Check for updates

RESEARCH NOTE

REVISED Importance of human demographic history knowledge in genetic studies involving multi-ethnic cohorts [version 2; referees: 2 approved]

Previously titled: H3Africa: crucial importance of knowledge on human demographic history in strategies for data exploitation – an analysis of the *Luhya in Webuye, Kenya* population from the 1000 Genomes Project

Benard W. Kulohoma 🆔

Centre for Biotechnology and Bioinformatics, University of Nairobi, Nairobi, Kenya

Abstract
Paucity of data from African populations due to under-representation in human genetic studies has impeded detailed understanding of the heritable human genome variation. This is despite the fact that Africa has sizeable genetic, cultural and linguistic diversity. There are renewed efforts to understand health problems relevant to African populations using more comprehensive datasets, and by improving expertise in health-related genomics among African scientists. We emphasise that careful consideration of the sampled populations from national and within-continental cohorts in large multi-ethnic genetic research efforts is required to maximise the prospects of identifying and fine-mapping novel risk variants in indigenous populations. We caution that human demographic history should be taken into consideration in such prospective genetic-association studies.

Keywords
Africa, GWAS, Population substructure, H3Africa

Open Peer Review

Referee Status: ✔✔

	Invited Referees	
	1	**2**
REVISED **version 2** published 18 Sep 2018	✔ report	✔ report
	↑	↑
version 1 published 06 Jul 2018	? report	? report

1 **Michèle Ramsay** 🆔 , University of the Witwatersrand, South Africa

2 **Nicola J. Mulder** 🆔 , University of Cape Town, South Africa

Discuss this article

Comments (0)

Corresponding author: Benard W. Kulohoma (bkulohoma@uonbi.ac.ke)

Author roles: Kulohoma BW: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

REVISED **Amendments from Version 1**

We have revised the analysis to include more populations in the analysis to verify our findings. We have made revisions to the analysis, and now include other populations that share recent genetic ancestry from the 1000GP that belong to the Niger-Kordofanian language group for comparison.

We have made revisions to the title and the text to encompass all studies, not just H3Africa. We have also revised the manuscript and reduced the number of figures and redrawn the structure plot.

We have amended the manuscript and discuss the importance of multi-disciplinary approaches that enlist the knowledge of anthropologists, linguists, geneticists, and historians to improve understanding on human history and migration of populations, genetics of complex traits and adaptive variations to modern environments, and language and cultural changes.

We agree that current analysis program that implement standard linear regression can use principal components from PCA analysis to avoid errors due to population stratification, as well as speed-up computation. We highlight the importance of multi-disciplinary approaches when sampling underrepresented populations of interest to put local populations in the right context and provide more detailed and accurate information for disease mapping efforts in Africa. This will provide a more granular understanding on the genetic traits associated with these populations. Although GWAS studies largely rely on self-reported data on ethnic background, which is the verified using genetic information to confirm ancestral backgrounds and exclude outliers. The exclusion of individuals may lead to insufficient representation of some populations, and disease-association studies of low prevalence or late onset conditions, such as Alzheimer's disease, would be underpowered. Taking population history into account during study design my help to alleviate these challenges.

We have made revisions to the manuscript to remove repetitions and improve meaning. We have amended the word "titling" to "tilting".

See referee reports

Introduction

The 1000 Genomes Project (1000GP) is an invaluable resource that has improved understanding of global human genetic variation and its contribution to disease biology across multiple populations of distinct ethnicity[1]. This catalogue of over 88 million high-quality variants from 26 populations has enhanced power to screen for common and rare variants that depict geographic and demographic differentiation[2]. This represents 80% (80 million) of all variants contributed or validated in the public dbSNP catalogue, with recent major enhancements for genetic variation within several South Asian and African populations (24% and 28% of novel variants respectively)[2]. Most of the low-frequency (< 0.5%) variants likely to be of functional significance are disproportionately present in individuals with substantial African ancestry, indicating bottlenecks in non-African populations[2,3]. The *"Luhya in Webuye, Kenya"* (LWK) population has the most accentuated number of these rare variants.

