and whole-exome sequencing (WES) of patients at the extremes of HIV disease progression;
4. Integrated genomic analyses of active TB progression and associated clinical outcomes using expression quantitative trait loci (eQTL) analysis.

These projects are being undertaken in the context of an extensive training and career development plan that will also see significant upgrades in African human capital and genomics infrastructure[16]. In so doing, *CAfGEN* creates a unique, highly synergistic African alliance that is contributing novel and important mechanistic insights to paediatric HIV and HIV-TB disease progression[17] while establishing sustainable genomics technology, expertise, and capacity on the African continent.

A genomics development framework for 21st century Africa

CAfGEN seeks "to create a collaborative, multi-disciplinary, multi-institutional, inter- and intra-country network of African scientists, clinicians, and researchers using genomics approaches to study gene/environment interactions for HIV/AIDS, its co-morbidities, and other diseases among diverse paediatric African populations." To meet the attendant challenges of accomplishing this mission, a highly collaborative, synergistic, network of institutions has been assembled.

Firstly, the project draws upon the extensive clinical and research experience of the Botswana, Swaziland and Uganda COEs - together the three COEs have >35 years of experience providing state-of-the-art care and treatment of paediatric HIV that extends to >20,000 HIV-infected children across Botswana, Swaziland and Uganda. The COEs are headquarters for country-wide research related to the care, treatment, and prevention of paediatric HIV/AIDS and co-occurring diseases and are affiliated with the Baylor International Pediatric AIDS Initiative (BIPAI) - a state-of the-art paediatric HIV/AIDS health care network that spans across 11 African nations, including major centres in Malawi, Lesotho, and Tanzania, which have expressed strong interest in collaborating with *CAfGEN*. The COEs are the clinical backbone of *CAfGEN*; their expertise substantially mitigates the challenges of proper phenotyping and sample numbers needed for large-scale genomics.

To this backbone, we appended molecular genetics expertise at two universities that are closely (geographically and academically) related to the COEs: Makerere University, Uganda, one of the most prestigious academic universities in East Africa[18], has extensive experience with infectious disease and genetic studies, including *Mycobacterium tuberculosis* and HIV; and the University of Botswana (UB), which has a growing expertise in human genetics and significant monetary and human investment in molecular genetics, bolstered by a multi-million dollar Medical Education Partnership Initiative (MEPI) grant, with which *CAfGEN* shared a Principal Investigator.

To further develop the genetic and genomic capabilities of the network, we partnered with Baylor College of Medicine in Houston, Texas, to afford trainees access to high-level genomics expertise, including hands-on laboratory experiences, didactic coursework, data analysis, and a variety of other educational activities[16]. These activities are then transferred to the home African countries and become rooted in the provision of new faculty with high-level training and expertise – North-South collaboration for the 21st century.

The culmination of these centres, as shown in Figure 1 below, is the genesis for a unique African alliance using a highly synergistic approach to contribute novel and important mechanistic insights to disease progression in paediatric HIV and HIV-TB co-infection.

Figure 1. *CAfGEN* collaborating institutions.

Specific aims of *CAfGEN*

Disproportionately few advanced genetic and genomic studies have involved the indigenous peoples of Africa, and even fewer have included African paediatric populations[19–21]. Yet, paradoxically, these populations collectively carry a large proportion of the human disease burden that results in significant mortality and morbidity. HIV/AIDS and TB – HIV's most frequent co-morbidity – exemplify this scientific disparity; together, HIV and HIV-TB result in more than 500,000 new childhood cases every year (see UN report on combatting HIV/AIDS, Malaria and Other Diseases). Studies of host genetic factors underlying Long-Term Non-Progressors (LTNPs) of HIV infection have led to new therapies through the identification of loci that are important to *in vivo* control of virus pathogenicity[22]. Similarly, a detailed understanding of the temporal *in vivo* host molecular events occurring in the progression to active TB disease in the face of HIV co-infection, could significantly impact development of effective therapeutic strategies. Although genomic approaches have been used to identify host response pathways that are important to TB-disease progression, almost all of these studies were undertaken in non-African, adult populations. HIV-infected Africans and particularly children - who have a different route of acquisition, clinical course, and pathophysiology from their adult counterparts - have not been included, although they potentially have more to ultimately contribute and gain from any therapeutic advances. *CAfGEN* envisaged a project that encompassed 5 inter-related aims:

Aim 1a: Recruit a cohort of well-phenotyped paediatric HIV and HIV-TB infected patients. Retrospectively recruit 500 LTNPs and 500 rapid progressors (RPs) controls through the Electronic Medical Record (EMR).

Aim 1b: Create a DNA and RNA bioarchive of HIV- and HIV-TB infected paediatric patients from blood and sputa

Aim 2a: Evaluate the role of 'established' HIV disease progression susceptibility loci in paediatric HIV by undertaking gene sequencing and allelotyping of candidate loci in RP and LTNP patients.

Aim 2b: Identify novel host alleles influencing paediatric HIV disease progression by conducting whole-exome sequencing to identify variants that are associated with either RPs or LTNP.

Aim 3a: Identify genes that show significant temporal differential expression with progression to active TB disease by performing RNA sequencing of paired samples taken from HIV co-infected children at baseline and again at the time of active TB disease progression.

Aim 3b: Identify genes key to the progression to active TB by combining differential gene expression with SNP genotyping to identify expression quantitative trait loci (eQTL) and performing integrated genomic analyses of active TB disease and related clinical outcomes.

Aim 4: Establish and enhance undergraduate, graduate, and faculty education in genetics/genomics and provide opportunities for long- and short-term training of scientists and technicians from African universities.

Aim 5: Establish genetic and genomic technologies and supporting laboratory and physical infrastructure for large-scale genetic/genomic analyses of common diseases in Africa.

