Evidence of population specific selection inferred from 289 genome sequences of Nilo-Saharan and Niger-Congo linguistic groups in Africa

Julius Mulindwa[1], Harry Noyes[2], Hamidou Ilboudo[9,11], Oscar Nyangiri[1], Mathurin Koffi[4], Dieudonne Mumba[5], Gustave Simo[6], John Enyaru[1], John Chisi[7], Martin Simuunza[8], Pius Alibu[1], Vincent Jamonneau[9], Annette Macleod[10], Bruno Bucheton[9,11], Christiane Hertz-Fowler[2], Issa Sidibe[3], Enock Matovu[1] for the TrypanoGEN Research Group, as members of The H3Africa Consortium.

Abstract

Background

There are over 2000 genetically diverse ethno-linguistic groups in Africa that could help decipher human evolutionary history and the genetic basis of phenotypic variation. We have sequenced 300 genomes from Niger-Congo populations from six sub-Saharan African countries (Uganda, Democratic Republic of Congo, Cameroon, Zambia, Ivory Coast, Guinea) and a Nilo-Saharan population from Uganda. Of these, we analysed 289 samples for population structure, genetic admixture, population history and signatures of selection. These samples were collected as part of the TrypanoGEN consortium project [1].

Results

The population genetic structure of the 289 individuals revealed four clusters, which correlated with ethno-linguistic group and geographical latitude. These were the West African Niger-Congo A, Central African Niger-Congo B, East African Niger-Congo B and the Nilo-Saharan. We observed a spatial distribution of positive natural selection signatures in genes previously associated with AIDS, Tuberculosis, Malaria and Human African Trypanosomiasis among the TrypanoGEN samples. Having observed a marked difference between the Nilo-Saharan Lugbara and Niger-Congo

populations, we identified four genes (*APOBEC3G*, *TOP2B*, *CAPN9*, *LANCL2*), which are highly differentiated between the two ethnic groups and under positive selection in the Lugbara population (_iHS –log p > 3.0, Rsb –log p > 3.0, Fst > 0.1 bonferroni p > 1.8x10e4).

Conclusion

The signatures that differentiate ethnically distinct populations could provide information on the specific ecological adaptations with respect to disease history and susceptibility/ resistance. For instance in this study we identified *APOBEC3G* which is believed to be involved in the susceptibility of the Nilo-Saharan Lugbara population to Hepatitis B virus infection.

Keywords

Whole genome sequencing, Sub-Saharan African genomes, population genetic variation, signatures of selection

Background

The African continent's ethno-linguistic groups have been classified into four major families, Afro-Asiatic, Nilo-Saharan, Niger-Congo, and Khoisan [2]. The Afro-Asiatic which includes the Semitic, Cushitic, and ancient Egyptian languages, is spoken predominantly by northern and eastern African pastoralists and agro-pastoralists; the Nilo-Saharan, which includes the Central Sudanic and Eastern Sudanic (Nilotic) languages, is spoken predominantly by eastern and central Saharan pastoralists; the Niger-Congo languages are subdivided into the Niger-Congo A in West Africa and the Niger-Congo B or Bantu in Central, Eastern and Southern Africa [3, 4]. Fourteen ancestral population clusters, which correlate with shared cultural and

linguistic affiliations, have been identified amongst these groups [5]. These 14 ancestral populations further subdivide into over 2000 ethnically diverse linguistic groups [6, 7].

The genetic diversity of ethno-linguistic groups can be used to study human evolutionary history and the genetic basis of phenotypic variation [5] and complement studies of African genotype variations [5, 8-10] that have contributed to the understanding of human origins and disease susceptibility markers. However, samples from sufficient individuals for population analysis have been sequenced from relatively few African populations. The 1000 genome project generated data from five Niger-Congo populations, The African Variome project added Afro-Asiatic populations and there have been small scale studies of the Khoisan hunter-gatherers [5, 8, 11, 12]. To date, no sequences of Nilotic populations have been published, although one previous study used 200,000 SNP loci to examine genetic diversity of the Nilo-Saharan speaking population of southern Sudan Darfurian and Nuba peoples [13]. In the present study we present the first genome sequences of a Nilo-Saharan population and genome sequences from six new Niger-Congo populations.