Paucity of data from African populations has restricted understanding of the heritable human genome variation. Although under-represented in human genetic studies, Africa has sizeable genetic, cultural and linguistic diversity (> 2000 distinct ethno-linguistic groups)[4]. African populations are more genetically diverse, with considerable population substructure, and lower linkage disequilibrium (LD) compared to non-African populations[4,5]. Inclusion of more African populations will improve understanding of genetic variation attributed to complex population history, variations in climate, lifestyles, exposure to infectious diseases, and diets[4,6]. Diverse multi-ethnic imputation panels will undoubtedly improve fine-mapping of complex traits and provide detailed insights on disease susceptibility, drug responses, and improve therapeutic treatments. One such integrated panel, consisting of the phase 1 1000GP and African Genome Variation Project (AGVP) whole genome sequence panels, has shown marked improvement in detecting association signals in specific African populations poorly represented in the 1000GP[7]. AGVP also present a new genotype array design that captures genetic variation in African populations.

The Human Heredity and Health in Africa (H3Africa) initiative is aimed at understanding health problems relevant to African populations, and tilting the scales of data deficit and lacking expertise in health-related genomics among African scientists[8,9]. The H3Africa consortium consists of over 500 members, from more than 30 of the 55 African countries. H3Africa projects are focused on establishing genetic and environmental determinants associated with infectious (human African trypanosomiasis, tuberculosis, HIV, and other respiratory tract infections) and non-communicable diseases (kidney disease, diabetes, and cardiovascular diseases)[10]. H3Africa is driven by African investigators, and is anticipated to close the gaps of 'missing' heritability by increasing the number of causal variants identified within genes, from a dataset of over 70,000 individuals collected using standardized protocols[8,10]. This presents a unique opportunity for the investigators to not only develop and direct their independent research agendas, but also enrich the datasets using their extensive knowledge of the continent's history. However, careful consideration of the sampled populations in similar projects is required to maximise the prospects of identifying and fine-mapping novel risk variants in indigenous populations. In order to translate genomic research findings to useful resources for clinicians and drug development, substantial knowledge about reference populations that are relevant to the individuals being treated alongside the actionable variants is required[10]. This is in addition to harmonised and well curated phenotype