These strategic aims were designed to create independent, sustainable genomics facilities in Africa that recruit and train new scientists in genomics, who can then apply these technologies to solve widespread relevant problems in human diseases in Africa.

Methods

Infrastructure development

The clinically important research goals of *CAfGEN* are set to uncover specific genetic markers and genes that reflect the greatest risk for progression in HIV disease and to gain new insights into the pathophysiology of HIV and HIV-TB coinfection, in order to ultimately facilitate development of new treatment strategies. The current lack of substantial work in these areas in the two main *CAfGEN* countries reflects a general maxim underlying the lack of sustainable genetic research in Africa - the dual deficiencies of too little expertise and supporting infrastructure. In keeping with the mission and vision of H3Africa, the *CAfGEN* approach to its primary research goals framed each project in the context of an opportunity to build sustainable genomics capacity and develop genomics infrastructure. Consequently, all *CAfGEN* research projects are interrelated and are deliberately designed to impart transferable expertise that stretches across the breadth of genetic and genomics studies: study design, Ethical Legal and Social Issues (ELSI), consent and sample collection, sample processing and storage, data generation and bioinformatics, statistical analysis of association studies and publication of results (*Figure 2: illustrates the core branches of the network*). Further, each project has a tangible upgrade in physical infrastructure and capacity. At the end of the grant period, *CAfGEN* researchers and clinicians will have been equipped with a solid understanding of how the rapidly changing world of genomics can be applied to answer the most pressing questions on African continent. Moreover, they are receiving the necessary equipment and facilities to design and implement future genomics studies in a culturally sensitive, sustainable fashion that engenders public trust, acceptance and avoids the pitfalls of some genomic research models of the past. The administrative personnel were trained in grants management and oversight equipping them with skills to administer large, multicenter research grants.

Ethics and informed consent

Each participating institution had to undergo ethical review processes by the respective institutional review boards (IRBs), followed by the submission of the research protocol by the main applying institution, being the Botswana-Baylor Children's Clinical Centre of Excellence in Gaborone, to the Botswana Ministry of Health Research and Development Committee for ethical approval. IRB approvals were obtained for all participating institutions.

Written informed consent was sought from the participant and/or their parents or legal guardians. Consent forms were prepared

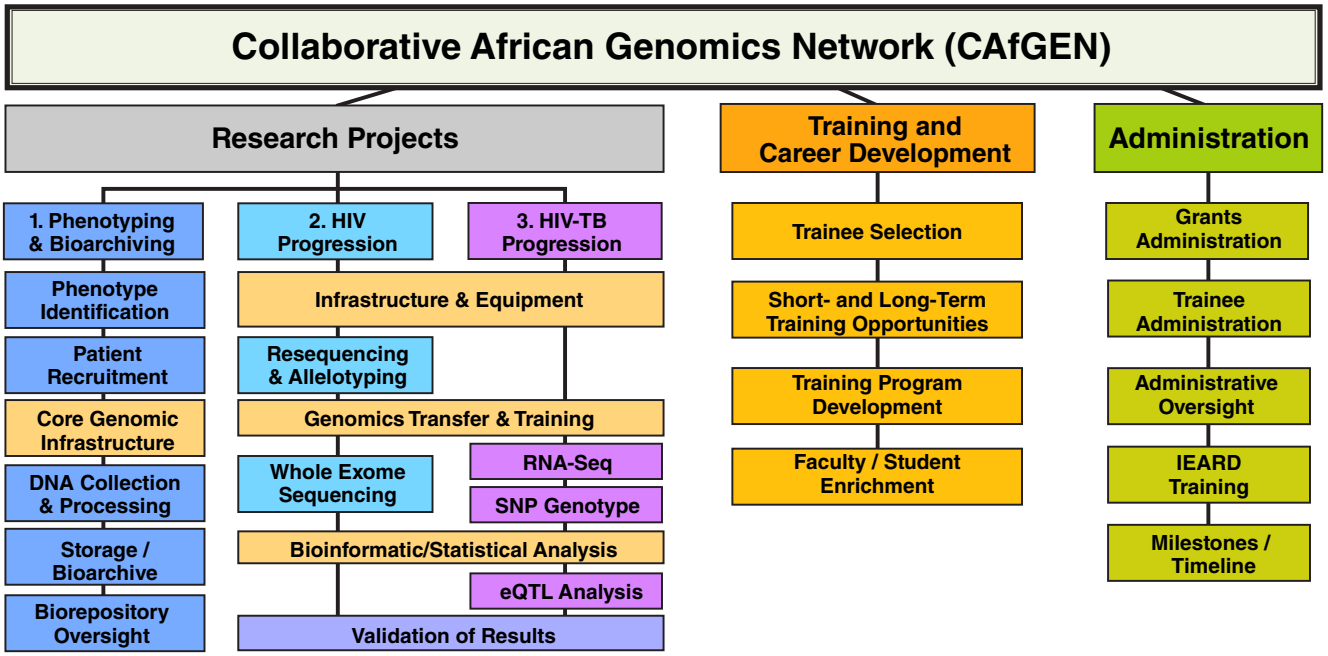

Figure 2. CAfGEN Activities.

in both English and the main local language at each study site (Luganda in Uganda and Setwana in Botswana). The parent or legal guardian of participants who were younger than the legal age of consent (18 years in Uganda and 21 years in Botswana) were provided written informed consent. Those who were older than the legal age of consent signed for themselves. Assent forms were prepared for children and adolescents aged between 12 and the legal age of consent (in addition to their parents/legal guardians' written informed consents). All subjects were free to decline participation in the study and it was made clear that non-participation would not affect any aspect of medical care they were receiving.