Results

Samples and sequencing

The samples used for this study were collected by the TrypanoGEN consortium and consisted of 300 individuals from 17 linguistic groups who were residents of Guinea, Ivory Coast, Cameroon, Democratic Republic of Congo, Uganda and Zambia (Table 1, Additional file 1: Table_S0). DNA was extracted from blood and genomes were sequenced on the Illumina 2500 platform at 10X coverage, except for the Zambia and Cameroon samples that were sequenced at 30X coverage.

77 Following mapping and SNP calling, we identified approximately 34.1 million single
78 nucleotide polymorphisms (SNPs) and 5.3 million insertion/deletion (Indel)
79 polymorphisms (Table 2). We identified 2.02 million variants that did not have rsIDs
80 and were considered 'novel'. The SNPs had a transition-transversion ratio of 2.0
81 (Additional file 2: Figure S1), implying good quality SNP calls [14, 15]. Prior to
82 population analysis, variants (SNPs and Indels) were filtered by removing loci with
83 >10% missing data, Minor Allele Frequency (MAF) < 0.05 or Hardy Weinberg
84 Equilibrium (HWE) P-value < 0.01. Eleven individuals with > 10% loci missing were
85 removed from the original 300 individuals dataset (Table 2), leaving 289 samples for
86 downstream analysis. An additional 504 samples from five African populations in the
87 1000 genomes project (Esan and Yoruba from Nigeria, Mende from Sierra Leone,
88 Mandinka from Gambia and Luhya from Kenya), were included in some of our
89 analyses.
90
91 **Population stratification by Multiple Dimensional Scaling**
92 Multiple Dimensional Scaling (MDS) implemented in Plink 1.9 software was used to
93 help visualise genetic distances between samples (Figure 1). All TrypanoGEN
94 samples clustered by country except those in Uganda, where the Nilo-Saharan
95 Lugbara samples formed a distinct cluster from the Niger-Congo B Basoga samples.
96 When the samples from the six TrypanoGEN and the four African 1000 genomes
97 project countries were merged, five groups representing five major geographic groups
98 were observed (Figure 1B): the Uganda Nilo-Saharan; East African Bantu speakers
99 from Uganda and Kenya; Central African Bantu speakers from Cameroon, DRC and
100 Zambia; Nigerian Niger-Congo A speakers (Esan and Yoruba); West African Niger-
101 Congo A speakers from the Ivory Coast, Gambia, Sierra Leone and Guinea. The

African and European samples were very distinct (Figure 1C). Since all samples except Ugandan Bantu and Nilo-Saharan clustered by country by MDS, samples were grouped by country for subsequent analyses except for the Uganda samples, which were grouped by both country and linguistic group.

Population Admixture and differentiation

The amount of shared genetic ancestry within the samples was estimated using the Admixture software [16]. Admixture was run on 2-8 population clusters (K) in triplicate; with K=4, K=5 and K=6 having the lowest cross validation errors and hence the most probable numbers of ancestral components represented in the data (Additional file 2: Figure S2). At K=6 the Niger-Congo populations exhibited 17-60% admixture with minor ancestries, whilst the Ugandan Nilo-Saharan population had a platry 7% admixture with Niger-Congo ancestries (Figure 2A). At K4 one European and three ancestral African populations were observed, which corresponded to Nilo-Saharan, Niger-Congo-B (East African) and Niger-Congo-A (West African). At K5 a homogeneous group of seven samples emerged within the Zambia population with no admixture with other populations in our data set and were also outliers on the MDS plot (Figure 1B). These seven were recorded as Soli/Chikunda speakers, which are Bantu languages but they had no admixture at (K=5 and K=6) with the other speakers of this language group from Zambia or any other group included in this study, suggesting that they had a quite distinct ancestry. At K6, a major group appeared that contributed ancestral components to both East African Niger-Congo B and West African Niger-Congo A but did not correspond to any existing linguistic group.

