Inferences of African Evolutionary History from Genomic Data

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Abstract

Africa is the origin of anatomically modern humans and a continent of linguistic, cultural, environmental, phenotypic, and genetic diversity. However, African populations remain underrepresented in genetic studies, which have largely focused on individuals with European and Asian ancestry. The expansion of high-throughput “omic” technologies to interrogate multiple tissue types across many biomolecules- DNA, proteins, epigenetic modifications, metabolites, and others- has heralded a new era of investigation into African history. In this review, we summarize how some of these recent advances have been applied to contemporary sub-Saharan African populations to inform studies on human origins and adaptation.

Introduction

Archaeological and genetic studies indicate that Africa is the origin of anatomically modern humans (AMH) within the past 200 thousand years (ky), the source of the worldwide range expansion of modern humans in the past 100 ky, and contains the greatest levels of human genetic variation on a global scale [1–4]. Africa contains substantial linguistic and cultural diversity, with populations that traditionally practice various dietary and subsistence methods (e.g. agriculturalists, hunter-gatherers, and pastoralists). Geographically and ethnically diverse African populations have been exposed to distinct selective pressures through dramatically different environments, climates, diet, and pathogen exposure. Some of these genetic adaptations are known to play roles in diet (e.g. amylase copy number variation, lactase persistence, bitter taste perception) and diseases (e.g. hemoglobinopathies) [2]. Despite the wide spectrum of phenotypic and genotypic diversity, Africans, particularly sub-Saharan Africans (SSA), remain underrepresented and understudied in analyses of adaptive traits and genetic variation. This

research deficit, in turn, has produced gaps in the understanding of African disease susceptibility and population history.

As the development and cost of sequencing technologies continues to decrease and the sophistication of computational models increases, researchers are able to decipher sub-Saharan genetic and biological structure and function to understand human evolutionary history on a finer and more cohesive scale than ever before. This review summarizes how omics-technologies (specifically genomics, methylomics and microbiomics) and analyses of SSA shed light on 1) AMH origins and archaic human introgression; 2) Patterns of diversity and substructure in contemporary SSA populations and 3) understanding SSA genetic adaptations and disease.

Using “-omics” to understand Human Origins in SSA

As AMH left Africa and migrated through Eurasia they encountered now-extinct archaic populations (i.e., Neanderthals and Denisovans), with gene flow events occurring between AMH and archaic populations at least once and potentially multiple times from 37-96 thousand years ago (kya) [5–8]. As a result, ~2-6% of the genomes of non-African individuals derive from Neanderthals [9] or Denisovans [10]. However, less is known about archaic admixture in early modern human groups residing in SSA. AMH fossils from the Herto [11,12] and Omo Kibish [13] sites date to ~160 kya (Figure 1). These AMH fossils overlap with other Pleistocene fossils from transitional *Homo* genera that show a range of archaic and modern traits. These transitional fossils support the coexistence of AMH with other, morphologically differentiated forms of archaic hominins until ~35 kya [14–16].

Buttressing fossil evidence [14,15], various genetic studies have proposed that anywhere from 2-5% of African genomes were contributed from a now-extinct taxon of the *Homo* genus

[17–21], and that archaic lineages may have persisted as late as 25-10 kya [15,19]. Direct comparison between a full archaic African reference sequence and modern African genomes would provide unambiguous evidence for interbreeding, but DNA from ancient samples remains challenging to obtain as fossils rapidly decay in the tropical environments found throughout much of SSA. Thus, indirect approaches to identify and model ancient admixture have been developed to discern signals of introgression within SSA populations [17,19,20,22]. Hammer and colleagues (2011)[19] examined multilocus DNA sequence polymorphism data from 61 non-coding regions in one West African population (Mandinka), a Central African rainforest inhabiting hunter-gatherer (CAHG) population (Biaka) and southern African San populations and detected signals of archaic introgression in the Biaka and San, but not the Mandinka. Furthermore, approximate-likelihood statistical models, with and without gene flow from archaic populations, were used to infer that ~2% of genetic material found in contemporary African populations was introgressed ~35 kya from a group of archaic hominins that separated from the ancestors of AMH ~700 kya during the Lower-Middle Pleistocene [19].