While HIV infection and its comorbidities remain the central foci of the project, the broader *CAfGEN* goal is to empower new investigators with a wide array of skills that are required to examine any clinical problem that would be suitable for a genomics approach. We envisioned a critical mass of well-trained, highly knowledgeable, African human geneticists and genomicists each with a broad spectrum of expertise, the knowledge and support to secure outside funding, the ability to educate future generations of researchers, and the experience to collaboratively empower clinicians and researchers in a multi-disciplinary manner.

Identification, selection and training of CAfGEN trainees

During the first award period of the grant (2014 to 2016), six trainees were identified - three each from Botswana and Uganda. Trainees were recruited through the University of Botswana and Makerere University via both internal and external advertising. Selection criteria included a postgraduate science degree, with preference given to those in the fields of genetics, cell biology, biostatistics, and computer science[16]. Candidates who were short-listed based on their academic background and an essay describing their motivation, as well as testimonials provided by three of their academic referees, were subsequently invited for oral interviews conducted by a panel of university staff. Potential scholarship recipients identified at this stage were required to successfully register with the university as PhD students and submit a research proposal based on the *CAfGEN* research objectives.

The selection process took approximately three months at each university. Trainees took graduate genomics and bioinformatics courses, seminars, and workshops that were not available at their home institutions. They also undertook laboratory rotations at selected BCM departments such as the United States Department of Agriculture (USDA)/Agricultural Research Service (ARS) Children's Nutrition Research Center, Human Genome Sequencing Center, Center for Statistical Genetics, RNA-Sequencing, as well as the Computational and Integrative Biomedical Research Center. In addition, they participated in the annual BCM's Department of Molecular and Human Genetics sponsored two-dayretreats at Galveston, Texas where they each presented their PhD concepts and proposals. The trainees have published a peer-reviewed report detailing their perspective on training the next generation of African genomic scientists[16]. All the six students successfully completed more than two years of training at BCM and returned to their respective universities to continue with their PhD studies. Their home universities will assimilate them into genomics faculty positions on completion of their PhDs.

The two participating African universities have enthusiastically embraced genomics training fulfilling *CAfGEN*'s long-term objectives. Tangible strides to nurture the era of genomics at the

collaborating institutions have been realized already such as the inauguration of a new department of Immunology and Molecular biology at Makerere University; this is a home for the genomics and bioinformatics graduate programs for which *CAfGEN* laid the foundation stone.

Recruitment of study participants

Initially, the COEs in Botswana and Uganda were involved in the study participant recruitment. A cohort of well-phenotyped paediatric HIV and HIV-TB infected patients was recruited retrospectively through the EMR, and consisted of 500 LTNPs and 500 RPs. In addition, we recruited HIV-positive children who also progressed to active TB disease. Swaziland COE was added later on to boost the enrolment in the TB case-control component of the study. Blood samples were drawn from the participants and sent to the two universities for the preparation of DNA and RNA.

Power considerations and replication of results

The *CAfGEN* study is divided into discovery and replication phases. To provide a broad approximation of the power of our study, we made a number of assumptions: a) variants with estimated minor allele frequencies > 0.05; b) an alpha (α) value for statistical significance of $p < 0.01$; c) a genetic relative risk (GRR) of between two and four for LTNP-associated variants d) a log additive disease model. Under these assumptions and using the methods of Gauderman implemented in Quanto 1.2.4, where a sample size of 200 cases and 200 controls had greater than 80% power to detect significant differences between groups at $\beta = 0.80$ discovery) of the study. Variants meeting an association threshold of $p < 0.01$ are carried forward for targeted allele typing in 300 LTNPs and 300 RPs in the replication phase.

Community engagement

Community engagement is a key requirement for the overall H3Africa goal of long-term sustainability and has been a prominent feature of the *CAfGEN* plan. We have used a variety of training and educational programs in the form of classroom lectures, workshops, and laboratory training as described below.

Classroom training:

a) Ethics in Research and Informed Consent: emphasis on African populations. This lecture targeted health care professionals who were actively engaged with recruited patients for genomics research studies. Special emphasis was given to the challenges that confront carrying out such studies in African populations including informed consent and assent, stigmatization, and effectively conveying the potential impact of such research on the current and future health. This course has been included in the new genomics graduate programs at Makerere University.

b) The importance of genomics to human health (community outreach). This lecture targeted community lay persons and emphasized basic concepts of heredity, genetics, and genomics, common community relevant human genetic conditions (e.g., albinism and Sickle cell disease), and the importance of genomics research to human health and research participants' protections.

Workshops: A variety of practical workshops were carried out by the COEs to provide training to individuals within and outside the *CAfGEN* network. The workshops were administered by the COEs including specific expertise such as; a) sputum induction in the children, b) Laboratory procedures for HIV testing, c) collection and processing of whole blood samples for research studies, d) effective use of EMR for research studies, and e) effective counselling to maximize patient compliance and research study participation.

Other innovative community engagement activities included the production of Genome Adventures comic books. In these series; Kitso, a young man takes a journey with the superheroes to explore the relations of heredity and genetics as shown in Figure 3 below. These series featured on a wide range of social media platforms such as Weebly, Facebook, Twitter, and Pinterest. These books have been translated into several languages such as Setswana, Swahili, Luganda, Arabic, Hausa, French, and Portuguese. This project was funded jointly by the Wellcome Trust and the US National Institutes of Health initiatives.

We also shared close working relations with the Botswana Harvard AIDS Institute Partnership (BHP), a world-renowned institution of excellence in research and education pertinent to HIV/AIDS and other emerging public health challenges. The more than 20 years of collaborative research and training experience at BHP was highly valuable to *CAfGEN*. Their research areas include virology, molecular biology, immunology, human genetics, epidemiology, and social and behavioral issues relevant to the AIDS epidemic in Botswana and southern Africa. We further maintained routine interactions with the community leaders and institutional review boards and community leaders through community advisory boards made up of local community leaders.