data that will allow easy integration and direct comparison of data outputs across different cohorts and phenotypes. Attentiveness to the considerable genetic substructure in African population may reveal uncaptured variation and distinct ancestry[11]. This extensive genetic diversity would benefit from strategies that explore genomics datasets that put local populations in context to provide more detail from disease mapping efforts in Africa. An example is the LWK in the 1000GP who do not represent all the *"Luhya people"*, a Bantu-speaking Niger-Congo population with a complex population history composed of 17 tribes, each with a distinct dialect (Figure 1A – C)[12,13]. We examined for possible substructure in LWK, from 1000GP, to establish its implication on association studies.
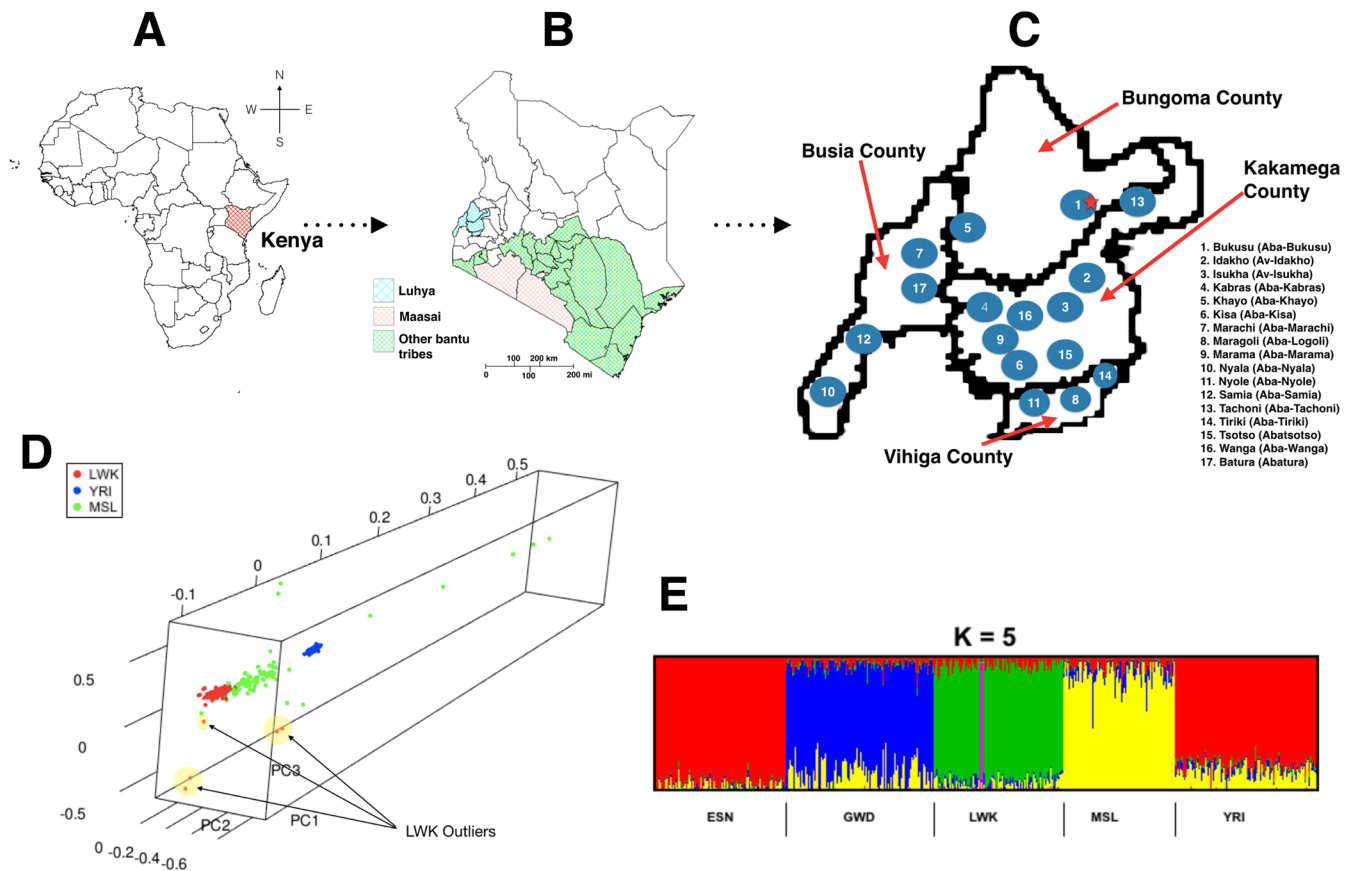
Figure 1. Population structure of the _Luhya in Webuye_, Kenya. (**A**) Map of Africa showing the location of Kenya. (**B**) Map of Kenya showing the location of Western Kenya Counties. (**C**) The four Counties in Western Kenya inhabited by the "_Luhya people_". The 17 tribes of the Luhya, and the locations they hail from in Western Kenya are shown with numbers 1 through 17. (**D**) The distribution of individuals from the LWK, YRI and MSL populations along the first three principal components. (**E**) Ancestry for K value 5 for the Luhya (LWK) from Webuye, Kenya (n=99); Yoruba (YRI) from Ibadan, Nigeria (n=108); Esan (ESN) from Nigeria (n=99); Mandika (GWD) from The Gambia (n=113); and the Mende (MSL) from Sierra Leone (n=85)) examining the same 193,634 variants. The plot of ancestry fractions shows population sub-structure in the LWK population, when compared to five other populations from the 1000 Genomes Project (1000GP).

Methods

We used principal component analysis (PCA) to examine relationships within the Luhya (LWK) from Webuye, Kenya, population (n=99) using 193,634 variants from the 1000GP phase 3[2]. We compared LWK to African populations from the 1000GP phase 3 (Yoruba (YRI) from Ibadan, Nigeria (n=108); Esan (ESN) from Nigeria (n=99); Mandika (GWD) from The Gambia (n=113); and the Mende (MSL) from Sierra Leone (n=85)) examining the same 193,634 variants, since these populations also speak the Niger-Kordofanian languages, and share recent genetic ancestry[12,14]. The 1000GP call set was already filtered down using VCFtools (v 0.1.12b) and PLINK (v1.90b6.2), and only contained biallelic, non-singleton SNV sites that are a minimum of 2KB apart from each other and a minor allele frequency > 0.05[2,15,16]. We considered just the first three principal components (PCs) computed to resolve the population substructure. We then used ADMIXTURE (v1.3) to estimate ancestry for K values from 2 through 20[17]. Distruct plots of the output ancestry fractions were generated using Genesis (v 0.2.6b)[18].