Lachance et al. (2012) [20] conducted high-coverage whole-genome sequencing on fifteen hunter-gatherers across three ethnic groups: CAHG from Cameroon characterized by a short-statured “pygmy” phenotype, Hadza hunter-gatherers from Tanzania, and the Sandawe, a recently settled former hunting and gathering group in Tanzania. Analysis of these populations revealed overlapping introgressed genetic regions in all three populations from at least one archaic population. Using the putative introgressed haplotypes, Lachance et al. (2012) [20] noted a median time to most recent common ancestor (TMRCA) of 1.2-1.3 million years ago (mya), which is similar to the TMRCA for introgressed haplotypes in Europeans (1.1-1.2 mya) and indicates a similar timeframe of divergence of archaic populations in Africa and Europe

(Neanderthals) from AMH. Hsieh and colleagues [23] used whole genomic sequences from seven hunter-gatherers (three Baka, four Biaka) to statistically model isolation and gene flow events. Their results revealed 265 candidate introgressed loci with a median TMRCA of 1.08 mya, which was compatible with prior ranges [20]. Furthermore, Hsieh et al. (2016)[23] found support for at least one, and possibly recurrent, admixture event(s) that favored a model of low level, frequent interbreeding between archaic and modern humans in Africa.

Future studies in this field would benefit from the inclusion of other ancient human genomes from SSA fossils and larger samples of modern human genomes from diverse SSA populations. Recently, the whole genome of a 4 kya AMH fossil from Ethiopia was sequenced [24]. While informative for reconstructing recent events, analyses of more ancient fossils will be necessary to infer ancient introgression events. Screening for introgressed variants that are adaptive, as has been done with Neanderthal variants in extant European and East Asian populations [7,8,25], may reveal if archaic alleles played a role in adaptation in SSA populations.

Patterns of diversity and substructure in SSA

The patterns of genetic diversity in SSA populations reflect their complex demographic history of short- and long-range migration events, subdivision and admixture, as well as local adaptation to a diverse array of environments. The genetic diversity of African populations parallels their linguistic diversity, with more than 2,000 languages spoken across the continent. The majority of these languages belong to four major families: Niger-Kordofanian, which includes Bantu languages and predominates throughout most of SSA; Afroasiatic, which is spoken in Saharan, northeastern and eastern Africa; Nilo-Saharan, which is spoken in portions of Saharan, eastern, and northeastern Africa; and Khoesan, a family of languages denoted by their

click consonants and spoken by the San in southern Africa and the Hadza and Sandawe in eastern Africa [26] (Figure 1).

The International HapMap Project [27] and the Human Genome Diversity Project (HGDP) [28] represent some of the earliest large-scale efforts by research consortiums to catalog human genetic diversity. More recently, the international “1000 Genomes Project” (2010) [3] has included an expanded range of populations to compile a global reference of human genetic variation. Although twelve African populations are represented between these projects, with overlap of Nigerian Yoruba in all three, most of the African populations are of recent Niger-Kordofanian ancestry and do not reflect the diversity present in Africa. The African Genome Variation Project (AGVP) has ongoing research to conduct dense genotyping and whole-genome sequencing across individuals belonging to ten language subgroups in sub-Saharan Africa; however, their low coverage genomic sequencing (4x coverage) risks misclassifying rare variants [29]. In addition, statistically imputing genetic variants from low coverage genome data, and from genetically sub-structured African populations, can be challenging. The largest high coverage whole genome sequencing data set, representing 15 hunter-gatherer individuals from three ethnic groups, identified millions of novel genetic variants [20]. Thus, more extensive high coverage whole genome sequencing of ethnically diverse Africans is warranted.

There have been efforts to expand studies of genome-wide genetic markers to include broader groups of sub-Saharan African populations. Analysis of genetic substructure based on genome-wide microsatellite and insertion/deletion markers in 121 geographically diverse African populations identified fourteen ancestral population clusters that were correlated with self-described ethnicity and shared cultural or linguistic features [26]. The results of this study and others suggested that African populations have maintained a large and subdivided population

structure throughout much of their evolutionary history (reviewed in Hirbo, Ranciaro, and Tishkoff 2012 [30]). More recent genome wide SNP genotyping studies have largely supported these observations. Principal components analysis indicates that, generally, SSA populations cluster based on geographic distribution [26,31–34] with some exceptions. For example, the CAHG cluster near the southern African San, suggesting that they may have ancient shared common ancestry [26,35]. There has also been extensive admixture in African populations [26,36]. Much of this admixture is driven by the Bantu expansion, which marked a major human migration in the last 4,000 years from west Africa throughout SSA (for a review see Pedro Soares et al. 2016 [37] (Figure 1). The Bantu migration initiated around the Nigeria/Cameroon highlands and moved to the east and then to the south of the continent (reviewed by Hirbo, Ranciaro and Tishkoff 2012 [30]), resulting in the expansion of agricultural practices, and the displacement, replacement, or admixture with other populations in many regions of SSA [26,32,38].