Genomic sample processing, bioarchiving, and shipping

Uniquely labeled blood samples were collected using PAXgene Blood DNA and RNA tubes at the COEs and transported to both the University of Botswana (Department of Biological Sciences, Faculty of Sciences) and Makerere University (Molecular Diagnostics laboratory, Department of Immunology and Molecular Biology) where genomic DNA and RNA was extracted. The team utilized the already existing molecular genetics expertise at these two universities to successfully perform extraction of high quality DNA and RNA for subsequent sequencing and genotyping. Samples were quantified using either a Nanodrop 2000 spectrophotometer or a Qubit 2.0 Fluorimeter. Sample quality was assessed by agarose gel electrophoresis. Samples were stored in a -80°C freezer at the H3Africa Integrated Biorepository at Makerere University. Genomic DNA and RNA were shipped, as per the requirements of the Centers for Diseases Control and Prevention (CDC) and the International Air Transport Association (IATA), to the Human Genome Sequencing

Figure 3. Genome adventures comics.

Center and the Children's Nutrition Research Center at BCM for WES and RNA sequencing, respectively. Aliquots of all samples are stored at the Biorepository at Makerere University.

Genomic data analyses and accomplishments

Following the completion of more than two years of didactic training in genomics and bioinformatics at BCM, *CAfGEN* trainees are currently analyzing the genomic data at their respective institutions utilizing local computing resources made possible by *CAfGEN* funding while they continue to receive both mentorship and supervision from their BCM mentors.

Trainees have focused on WES data analysis as part of their PhD requirement at the Universities in Uganda and Botswana. Analysis has revealed uncaptured genetic variation and distinct ancestry in the southern African population of Botswana[23]. Trainees contributed to a number of studies such as "Whole-exome sequencing of SCD patients with hyperhemolysis syndrome (HHS) suggests a role for rare variation in disease predisposition". This study highlights a potential role for rare genetic defects in the development of HHS among adult SCD patients[24], a disease important to African populations. This work was funded by the US National Blood Foundation and National Human Genome Research Institute. Trainees continue to analyse *CAfGEN* genomic data to answer their respective study objectives including roles of chemokines & their ligands variant in HIV/AIDS disease progression from which the preliminary results suggest no role for chemokines and their cognate ligands in pediatric AIDS disease progression (unpublished data), interrogation of unmapped reads, rare and common variants in paediatric HIV/AIDS disease progression as well as RNA-Seq data analyses.

CAfGEN trainees at Makerere University coordinated H3ABioNet's Introduction to Bioinformatics three months' on-line course. This course provides an introduction to the field of bioinformatics, with a focus on important bioinformatics tools and resources. The course attracted 56 participants and this was the first time it was offered at the University. *CAfGEN* trainees have made both oral and poster presentations during the International Society for Computational Biology (ISCB) and African Society for Bioinformatics and Computational Biology (ASBCB) at Entebbe, Uganda; from October 10 – 13, 2017[25].

The trainees have also cultivated collaborations and networks that are important for sustaining and development of genomics capacity in Africa. These include; Bridging Biobanking and Biomedical Research across Europe and Africa (B3Africa), this collaboration enabled Makerere University to receive eB3Kit -mini-computational server. The eB3Kit consists of 3 main components: A bioinformatics platform, an ethical and legal framework, a training component. This server has been very useful in the genomics trainings/workshops held at Makerere. It provides a linux environment which is remotely utilized by workshop participants. *CAfGEN* trainees have been able to apply for collaborative funding opportunities and awarded a fellowship by the THRiVE Consortium (Training Health Researchers into Vocational Excellence in East Africa) also funded by the Wellcome Trust. The support covers the trainee's PhD related activities and six months of training in the department of Genetics at the University of Cambridge, in the UK.

Other networks that have been established by the trainees include the Makerere University – Uganda Virus Research Institute (UVRI) Centre of Excellence for Infection and Immunity

Research and Training (*MUII-Plus*). The MUII is passionate about the development of bioinformatics in Uganda. They have offered both bioinformatics grants and travel scholarships to the trainees to present their PhD work during the ISCB/ASCB at Entebbe in October, 2017. Trainees are also taking part in grant application processes at their home institutions. This is critical for sustainability of the genomics milestones attained through *CAfGEN* funding; trainees also continue to assist in external sequencing projects and genomic data analyses at their home institutions.

To develop pedagogical skills, trainees are equally involved in teaching undergraduate courses such as introduction to bioinformatics, fundamental genetics & molecular biology for non-medical students and introduction to bioinformatics and medical genomics for MBChB (medical) students. This is an important strategy to ensure trainees retention as faculty as well as knowledge and skills transfer.

Capacity building and infrastructure development

The *CAfGEN* project has enabled six PhD students to successfully complete their intensive genomics training at BCM. They are all being supported to complete their PhDs at their home institutions. The project allowed the purchase of both an ABI 3500 Capillary sequencer (Life Technologies, CA) and a MiSeq System (Illumina, San Diego, CA) for the University of Botswana and Makerere University, respectively. The acquisition of these instruments has already spurred new opportunities for genomics training and research at these institutions. In particular, the "Introduction to Bioinformatics and Next Generation Sequencing Techniques" workshop was funded by the World Bank through the African Higher Education Centres of Excellence Project (MAPRONANO ACE II) and attended by 52 researchers from Rwanda, Tanzania, Zambia, Ethiopia, Kenya, Malawi, and Uganda, the Bioinformatics RNA sequence data analysis workshop was funded by the Alliance for Global Health Science also attended by 19 participants from Uganda and Zimbabwe and one-year training program funded through the University of Georgia's "Computational and Molecular Epidemiology in TB and HIV in Uganda". This mentorship program offers a unique training arrangement to students who have special interest in genomics and bioinformatics to attend weekly tutorials and practical sessions offered by faculty genomics mentors throughout the year. The program is currently training four students at Makerere University (D43TW010045-03). *CAfGEN* trainees are at the centre of these trainings and workshops.