Results and discussion

Our PCA analyses reveal that all individuals in the LWK population cluster closely except five individuals along PC2 (n=2) and PC3 (n=3), possibly suggesting that the outliers are individuals from different Luhya tribes (Figure 1D, & Supplementary Figure 1 & Supplementary Figure 2). We suggest that whereas the first principal component, PC1, distinguished individuals primarily on genetic ancestry, PC2 and PC3 may reflect genetic diversity associated with differences in the geographic distribution and linguistic differences of the individuals. We propose that although a huge proportion of individuals in the LWK population are actually from Webuye, which predominantly inhabited by the Bukusu tribe, the outliers hail from various other settlements associated with other Luhya tribes (Figure 1C). Unsupervised ADMIXTURE analysis suggests minimal substructure (Figure 1E, & Supplementary Figure 3).

In sub-Saharan African (SSA), there are nearly 500 closely related but distinct languages distributed over a total area of approximately 500 000 km[214]. These languages are spoken by

approximately one quarter of the SSA population (~200 million people)[14,19]. The Bantu languages fall into this category, and consist of separate groups that constitute part of the Niger-Congo language phylum[20]. The spread of Bantu-speaking populations in SSA is primarily due to historical migration of populations, approximately 3000–5000 years ago, and not solely due to diffusion of language[14]. This demographic history is associated with admixture and changes in population structure, resulting in complex patterns of genetic variation in present day populations[21,22]. An example is the identification of haplotypes among Nilo-Saharan language speakers of the Luo community that neighbours the Luhya of Western Kenya, which were previously thought to be private in Bantu populations, that are now associated with interactions between these distinct populations during the migration of the Bantu farmer populations[22]. Previous studies on Bantu expansion and migration suggest populations first moved south from their homeland, near the Nigeria-Cameroon border, through the rainforest and split into two groups: one branched south and west; while another moved east towards the Great Lakes[14,23]. The East Bantu languages, which also include the Luhya language, are distributed in East and Southern Africa[23]. In Kenya, these Eastern Bantu speaking populations are further categorised into two based on their migratory routes to present day Kenya: the Eastern Kenya Bantus (Kamba, Kikuyu, Meru, Embu, Taita, Giriama, Kombe, Chonyi, Digo, Rabai, Jibana, Pokomo, Duruma, Kauma and Ribe) and Western Kenya Bantus (Kisii, Luhya, Kuria, Suba and Khene)[24,25].

Multi-disciplinary approaches that enlist the knowledge of anthropologists, linguists, geneticists, and historians would significantly improve understanding on human history and migration of populations, genetics of complex traits and adaptive variations to modern environments, and language and cultural changes[26–28]. Previous studies on intricate languages in China, and Australia suggest consistency of genetic and linguistic evolution, with striking evidence of compatible phylogenetic signal and phonological evolution[29,30]. In SSA such studies are hindered by paucity of data with only a limited number of reasonably close populations available, impeding more detailed analysis[18,31]. A recent study highlights population differentiation between two South Eastern Bantu groups in South Africa, which were assumed to be genetically homogenous, further emphasising the importance of having a clear perspective of population structure in disease-association studies[18]. This result was arrived at by understanding ethnolinguistic divisions within the present-day population, and purposely recruiting from rural areas or regions with little ethnolinguistic diversity[18].