Several studies of SSA populations have included analysis of African groups that currently, or until recently, have practiced hunting and gathering subsistence methods. Autosomal sequencing and SNP array data indicate that the genetic lineages of Khoesan populations of southern Africa are basal to other populations, with an estimated divergence time around 157 - 108 kya [20,35,39–41]. Furthermore, genome-wide SNP array studies indicate extensive population substructure in southern Africa [33,34,41]. A recent analysis of genome-wide SNPs from 21 southern African populations indicate that this structure was the byproduct of geographical and ecological barriers around the Kalahari Basin [33]. Pickrell et al (2012) [41] inferred that the southern Khoesan speaking San split into two geographic groups in the northwestern and southeastern Kalahari ~30 kya. Challenging this observation, a recent study of

whole-genome SNP data from 46 African populations found evidence for at least three genetically divergent Khoesan groups (northern, central, and southern Kalahari), dating their divergence to ~33 kya [34]. The CAHG populations diverged from the San ~48 - 60 kya [35,42] and the Hadza and Sandawe diverged from other populations > 35 kya [43].

Adaptation in sub-Saharan Africa

African populations have adapted to a broad range of environments and diets and are likely to have regional- or population-specific adaptive traits. Many statistical approaches have been developed to detect recent selective sweeps (i.e. iHS and EHH) or local adaptation (i.e. F_{ST} , LSDL, PSB, XPCLR) (reviewed in Scheinfeldt and Tishkoff, 2013 [44] and Fan *et al.*, 2016 [45]). If these targets of selection fall within a genomic region associated with a putative function, the adaptive phenotype can potentially be inferred. Functional significance of an adaptive variant can be determined by *in vitro* studies of gene expression, in the case of regulatory variants, *in vivo* analyses in model organisms (for example, a genetic variant at *SLC24A5* associated with light skin in non-Africans was identified in zebrafish [46]), or by genotype/phenotype association studies. Some prominent examples which integrate genome-wide scans of selection with genotype/phenotype association studies or other functional assays in Africans include bitter taste perception [47], adaptation to diverse diets [43,48–50], high-altitude adaptation [51,52], short stature in CAHG [20,53,54], and immune response [39,55–60], a few of which are discussed here in greater detail.

Genome-wide scans for selection have revealed extended haplotype homozygosity on chromosome 2 in the region containing lactase persistence (LP) associated alleles in the genomes of Europeans and Africans [61,62]. LP confers the ability to digest lactose (the sugar present in

milk) during adulthood, giving a selective advantage to those individuals who can consume dairy products as a source of nutrition and hydration. Four alleles associated with LP have been demonstrated to be functional, and they are located in a regulatory region in intron 13 of the *MCM6* gene, an enhancer of the lactase gene (*LCT*) [63,64]. The variant *T-13910* is common in Europe but is also present at low levels in the Middle East and in Central and Western Africa where it was introduced due to migration [49,61]. In eastern/northeastern Africa, three functionally relevant genetic variations have been identified: *C-14010*, *G-13915* and *G-13907* [50,65–67]. The presence of African and European LP-associated variants on different haplotype backgrounds shows that the LP trait has evolved independently multiple times, a classic example of convergent evolution [66].

Genomic scans for selection have also been applied to complex traits that are influenced by multiple genetic variants and environment, such as stature. For example, the short stature phenotype of CAHG populations has been proposed as an adaptive phenotype for a tropical forest environment [20,53,54,68–70]. Jarvis et al (2012) [53] genotyped CAHG from Cameroon and neighboring Bantu groups using a genome-wide SNP array and identified signatures of positive selection in CAHG for regions enriched in genes involved with immune response, reproduction, thyroid function, and body size. A subset of these loci (e.g. *DOCK3* and *CISH*) was previously associated with stature. Lachance et al (2012) [20] looked for differentiated regions of the genome in CAHG using high coverage whole genome sequence data. Genes in the top differentiated regions include *TRHR*, which is expressed on the surface of anterior pituitary cells and has an important role in thyroid function, and *HESX1*, which encodes a homeobox-containing transcriptional repressor that plays a critical role in development of the anterior pituitary, the site of growth hormone synthesis and secretion. Other targets of selection were near

genes involved in immunity, metabolism, fertility, and olfaction. Perry et al. (2014) [54] genotyped Batwa CAHG and neighboring Bakiga agriculturalists from Uganda using a genome-wide SNP array. Consistent with results from Jarvis et al. (2012) [53] and Lachance et al. (2012) [20], Perry and colleagues (2014) [54] found several potential signals of selection, including a 15 Mb region on chromosome 3. They identified sixteen genomic regions marginally associated with short stature. These results did not replicate in a group of western CAHG (Baka), leading Perry et al. (2014) to suggest that the “pygmy” phenotype may be an example of convergent evolution in western and eastern CAHG groups [54]. The identification of loci associated with short stature in CAHG, and the question of convergent evolution in western and eastern CAHG populations remains an ongoing area of investigation.