The *CAfGEN* project has also procured and installed a server for bioinformatics at the Makerere University College of Health Sciences. The server is being used by students in the genomics mentorship programs and will soon be utilized by the genomics graduate programs at Makerere University. *CAfGEN* has already fostered many endeavors that are sustainable, productive, impactful, and transformative in both the satellite countries and the continent at large in constructing strong networks and strategies to achieve the goals of the H3Africa initiative. In addition to the above, other highlights include;

1. To ensure long-term, high-quality, sustainable human genetic and genomics studies in Africa, one of our principal investigators (Dr. Moses Joloba) received funding and set up the largest biorepository in East Africa, the Integrated Biorepository of H3Africa Uganda - IBRH3AU.

2. To ensure best practices governing *CAfGEN* and future genomics studies in Africa, our center at Makerere University was awarded an NIH U01 grant entitled "Ethical and social issues in informed consent processes in African genomic research funding (Principal investigator: Dr. Erisa Sabakaki Mwaka, Makerere University College of Health Sciences, Uganda). This project aims to increase awareness and protection of the interests of research participants regarding ELSI (U01-HG009810-01).

3. The "Nurturing Genomics and Bioinformatics Research Capacity in Africa (BRecA)" program, which builds on the momentum of *CAfGEN*, was awarded to Dr. Kateete David and Dr. Graeme Mardon by the Fogarty International Center to establish sustainable genomics and bioinformatics graduate programs at Makerere University (U2RTW010672). Both masters and PhD graduate programs in Genomics and Bioinformatics are in the final stages of approval at Makerere. *CAfGEN* alumni are also highly active in these training programs.

4. *CAfGEN* trainees continue to be outstanding in so many ways including winning travel scholarship awards for both oral and poster presentations at different H3Africa consortium meetings, trainings, and workshops, in 2017 during this 10th H3A consortium meeting, a *CAfGEN* trainee excelled and was awarded the prize for the best poster presenter. Another trainee made both an oral and poster presentation during the ISCB/ASCB conference. They have presented their work at several other conferences such as the UCSF-Gladstone - Centers for AIDS Research (CFAR) 9th East Africa Collaborative Scientific Symposium at the Infectious Diseases Institute in Kampala, Uganda on the 19 – 20th of January 2017. At the end of their training, we envisage a team of African human geneticists and genomicists who have a broad spectrum of expertise and knowledge that will enable them compete for and secure outside funding while they continue the cycle of educating future generations of researchers.

Challenges encountered

Implementing the *CAfGEN* project has not been without challenges, through which we have learnt valuable lessons. Retention of personnel has continued to be a problem at all levels of academia and *CAfGEN* has been no different as some of the *CAfGEN* personnel have since moved on to more lucrative job offers; delayed project approvals/renewals have also been a fact of life - the project required ethics approvals to be in place at all the involved institutions, although genomics studies are new territory for any of the local ethics/Institutional Review Boards. The broader H3Africa consortium was faced with similar issues, and this ultimately led to sponsored invitations to Head of Ethics committees/IRBs to attend H3Africa meetings in order to voice their concerns and engage in dialogue to mitigate any grievances.

This process has paid significant dividends - green-lighting projects that were on hold for some time, and gaining a wider audience and appreciation for H3Africa protocols at the University and Ministry levels. Finally, communication and data transfer issues due to slow or absent internet connections continue to be a significant problem without an obvious solution thus far. Genomics generates large amounts of data, necessitating fast connections for analyses and data transfer; although increased bandwidth and speed are a priority for many African governments, progress on this front has been slow.

Dissemination of findings

The findings of the *CAfGEN* project will be disseminated in open access peer reviewed journals as required by H3Africa publications policy. All data will be made available to the research community upon request as per the H3Africa Consortium Data Sharing, Access and Release (DSAR) Policy.

Study status

The *CAfGEN* trainees are currently analyzing the genomic data (exome and RNA-Seq) as well as writing manuscripts as part of their PhD requirements at African institutions.

Conclusions

The opportunity offered by studying HIV and HIV-TB coinfection in children at the COEs and integrated genomics training in both Botswana and Uganda has permitted us to pioneer a new method of undertaking genomics and biomedical research in Africa whilst building sustainable expertise and infrastructure. This innovative approach has registered significant gains in achieving the goal of H3Africa. Furthermore, we have successfully ensured that the acquired genomics expertise is transferred to Africa as trainees returned to their home countries to take faculty positions, transfer skills and participate in genomics-driven research pertinent to African populations with a goal of improving health.

CAfGEN will continue to work hand in hand with the African academia, funding agencies, and governments to promote genomics training, research, and support genomics policy formulation, in pursuit of its long-term goal of becoming a major centre for large-scale genomic studies of paediatric HIV and associated co-morbidities in sub-Saharan Africa.

Data availability

All data underlying the results are available as part of the article and no additional source data are required

Competing interests

Outside the submitted work, Misaki Wayengera works as a chief scientific officer at Restrizymes Biotherapeutics (U) LTD and Graeme Mardon is a 50% owner and President of GenetiVision Corporation. There are no competing financial interests. The other authors declare no conflict of interest.