The multi-ethnic genetic-association studies, like those in the H3Africa initiative, now offers a unique opportunity to resolve this challenge using multiple large scale GWAS analyses of important genetic traits from diverse populations across Africa. GWAS studies largely rely on self-reported data on ethnic background. Genetic information is then used to confirm ancestral backgrounds and exclude outliers. However, this may lead to insufficient representation of some populations, and disease-association studies of low prevalence or late onset conditions, such as Alzheimer's disease, would be underpowered. Thus, in order to understand complex traits in say the entire "*Luhya people*", adequate sampling of underrepresented tribes would provide a high-resolution view of their ancestral history. Haphazard sampling would significantly reduce power to detect signal due to population substructure, even within this single community. We speculate that this was largely circumvented at recruitment when sampling LWK in the 1000GP by asking the participants whether all four of their grandparents were of the Bukusu tribe. Whereas projects covering relatively small geographical areas are able to overcome such challenges, national and within-continental cohorts in large multi-ethnic genetic research efforts must have well thought out documented protocols that carefully consider human demographic history.

Data availability
The LWK dataset was obtained from the European Bioinformatics Institute 1000 Genomes Project website http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/supporting/admixture_files/

Supplementary material
Supplementary Figure 1
The distribution of individuals from the LWK, YRI, MSL, ESN and GWD populations along the first three principal components.

Click here to access the data

Supplementary Figure 2

The distribution of individuals from only the LWK, and YRI populations along the first three principal components.

Click here to access the data

Supplementary Figure 3.

Ancestry for K values 2 through 7 for: (A) The Luhya (LWK) from Webuye, Kenya (n=99); Yoruba (YRI) from Ibadan, Nigeria (n=108); and the Mende (MSL). (B) The Luhya (LWK) from Webuye, Kenya (n=99); Yoruba (YRI) from Ibadan, Nigeria (n=108); Esan (ESN) from Nigeria (n=99); Mandika (GWD) from The Gambia (n=113); and the Mende (MSL) from Sierra Leone (n=85)). The same 193,634 variants were examined in all analyses. The plot of ancestry fractions shows population sub-structure in the LWK population, when compared to five other populations from the 1000GP.

Click here to access the data

References

1. Sudmant PH, Rausch T, Gardner EJ, *et al.*: **An integrated map of structural variation in 2,504 human genomes.** *Nature.* 2015; **526**(7571): 75–81.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

2. 1000 Genomes Project Consortium, Auton A, Brooks LD, *et al.*: **A global reference for human genetic variation.** *Nature.* 2015; **526**(7571): 68–74.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

3. Marth G, Schuler G, Yeh R, *et al.*: **Sequence variations in the public human genome data reflect a bottlenecked population history.** *Proc Natl Acad Sci U S A.* 2003; **100**(1): 376–381.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

4. Campbell MC, Tishkoff SA: **African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping.** *Annu Rev Genomics Hum Genet.* 2008; **9**: 403–433.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

5. Tishkoff SA, Dietzsch E, Speed W, *et al.*: **Global patterns of linkage disequilibrium at the CD4 locus and modern human origins.** *Science.* 1996; **271**(5254): 1380–1387.
    PubMed Abstract | **Publisher Full Text**

6. Gomez F, Hirbo J, Tishkoff SA: **Genetic variation and adaptation in Africa: implications for human evolution and disease.** *Cold Spring Harb Perspect Biol.* 2014; **6**(7): a008524.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

7. Gurdasani D, Carstensen T, Tekola-Ayele F, *et al.*: **The African Genome Variation Project shapes medical genetics in Africa.** *Nature.* 2015; **517**(7534): 327–332.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

8. H3Africa Consortium, Rotimi C, Abayomi A, *et al.*: **Research capacity. Enabling the genomic revolution in Africa.** *Science.* 2014; **344**(6190): 1346–1348.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

9. Mulder NJ, Adebiyi E, Alami R, *et al.*: **H3ABioNet, a sustainable pan-African bioinformatics network for human heredity and health in Africa.** *Genome Res.* 2016; **26**(2): 271–277.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

10. Mulder N, Abimiku A, Adebamowo SN, *et al.*: **H3Africa: current perspectives.** *Pharmgenomics Pers Med.* 2018; **11**: 59–66.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

11. Retshabile G, Mlotshwa BC, Williams L, *et al.*: **Whole-Exome Sequencing Reveals Uncaptured Variation and Distinct Ancestry in the Southern African Population of Botswana.** *Am J Hum Genet.* 2018; **102**(5): 731–743.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