Genetic variation within the populations that are part of the TrypanoGEN project was estimated using the pairwise F_{ST} [17] (Figure 2B, Additional file 2: Figure S3). F_{ST}

was relatively high between the Nilo-Saharan Lugbara samples and the African Bantu populations (Figure 2B) except the East African Basoga (population mean F_{ST} = 0.012) and Luhya (population mean F_{ST} = 0.011), presumably due to the 30% admixture of Nilo-Saharan origin within these populations. The pattern of the observed genetic variation was consistent with the relative geographic distance from the Nilo-Saharan population (Figure 2C). In addition, a classification based on the genetic distances between populations (F_{ST}) showed clustering of populations by geographic region on the African continent (Figure 2D).

Population size over time and timing of population isolation.

The Multiple Sequentially Markovian Coalescent (MSMC) method was used to estimate population sizes over time and times at which populations became isolated (Figure 3). Effective population sizes (N_e) were relatively stable at around 13,000 in all populations tested from 100 thousand years ago (kya) until about 50kya when they started to decline reaching a nadir of about 8,000 about 13kya coinciding with the dry period at the end of the last ice age (Figure 3A, Additional file 3: Table S1). All population sizes increased rapidly thereafter but the Niger-Congo populations increased to an N_e of around 200,000, whilst the Nilotic population only increased to 60,000. The Ugandan Bantu population was intermediate in N_e presumably due to admixture with the Nilotics. This post glacial population increase was briefly reversed in the Central and West African populations which suffered declines of 6-23% between 1500 and 750 years ago before recovering to even higher levels at the present time. This absolute decline in N_e was not observed in the Ugandan Bantu population, although the growth rate declined. In the Nilotic population, a decline was observed at a later time point after 750 years ago.

152 Population separation data is less clear and may be more sensitive to admixture
153 (Figure 3B). The Guinea and Ivory Coast populations were the least admixed and
154 appeared panmictic until about 10kya, and had become isolated by about 3kya. The
155 Ugandan Bantu and Ugandan Nilotic appeared to begin separating from other
156 populations about 23 and 47kya, respectively and became isolated about 3kya but
157 these estimates may be confounded by admixture.
158
159 **Genome-wide screen for extended haplotypes under selection**
160 ***Signatures within population***
161 In order to identify alleles under selection pressure, we used the within population
162 Extended Haplotype Homozygosity (EHH) test [2, 18]. Similar patterns of loci with
163 extreme positive and negative iHS scores were observed across all groups (Additional
164 file 4: Figure S4A). The iHS values for all groups had an approximate normal
165 distribution (Additional file 4: Figure S4C) implying that the sizes of iHS signals
166 from different SNPs in all the populations were comparable [3, 4, 19]. The mean
167 number of loci with extreme positive and negative iHS score (–log p > 3) from all
168 groups was 8,984, Guinea had the largest number of loci with extreme iHS score
169 (11,401) and Zambia had the least (5,570) (Table 3, Additional file 5: Table S2).
170 These extreme loci were classified by the Ensembl annotation of the nearest gene.
171 Approximately 34% of these annotations were for protein coding genes; a mean of
172 3,058 SNPs in protein coding genes per population were associated with extreme iHS
173 scores. Some protein coding genes with extreme iHS SNP loci were shared between
174 different countries whereas some occurred only in a single country population
175 (Additional file 5: Table S2, sheet 'ALLpop.protein_coding'). We observed strong
176 iHS signatures in genes that have been previously identified in other African