Grant information

CAfGEN is funded by NIH grant 1U54AI110398. Gerald Mboowa is also supported through the DELTAS Africa Initiative grant #DEL-15-011 to THRiVE-2 (the Training Health Researchers into Vocational Excellence in East Africa). The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust (WT) grant #107742/Z/15/Z and the UK government. Genome Adventure Comic Books were sponsored through the *CAfGEN* NIH grant and WT grant # 105057/z/14/z. The views expressed in this publication are those of the author(s) and not necessarily those of AAS, NEPAD Agency, or Wellcome Trust.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We appreciate the contributions of the following *CAfGEN* institutions: Baylor College of Medicine, Houston, Texas; Baylor Children's Clinical Centers of Excellence in Botswana, Swaziland, Uganda; Makerere University, Kampala; and University of Botswana, Gaborone. We are also grateful to the H3Africa Consortium.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 21 May 2018

https://doi.org/10.21956/aasopenres.13898.r26408

✔ **Syntia Nchangwi Munung**
University of Cape Town (UCT), Cape Town, South Africa
Jantina de Vries
Department of Medicine, University of Cape Town (UCT), Cape Town, South Africa

In this paper, Mbwoowa *et al* present the CafGEN project, and describe how it operates, what the pertinent challenges are, and how it has gone about community engagement. The paper illustrates that a well organised and funded collaborative genomics project in Africa can, in a short period of time, achieve a lot in terms of research capacity building. That is quite an encouraging and important message in the manuscript.

Major Comments:

In both the abstract and in the introduction, you first mention H3Africa and then CafGEN, and we would suggest that you reverse that order. In doing that, you'll give more prominence to your own work – a small but important change of focus. In the way you present your work, we think you should make it clear that CafGEN derives legitimacy from the pertinent scientific questions in this field, and not from H3Africa. H3Africa is the facilitator of CafGEN through funding, network building, and infrastructure – it is not the reason that CafGEN exists though.

- In the same vein, it is not that interesting for an audience to know that 'a call for applications was issued'. Rather, why don't you speak about the most important challenges in the field of (paediatric) HIV/AIDS research that motivated you to set up CafGEN? With a follow up section on how CafGEN will systematically tackle those questions.
    - So in short, what we would suggest is that you review your entire first page of the manuscript, and cut out much of the current text, and rather introduce the context pertinent to CafGEN specifically. In a way, we would suggest re-writing your text to demonstrate (rather than state) that CafGEN is a 'unique, highly synergistic African alliance'
    - One example of that is that it's only on pg 5 of the current manuscript that you introduce the three Centers of Excellence that CafGEN work is premised on – but isn't the collective experience in those centres (and a detailed understanding of the knowledge gaps derived thereof) the backbone to the collaboration? If so, then this should take centre stage in the article.

- In a way, we propose that the text on Pg 6 achieves some of our suggestions above.

Minor comments:

1. One of the 6 CAFGEN sites is the Swaziland Children's Clinical Centres of Excellence (Page 4). But the article is silent on research and capacity building activities in this country/site, except that the Swaziland COE was added later on to boost the enrolment in the TB case-control component of the study (see page 8). It therefore appears that much of the capacity building and research activities took place in Uganda and Botswana and not Swaziland. Maybe the authors can add a few sentences on the capacity building and research activities at this site. If none, then maybe explain why that wasn't the case and the challenges they had in implementing capacity building and research in Swaziland.

2. Page 4: I think the use of heart disease as an example of an inherited disease may not be the best. Perhaps it is the way it is used in the sentence – we would encourage the authors to review this sentence and the example and to perhaps to use another example or to reword the sentence.
3. Page 7. Consent forms where translated to Luganda and Setswana. Again, information for the Lesotho site is missing, though there was participant enrolment at the site. Please can you add information on consent, Recruitment/CE for the Lesotho site or otherwise explain how this site fits in with the other sites.
4. Page 8. Community engagement activities:The CAFGEN comic series is described as an innovative (maybe creative?) CE method (and we agree). The authors can maybe add a sentence or two on why they consider it innovative and also a few lines, in the discussion, on the advantages of this CE method for genomics research in Africa. This will benefit readers who are not familiar with CE in health research in Africa or the use of comics and social media for CE in health research in Africa.
The first community engagement activity -- classroom training for health care workers may fit better in the section on "Capacity building and infrastructure development". This is perhaps more an activity concerning training in research ethics for health care workers than a community engagement activity?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 17 Jun 2018

Gerald Mboowa, Makerere University, Kampala, Uganda

Major Comments:

In both the abstract and in the introduction, you first mention H3Africa and then CAfGEN, and we would suggest that you reverse that order. In doing that, you'll give more prominence to your own work – a small but important change of focus. In the way you present your work, we think you should make it clear that CAfGEN derives legitimacy from the pertinent scientific questions in this field, and not from H3Africa. H3Africa is the facilitator of CAfGEN through funding, network building, and infrastructure – it is not the reason that CAfGEN exists though.

We have addressed the order of first mention of "*CAfGEN" in* the abstract.

Here, we describe how the Collaborative African Genomics Network (*CAfGEN*) of the Human Heredity and Health in Africa (H3Africa) consortium is using genomics to probe host genetic factors important to the progression of HIV and HIV-tuberculosis (TB) coinfection in sub-Saharan Africa. The H3Africa was conceived to facilitate the application of genomics technologies to improve health across Africa.

- In the same vein, it is not that interesting for an audience to know that 'a call for applications was issued'. Rather, why don't you speak about the most important challenges in the field of (paediatric) HIV/AIDS research that motivated you to set up CAfGEN? With a follow up section on how CAfGEN will systematically tackle those questions.

We have included a section on Challenges and opportunities of pediatric HIV/AIDS and TB genomics research.