12. Coriell Institute: **Luhya in Webuye, Kenya [LWK].** 2018; **2018**.
    Reference Source

13. Wanjala WB: **Inter-dialect maintenance and shift in the contact of lubukusu and lutachoni.** MA (English and Linguistics) thesis, Kenyatta University, 2014.
    Reference Source

14. Li S, Schlebusch C, Jakobsson M: **Genetic variation reveals large-scale population expansion and migration during the expansion of Bantu-speaking peoples.** *Proc Biol Sci.* 2014; **281**(1793): pii: 20141448.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

15. Chang CC, Chow CC, Tellier LC, *et al.*: **Second-generation PLINK: rising to the challenge of larger and richer datasets.** *GigaScience.* 2015; **4**: 7.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

16. Danecek P, Auton A, Abecasis G, *et al.*: **The variant call format and VCFtools.** *Bioinformatics.* 2011; **27**(15): 2156–2158.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

17. Alexander DH, Lange K: **Enhancements to the ADMIXTURE algorithm for individual ancestry estimation.** *BMC Bioinformatics.* 2011; **12**: 246.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

18. Choudhury A, Ramsay M, Hazelhurst S, *et al.*: **Whole-genome sequencing for an enhanced understanding of genetic variation among South Africans.** *Nat Commun.* 2017; **8**(1): 2062.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

19. Bakilana AM: **7 facts about population in Sub-Saharan Africa.** *African can end poverty.* World Bank, 2015.
    Reference Source

20. Batai K, Babrowski KB, Arroyo JP, *et al.*: **Mitochondrial DNA diversity in two ethnic groups in southeastern Kenya: perspectives from the northeastern periphery of the Bantu expansion.** *Am J Phys Anthropol.* 2013; **150**(3): 482–491.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

21. Campbell MC, Tishkoff SA: **The evolution of human genetic and phenotypic variation in Africa.** *Curr Biol.* 2010; **20**(4): R166–173.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

22. Castrì L, Garagnani P, Useli A, *et al.*: **Kenyan crossroads: migration and gene flow in six ethnic groups from Eastern Africa.** *J Anthropol Sci.* 2008; **86**: 189–192.
    PubMed Abstract

23. Currie TE, Meade A, Guillon M, *et al.*: **Cultural phylogeography of the Bantu Languages of sub-Saharan Africa.** *Proc Biol Sci.* 2013; **280**(1762): 20130695.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

24. Munro JF: **Migrations of the Bantu-Speaking Peoples of the Eastern Kenya Highlands: A Reappraisal.** *J Afr Hist.* 1967; **8**(1): 25–28.
    Publisher Full Text

25. Owili J: **The peoples of Kenya up to the 19th century.** *Offline Digital Library.* 1–5; (Accessed 15th August 2018).
    Reference Source

26. Comas D, Bosch E, Calafell F: **Human Genetics and Languages.** *eLS.* John Wiley & Sons Ltd. 2008.
    Publisher Full Text

27. Quintana-Murcim L: **Genetic, Linguistic and Archaeological Perspectives on Human Diversity in Southeast Asia.** *Am J Hum Genet.* 2002; **71**(5): 1253–1255.
    Publisher Full Text | **Free Full Text**

28. Shiue I, Samberg L, Kulohoma B, *et al.*: **2014 Future Earth Young Scientists Conference on integrated science and knowledge co-production for ecosystems and human well-being.** *Int J Environ Res Public Health.* 2014; **11**(11): 11553–11558.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

29. Sun H, Zhou C, Huang X, *et al.*: **Correlation between the linguistic affinity and genetic diversity of Chinese ethnic groups.** *J Hum Genet.* 2013; **58**(10): 686–693.
    PubMed Abstract | **Publisher Full Text**

30. Reesink G, Singer R, Dunn M: **Explaining the linguistic diversity of Sahul using population models.** *PLoS Biol.* 2009; **7**(11): e1000241.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

31. Pierron D, Razafindrazaka H, Pagani L, *et al.*: **Genome-wide evidence of Austronesian-Bantu admixture and cultural reversion in a hunter-gatherer group of Madagascar.** *Proc Natl Acad Sci U S A.* 2014; **111**(3): 936–941.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Open Peer Review

Current Referee Status: ✔ ✔

Version 2

Referee Report 24 October 2018

doi:10.21956/wellcomeopenres.16146.r33899

✔ **Nicola J. Mulder** iD

Computational Biology Group, Department of Integrative Biomedical Sciences, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

I am still concerned that the content is a bit thin for a research paper, but as a short research note perhaps it is sufficient. I suggest the abstract is updated to not just mention cautions but to add a couple of sentences about what the paper aimed to do and the key findings before the cautionary comments.