Two recent studies [70, Soi *et al.*, unpublished] investigated patterns of methylation across the genome (e.g. “methylome”) of CAHG and Bantu groups. These studies revealed differentially methylated loci near genes that play a role in immunity, glucose and lipid metabolism, fatty acid, bone growth, and stature. They also identified genetic variants influencing differential methylation (known as methylation quantitative loci, or meQTLs) in genes involved in insulin metabolism, bone-mineral density, and height. Gopalan et al. (2016) [72] found patterns of methylation in African hunter-gatherers (Baka CAHG and San) that could be useful for inferring age, particularly in geographic regions where age is often unknown. Taken together, these epigenomic studies highlight how genetic variation and environment can influence methylation patterns, which in turn can influence adaptive trait variation.

Lastly, gut microbiome studies in SSA are informative for distinguishing how humans and bacteria have co-evolved. These studies have indicated that gut microbiomes of hunter-gatherers are generally distinct from western populations and are abundant in microbiota that

may be particular to their traditional diets [73–75]. Metagenomic analyses from Tanzanian Hadza hunter-gatherer gut microbiomes showed an enrichment of broad-spectrum carbohydrate metabolizing microbes, which could be an adaptation to their diet which is high in tubers [76]. In CAHG populations from Cameroon, Morton et al. (2015) [77] found associations between infection with gut parasites and particular gut microbial composition. Gomez et al. (2016) [78] analyzed metabolites and gut microbiota samples from Central African Republic populations, and showed that while the gut microbiomes of CAHG (BaAka) and Bantu generally looked distinct from those of U.S. Americans, the settled, agriculturalist Bantu had gut microbiome profiles that were more similar to U.S. guts than those of the BaAka [78]. While these studies indicate that subsistence practice and pathogen infection shape the microbial composition of the gut, expanding this research to include more studies of ancient human microbial communities could elucidate differences between modern and ancient microbe structure and function, evidencing adaptations to changing environmental pressures and dietary shifts during long term human evolution.

Conclusion and Future Directions

Advances in “omics” technologies are generating an unprecedented level of knowledge about human genomes and physiology, disease susceptibility, and evolutionary history, but their application to SSA populations is still disproportionately small. The analysis of large sample sizes in diverse African populations is becoming increasingly accessible and financially feasible with next-generation “omics” approaches. Integrating multiple methods to detect local adaptation to diverse environments, such as GWAS and genome-wide scans for selection takes advantage of the variable environments, demography, and population structure present in SSA. There is a

substantiated need to develop better demographic models that provide accurate estimates of divergence times, migration events, and fluctuations in population size in African populations as well as the ability to discern these genome-wide demographic effects from locus-specific selection. Combining these approaches, together with novel integrative genomics studies that incorporate data from proteomics, metabolomics, transcriptomics, epigenomics and microbiomics, will shed light on the complex demographic and adaptive history of sub-Saharan African populations.

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Figure

Figure 1. Map of Africa showing the distribution of the major language families, the location of hominid remains discussed in the text, and major migration routes of AMH through the continent within the past 10 ky.

The Bantu expansion started around 5-4 kya from Cameroon/Nigeria border, initially from west to east, either north or south of the rainforest, and to the Great Lakes of Uganda by around 3 kya and then from east to south in the last 2.5 ky, rapidly expanding into central and southern Africa, reaching Mozambique ~1.8 kya and South Africa ~1.5 kya. Another dispersion occurred ~3.5 kya from Cameroon, moving south to Angola.

The spread of pastoralism into sub-Saharan Africa occurred around 4.5 kya (for a review see Pedro Soares et al. (2016) [36]. Agropastoralist, Afroasiatic populations migrated from Ethiopia into Kenya and Tanzania within the past 5 ky [42]. After admixing with Bantu groups, pastoralist populations from eastern Africa migrated through Tanzania to southern Africa around 2.4 kya [77].

Migrations through the Sahel occurred bidirectionally between east and west Africa in the past 8 ky [27,30].

There are many fossil sites of archaic and early AMH populations in Africa. A handful of sites important to discussions on human origins and archaic introgression are listed here.

Herto: The well-preserved cranial remains of three individuals, dated to ~160 kya, were recovered at Herto Bouri, near the Middle Awash of Ethiopia. The crania are robust, consistent with other large archaic subspecies of *Homo* fossils found in Africa, but have endocranial morphology and facial features typical of modern *Homo sapiens*; thus they're thought to represent an extinct subspecies of *Homo sapiens*.

Mota: The first ancient DNA analysis from Africa came from a ~4.5 ky old fossil found in the Mota Cave in the Ethiopian highlands. This fossil set the timing of reverse gene flow from Eurasia into Eastern Africa AMH ~1.5 ky earlier than prior estimates.

Omo Kibish: Two crania, named Omo 1 and Omo 2, as well as post-cranial skeletal elements were found near the Omo River in Ethiopia. Dated to 195 kya, they possess a cranial vault height similar to AMH, situating them as early members of the *Homo sapiens* lineage. Of the two crania, Omo 1 has stronger morphological similarity to modern humans.