177 populations as being under strong selection [5, 8, 19, 20]. These included ***SYT1,*** a
178 synaptosomal protein implicated in Alzheimer's disease [6, 7, 21] that was found in
179 all country populations; ***LARGE***, a glycosylase involved in Lassa fever virus binding
180 [5, 22] (Zambia, Cameroon, Ivory Coast); ***CDK5RAP2***, a microcephaly gene
181 controlling brain size [5, 8-10, 23] (Ugandan Bantu); ***NCOA1***, a transcriptional co-
182 activator associated with Lymphoma (Guinea, Ivory Coast, DRC); ***SIGLEC12***;
183 involved in immune responses [5, 8, 11, 12, 24] (Zambia, Cameroon). Using the
184 DAVID annotation database [13, 25] we observed that all of the country populations
185 had strong signals that have been implicated in communicable diseases such as
186 HIV/AIDS, Malaria and Tuberculosis which have high prevalence in Africa [14, 15,
187 26] (Table 4), suggesting an adaptive role of these genes to infection.
188 Having collected samples from Human African Trypanosomiasis (HAT) endemic
189 regions, we identified signatures that have been implicated in trypanosome infection.
190 These signatures were observed in genes overlapping the KEGG calcium signalling
191 pathway [16, 27, 28] that were previously identified mainly from mouse studies [29];
192 ***F2RL1*** (Guinea, Ivory Coast), ***GNA14*** (Zambia), ***GNAQ*** (Cameroon), ***GNAL***
193 (Guinea, Cameroon), ***GNAS*** (Zambia). The calcium-signalling pathway regulates
194 permeability of the blood brain barrier to trypanosome parasites during central
195 nervous system (CNS) disease [30]. In addition, we observed signatures in genes
196 overlapping the Mitogen-activated protein kinase MAPK pathway ***MAPK1***
197 (Cameroon), ***MAPK10*** (Ugandan Nilo-Saharan, DRC, Ugandan Bantu), ***MAPK9***
198 (Zambia); which is targeted by trypanosomatids in order to modulate the host's
199 immune response [17, 31]. These host signalling pathways have been shown to play a
200 role in host immunity against trypanosome infection in mice and cattle [32-34].
201

202 ***Signatures unique to Nilo-Saharans***

203 In order to determine which signatures are unique to the Nilo-Saharan Lugbara, we
204 first ascertained which extreme iHS loci (-log p > 3) were common to the Nilo-
205 Saharan and one or more Niger-Congo groups. We observed that approximately 15%
206 of the protein coding gene associated extreme iHS SNPs of the Ugandan Bantu, DRC,
207 Ivory Coast and Guinea groups were common with the Nilo-Saharan group, whereas
208 Cameroon and Zambian groups had 2.7% in common (Table 3, Additional file 4:
209 Figure S4B). We identified 149 extreme SNPs associated with protein coding genes
210 and were unique to the Uganda Nilo-Saharan (Additional file 6: Table S3). Using the
211 PANTHER Gene ontology database [35], we observed that these unique genes were
212 mainly enriched for cellular and metabolic process proteins (approximately 50.8%)
213 (Additional file 7: Figure S5). Amongst these were SNPs associated with genes that
214 have also been shown by other studies to be under positive selection, including
215 ***APOBEC3G,*** which is involved in innate anti-viral immunity [36, 37], with
216 demonstrated protective alleles against HIV-1 in Biaka and Mbuti pygmies of Central
217 African Republic and DRC respectively [38]; ***IFIH1*** (also called ***MDA5***), a
218 cytoplasmic RNA receptor that mediates antiviral responses by activating type I
219 interferon signalling [39] but is also implicated in protection against type 1 diabetes
220 ([40, 41]; ***OR2L13,*** an olfactory receptor involved in activation of signal transduction
221 pathway for odorant recognition and discrimination [42], and is associated with
222 Diabetic nephropathy in African Americans [43].