Other challenges of pediatric HIV/AIDS and TB genomics research included:

1. **Immature immune system that prompts HIV/AIDS to develop more rapidly than in their adult counterparts**
2. **Technical expertise in HIV testing algorithm for infants born to HIV- positive women includes HIV RNA/DNA - polymerase chain reaction test which is more technically demanding and has a relatively longer turnaround time for HIV diagnosis unlike the routine rapid immunochromatographic screening HIV test for detection of antibodies to HIV- 1**
3. **Adherence to antiretroviral therapy and retention for HIV-infected adolescents, social stigma in both communities and schools affecting their school performance, health professionals required to care for HIV-infected children are in short supply generally in resource limited settings**
4. **New policies such as HIV 'test and treat' strategy and isoniazid prophylactic therapy for the prevention of tuberculosis in HIV-infected children**
5. **The challenges of TB case finding amongst HIV infected children.**

***CAfGEN* and the COEs addressed some of the above challenges. The COEs do perform HIV RNA PCR routine testing for infants born to HIV-positive women and ensure a short turnaround time for this diagnosis. The COEs have both nationally and internationally well–trained health professionals who manage the ART and TB clinics, ensure adherence to treatment, retention for HIV-infected children, and psychosocial well being.**

- So in short, what we would suggest is that you review your entire first page of the manuscript, and cut out much of the current text, and rather introduce the context pertinent

to CAfGEN specifically. In a way, we would suggest re-writing your text to demonstrate (rather than state) that CAfGEN is a 'unique, highly synergistic African alliance'

We have reviewed the entire first page of the manuscript making it *CAfGEN* pertinent demonstrating that CAfGEN is a 'unique, highly synergistic African alliance'
In 2011, a group of scientists came together and conceived the white paper "Harnessing Genomic Technologies Toward Improving Health in Africa: Opportunities and Challenges" [1]. Currently, much research in Africa is funded through foreign granting mechanisms [2] and many PhD level scientists in Africa have received their training at foreign institutions [3]. Unfortunately, scientists often leave for training and do not return to Africa because of limited opportunities on the continent [4]. The Human Heredity and Health in Africa consortium (H3Africa) was initiated, in part, to reverse this 'brain drain' and has already made substantial strides towards this goal and the Collaborative African Genomics Network (*CAfGEN*) is an H3Africa collaborative centre.
The clinically important research goals of *CAfGEN* are providing us new insight into the pathophysiology of paediatric HIV and HIV-TB in SSA in order to ultimately facilitate development of new treatment strategies. *CAfGEN* continues to support sustainable genetic research in Africa to overcome the dual deficiencies of too little genomics expertise and infrastructure. Subsequently, all *CAfGEN* research projects are deliberately designed to impart transferable expertise that stretches across the breadth of genetic and genomics studies – study design, Ethical, Legal and Social Implications (ELSI), informed consent and sample collection, sample processing and storage, data generation and bioinformatics, statistical analysis of association studies, and reporting and publication of results. *CAfGEN* researchers and clinicians have a solid understanding of how the rapidly changing world of genomics can and should be applied to answer the most pressing questions in the African settings.

- One example of that is that it's only on pg 5 of the current manuscript that you introduce the three Centers of Excellence that CAfGEN work is premised on – but isn't the collective experience in those centres (and a detailed understanding of the knowledge gaps derived thereof) the backbone to the collaboration? If so, then this should take centre stage in the article.

Agreed, we shall be working on a separate manuscript to cover this important aspect of collective experience in COEs also detailing the knowledge gaps and collaboration.

- In a way, we propose that the text on Pg 6 achieves some of our suggestions above.

Minor comments:

1. One of the 6 CAfGEN sites is the Swaziland Children's Clinical Centres of Excellence (Page 4). But the article is silent on research and capacity building activities in this country/site, except that the Swaziland COE was added later on to boost the enrolment in the TB case-control component of the study (see page 8). It therefore appears that much of the capacity building and research activities took place in Uganda and Botswana and not Swaziland. Maybe the authors can add a few sentences on the capacity building and research activities at this site. If none, then maybe explain why that wasn't the case and the challenges they had in implementing capacity building and research in Swaziland.

We have added a line regarding the capacity building activities done at the Swaziland

COE.

Capacity building and research activities undertaken by CAfGEN at the Swaziland COE included training the investigators in implementation of pediatric tuberculosis intensive case finding among the HIV infected children and held genomics community outreach workshops.

2. Page 4: I think the use of heart disease as an example of an inherited disease may not be the best. Perhaps it is the way it is used in the sentence – we would encourage the authors to review this sentence and the example and to perhaps to use another example or to reword the sentence.

We have rewritten the sentence as below:
Some inherited diseases, such as Sickle cell disease (SCD), are single gene or Mendelian disorders arise as a result of mutations in one or both alleles of a gene while others, such as Chronic Kidney Disease, are the result of a complex interplay of factors that can include a multitude of both genetic and environmental influences, acting to both increase or decrease susceptibility.

3. Page 7. Consent forms where translated to Luganda and Setswana. Again, information for the Lesotho site is missing, though there was participant enrolment at the site. Please can you add information on consent, Recruitment/CE for the Lesotho site or otherwise explain how this site fits in with the other sites.

We have included the statement regarding Swaziland informed consent process.

Consent forms were prepared in both English and the main local language at each study site (Luganda in Uganda, Setwana in Botswana, and Swati in Swaziland). The parent or legal guardian provided written informed consent on behalf of participants who were younger than the legal age of consent (18 years in Uganda, Swaziland and 21 years in Botswana).

4. Page 8. Community engagement activities: The CAfGEN comic series is described as an innovative (maybe creative?) CE method (and we agree). The authors can maybe add a sentence or two on why they consider it innovative and also a few lines, in the discussion, on the advantages of this CE method for genomics research in Africa. This will benefit readers who are not familiar with CE in health research in Africa or the use of comics and social media for CE in health research in Africa.

The first community engagement activity -- classroom training for health care workers may fit better in the section on “Capacity building and infrastructure development”. This is perhaps more an activity concerning training in research ethics for health care workers than a community engagement activity?

We have moved the classroom training to capacity building section.

Competing Interests: No competing interests were disclosed.