Competing Interests: The author mentions that he was funded by H3ABioNet at the time of the work. I am PI of the H3ABioNet network, though was not aware of this specific project prior to the review.

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.

Referee Report 02 October 2018

doi:10.21956/wellcomeopenres.16146.r33898

✔ **Michèle Ramsay** iD

Sydney Brenner Institute for Molecular Bioscience, Division of Human Genetics, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

The new title is appropriate and the paper is much improved.

This is a single author paper so the attribution to "we" should be "I". Please check throughout or revise sentences.

Introduction:
First paragraph: It is unclear what "80% (80 million) of all variants" in dbSNP is based on. The previous sentence refers to 88 million variants. Reference 2 was published in 2015 and therefore is no longer current as dbSNP has increased considerably.

Some minor editing would be beneficial
Introduction second paragraph: Second last sentence requires revision.

Third paragraph: line 11 from the bottom of the page – "population" should be "populations" may reveal…

The multi-ethnic genetic-association studies, like those in the
H3Africa initiative, now offers (should be "offer") a unique opportunity…..

Data availability: Also include the other populations from 1000GP that were used in the study.

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.

- -

Version 1

Referee Report 06 August 2018

doi:10.21956/wellcomeopenres.15998.r33497

?

Nicola J. Mulder (iD)

Computational Biology Group, Department of Integrative Biomedical Sciences, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

This paper uses the LWK population from the 1000 Genomes dataset to demonstrate the need for considering demographic data when sampling. The paper is very thin on results for a research paper as it only really includes a PCA on a small sample set. In order for it to be a reasonable research paper it would need to include other populations and data to verify the findings. It is also not clear to me whether there is support for the ethnic origin of the samples, is this information available?

Additional comments:
- The title refers to H3Africa but the paper is about the 1000 Genomes data so I recommend that the title changes, the results are relevant to all studies, not just H3Africa.
- In the abstract and later there is mention of "titling" was this supposed to "tilting"?
- There are too many figures to demonstrate a simple point.

Though the message of the paper is important, I think it needs more work. The PCAs and the paper in general need to include more populations to verify the point.

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

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

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?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

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, however I have significant reservations, as outlined above.

Author Response 10 Sep 2018
Benard Kulohoma,

We have made the following changes to this research note paper:

We have revised the analysis to include more populations in the analysis to verify our findings. We now also include anthropology and population genetics references on the history of the Bantu migration, and the Luhya population of Western Kenya. We have made revisions to the analysis, and now include other populations that share recent genetic ancestry from the 1000GP that belong to the Niger-Kordofanian Bantoid language group for comparison.

We have made revisions to the title to encompass all studies, not just H3Africa.

We have amended the word "titling" to "tilting". We have also revised the manuscript and reduced the number of figures.

We have made revisions to the analysis, and include other populations that share recent genetic ancestry from the 1000GP that belong to the Niger-Kordofanian language group for comparison.

Competing Interests: None

? **Michèle Ramsay** iD
Sydney Brenner Institute for Molecular Bioscience, Division of Human Genetics, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

This study uses genotype data from the 1000 Genomes Project to examine population structure among the 99 individuals of the Luhya people sampled from Webuye in Kenya. It emphasizes the need for careful participant selection in disease-related genome-wide studies in African populations. Principal component analysis (PCA) and ADMIXTURE are used. I fully agree that we need to consider demographic histories when analyzing genomic data, but found the title misleading in two ways: Why focus on H3Africa as the target audience and not more generally research among Africans (and other populations) and, secondly,

how does selection of this group of Luhya people demonstrate that the sampling was less than optimal. The only suggestion in the paper, related to the title, is to request data on the ethnolinguistic origins of the grandparents during the participant selection phase – I was expecting more insight into how to include knowledge of demographic history into a sampling framework (e.g. collaborate or consult with anthropologists, linguists and historians; select participants from rural areas that have less ethnolinguistic diversity and admixture; request ethnolinguistic identification of grandparent; etc.). Sampling frameworks will of course be influenced by the aims and objectives of the research.

The study focused on a single population and no additional or neighboring populations were included in the PCA and ADMIXTURE analyses. It is therefore unclear what the three ancestral components (K=3) are likely to be and what the origins of the 5 outliers may be. The sampling appears to have been good in identifying a closely related group of individuals, which is not unexpected as they were from a single ethnolinguistic origin and geographic location. It would be very interesting to sample all 17 Luhya tribes to determine whether they are genetically distinguishable on PCA, but I appreciate that these data are not available. An understanding of their demographic history could provide some clues as to what one may expect. Perhaps refer to the recent paper on South African genomes by Choudhury et al.[1].

Currently there are good analysis programs that either rely on adjusting for ancestral component diversity or using meta-analysis when participants are from significantly different populations (e.g. GEMMA, METAL, BOLT-LMM and others). Since there is a vast amount of data on every participant in GWAS studies, PCA can effectively be used to identify outliers and to exclude them, if appropriate, or adjust for population sub-structure.

The paper would be strengthened by providing other examples and illustrating how some studies/populations have been successful at using demographic history in their sampling strategy whereas others fall short, as they have ignored this. The figures are numerous and do not all add additional value, no need to show structure plots at high K values. The structure plots would be enhanced by using a tool such as Genesis (www.bioinf.wits.ac.za/software/genesis) to ensure that the individuals are shown in the same order and that the same color is used throughout to represent the same ancestral component for the different K values.

There are several sentences that could be rewritten to improve meaning and direct repetition of whole sentences should be avoided between the abstract and paper content. Presumably the author meant "tilting the scales" and not "titling the scales"?

References
1. Choudhury A, Ramsay M, Hazelhurst S, Aron S, Bardien S, Botha G, Chimusa ER, Christoffels A, Gamieldien J, Sefid-Dashti MJ, Joubert F, Meintjes A, Mulder N, Ramesar R, Rees J, Scholtz K, Sengupta D, Soodyall H, Venter P, Warnich L, Pepper MS: Whole-genome sequencing for an enhanced understanding of genetic variation among South Africans.*Nat Commun*. 2017; **8** (1): 2062 PubMed Abstract | Publisher Full Text

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

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

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

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

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: Genetics, genomics and population genetics.

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, however I have significant reservations, as outlined above.

Author Response 10 Sep 2018
Benard Kulohoma,

We have revised the title to make it more general to encompass all studies, not just H3Africa. We have made revisions to illustrate that these considerations should be made to all multi-ethnic GWAS studies.

We have amended the manuscript and discuss the importance of multi-disciplinary approaches that enlist the knowledge of anthropologists, linguists, geneticists, and historians to improve understanding on human history and migration of populations, genetics of complex traits and adaptive variations to modern environments, and language and cultural changes.

We have made revisions to the analysis, and include other populations that share recent genetic ancestry from the 1000GP that belong to the Niger-Kordofanian language group for comparison.

We agree that current analysis program that implement standard linear regression can use principal components from PCA analysis to avoid errors due to population stratification, as well as speed-up computation. Essentially, our manuscript is aimed at highlighting population stratification; and the importance of multi-disciplinary approaches when sampling underrepresented populations of interest to put local populations in the right context and provide more detailed and accurate information for disease mapping efforts in Africa. This will provide a more granular understanding on the genetic traits associated with these populations. Although GWAS studies largely rely on self-reported data on ethnic background, which is the verified using genetic information to confirm ancestral backgrounds and exclude outliers. The exclusion of individuals may lead to insufficient representation of some populations, and disease-association studies of low prevalence or late onset conditions, such as Alzheimer's disease, would be underpowered. Taking population history into account during study design my help to alleviate these challenges.

We have revised the manuscript to provide examples of how multi-disciplinary approaches could

improve research findings.

We have revised the manuscript to scale down the number of figures. We have also redrawn the structure plot.

We have made revisions to the manuscript to remove repetitions and improve meaning. We have amended the word "titling" to "tilting".

Competing Interests: None