Author Response 18 Jun 2018

Gerald Mboowa, Makerere University, Kampala, Uganda

One example of that it's only on pg 5 of the current manuscript that you introduce the three Centers of Excellence that CAfGEN work is premised on – but isn't the collective experience in those centres (and a detailed understanding of the knowledge gaps derived thereof) the backbone to the collaboration? If so, then this should take centre stage in the article.

Agreed, At the end of the grant period, we shall be working on a separate manuscript to cover this important aspect of collective experience in COEs also detailing the knowledge gaps and collaboration.

Competing Interests: No competing interests were disclosed.

Reviewer Report 30 April 2018

https://doi.org/10.21956/aasopenres.13898.r26342

Edward J Hollox
Department of Genetics and Genome Biology, University of Leicester, Leicester, UK

This paper describes the construction and aims of the Collaborative African Genomics Network (CAfGEN), with a focus on the training on a cohort of African genomics researchers, as well as investigating the host genetic factors that influence progression of HIV and HIV-tuberculosis co-infection in sub-Saharan Africa.
This is a very useful document that describes the development of CafGEN, and provides information to other African genomics partnerships in approaches to build a collaboration that includes training and research. In particular, it highlights infrastructure and community engagement approaches, and identifies roadblocks to this, and potentially other, capacity-building programmes.
I only have a few minor suggestions that would improve the manuscript.

1. Page 4, paragraph 2. "Key to this, is identifying". No comma needed.
2. Page 4, paragraph 2. "relatively straightforward variation in a single gene". This sentence needs to be clarified, reworded, and perhaps this point should be expanded. Sickle cell disease is, as the authors will know, caused by a single variant in a single gene, there are other genetic disease that are caused by different variants in a single gene, and the complexity increases in a continuum to complex multifactorial disease. The sentence seems to suggest that it is either a single gene disease, or a complex disease. It also seems to suggest that heart disease is an inherited disease – I'm sure this was not the intention of the authors but the sentence can be read this way.
3. Page 4, paragraph 3. "… DNA variation both directly and indirectly impacts disease outcomes". The previous paragraph discusses how DNA variation can directly affect disease outcomes, but the authors need to be more explicit how DNA variation indirectly affects disease outcomes.
4. Page 4, column 2, paragraph 1. "classically considered to be the wellspring of modern humanity". This sentence is vague and should be rewritten. In particular, reconsider the use of the term

"classically" as I don't think this is the case, and try to avoid the circularity in this sentence – evidence for Africa being the origin of modern humans comes from the observed high levels of genetic diversity, while this sentence seems to suggest the inference is the other way around.
5. Page 4, column 2, paragraph 2. "After" is clearer than "Subsequent to".
6. Page 6 column 1. It is not clear why some aims are split into 2 – a and b. Either combine these to make five aims, or give them different numbers to make 8 aims.
7. Page 6, aim 2a. It is not clear why "established" is in scare quotes. Do the authors mean that these loci are established in Europeans but not Africans? The authors should clarify this.
8. Page 7, paragraph 1. The second sentence is not clear. I'd suggest rewording: "The parent or legal guardian provided written informed consent on behalf of participants who were younger than the legal age of consent (18 years in Uganda and 21 years in Botswana)."

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 17 Jun 2018

Gerald Mboowa, Makerere University, Kampala, Uganda

1. Page 4, paragraph 2. "Key to this, is identifying". No comma needed.

Comma removed.

1. Page 4, paragraph 2. "relatively straightforward variation in a single gene". This sentence needs to be clarified, reworded, and perhaps this point should be expanded. Sickle cell disease is, as the authors will know, caused by a single variant in a single gene, there are other genetic disease that are caused by different variants in a single gene, and the complexity increases in a continuum to complex multifactorial disease. The sentence seems to suggest that it is either a single gene disease, or a complex disease. It also seems to suggest that heart disease is an inherited disease – I'm sure this was not the intention of the authors but the sentence can be read this way.

Sentence re-written as below:
Some inherited diseases, such as Sickle cell disease (SCD), are single gene or Mendelian disorders arise as a result of mutations in one or both alleles of a gene while others, such as Chronic Kidney Disease, are the result of a complex interplay of factors that can include a multitude of both genetic and environmental influences, acting to both increase or decrease susceptibility.

1. Page 4, paragraph 3. "… DNA variation both directly and indirectly impacts disease outcomes". The previous paragraph discusses how DNA variation can directly affect disease outcomes, but the authors need to be more explicit how DNA variation indirectly affects disease outcomes.

DNA variation can directly affect disease outcomes leading to variations in disease susceptibility, resistance, and rate of progression.

1. Page 4, column 2, paragraph 1. "classically considered to be the wellspring of modern humanity". This sentence is vague and should be rewritten. In particular, reconsider the use of the term "classically" as I don't think this is the case, and try to avoid the circularity in this sentence – evidence for Africa being the origin of modern humans comes from the observed high levels of genetic diversity, while this sentence seems to suggest the inference is the other way around.

Sentence re-written "Africa is the evolutionary *home* of *modern humans".*

1. Page 4, column 2, paragraph 2. "After" is clearer than "Subsequent to".

"Subsequent to" replaced by "After".

1. Page 6 column 1. It is not clear why some aims are split into 2 – a and b. Either combine these to make five aims, or give them different numbers to make 8 aims.

All aims renumbered 1 to 8.

1. Page 6, aim 2a. It is not clear why "established" is in scare quotes. Do the authors mean that these loci are established in Europeans but not Africans? The authors should clarify this.

HIV disease progression susceptibility loci that will be identified in the CAfGEN paediatric HIV infected cohorts. We have removed the scare quotes.

1. Page 7, paragraph 1. The second sentence is not clear. I'd suggest rewording: "The parent or legal guardian provided written informed consent on behalf of participants who were younger than the legal age of consent (18 years in Uganda and 21 years in Botswana)."

We have re-worded the sentence as requested.

Competing Interests: No competing interests were disclosed.