223

224 ***Nilo-Saharan versus Niger-Congo cross population signatures***

225 There were 299 SNPs with high F_{ST} (above 99th percentile) and XPEHH (Rsb –log p
226 > 3) in the regions of protein coding genes that were also highly differentiated

227 between the Nilo-Saharan and Niger-Congo populations (Additional file 8: Table S4).
228 We then compared SNP loci with derived alleles that are unique to the Nilo-Saharan
229 Lugbara and occur in highly differentiated genes (extreme Rsb, high Fst) between the
230 Nilo-Saharan and Niger-Congo groups. From this we identified 12 genes (Table 5,
231 Additional file 9: Figure S6D) including the ***APOBEC3G*** gene, that are highly
232 differentiated between the Nilo-Saharan Lugbara and Niger-Congo groups (mean F_{ST}
233 0.11, Rsb –log p = 4.1). *APOBEC3G* also contains the SNP rs112077004, which was
234 observed to be under positive selection in the Nilo-Saharan Lugbara (Figure 4,
235 Additional file 10: Figure S7, Additional file 11: Table S5).
236
237 **Discussion**
238 We have analysed the genomes of 289 individuals from seven populations from six
239 Sub-Saharan Africa Countries, investigating their admixture profile, demographic
240 histories and signatures of selection that differentiate the major linguistic groups. The
241 MDS analysis identified five major clusters: Nilo-Saharan, two Niger-Congo A
242 groups from Nigeria and West Africa and two groups of Niger-Congo B (Bantu
243 speakers) from Central and East Africa, which were consistent with previous studies
244 [5, 8, 44]. The samples represented three of the five major linguistic groups in Africa,
245 omitting the Afro-Asiatic and Khoisan speakers. Afro-Asiatic speakers are found
246 across North and North-East Africa in regions adjacent to Nilo-Sharan and Bantu
247 speakers. Afro-Asiatic reference populations were not included in this study and we
248 therefore could not detect any admixture from this source. A SNP genotype based
249 analysis of Nilotic populations indicated that Nilotic populations only contain a trace
250 of Afro-Asiatic ancestry and therefore our observations on East African populations
251 may not be significantly limited by the absence of Afro-Asiatic data [13].

Linguistic analyses suggest that Niger-Congo speaking hunter-gathers originated from the Kordofanian speakers of the Nuba mountains of Sudan and then traversed the Sahel to Mali (Figure 5). They colonised the coast from Senegal to Nigeria and Cameroon over several thousand years, forming multiple linguistic groups. The Bantu (Niger-Congo-B) speaking people emerged as another linguistic group amongst the greater than 60 Niger-Congo-A groups in the Nigeria/Cameroon region about 3,000 years ago. Bantu speaking peoples then spread South-East along savannah corridors through the Congo basin and emerged in the Great Lakes region and spread North to the Lake Victoria region and South down the East Side of Africa[10, 45, 46]. This rapid expansion is believed to have been enabled by the development of agriculture and later enhanced by the acquisition of iron tools [5].

Our admixture analysis at K=4 was consistent with this linguistic history and recent genetic analyses [8, 10] with three African ancestral allele clusters (AAC) which can be interpreted as representing Niger-Congo A languages in West Africa, Niger-Congo B (Bantu) in Central and East Africa and Nilo-Saharan in Northern Uganda. The Niger-Congo-A speakers in extreme West Africa appear to have approximately 10% Nilo-Saharan ancestry and this declines towards the East. On the other hand, the Bantu speakers are a mix of Niger-Congo-A and a distinct putative Bantu ancestral cluster that it at highest frequency in Nigeria and Cameroon, despite the Nigerian Yoruba and Esan not being Bantu languages. The Niger-Congo-A component is displaced by a Nilotic component with easterly latitude whilst the “Bantu” component remains constant. At K=5 a small AAC of 7 Bantu speakers from Zambia emerges, who evidently have a genetic heritage that does not match their self-declared linguistic affiliation, and are of unknown descent. At K=6 a fourth major African AAC appears (green in Figure 2) with strongest representation in the Nigerian Yoruba

277 and Esan then tapering off east and west into Central and West Africa. This does not
278 correspond to any linguistic group and displaces the Niger-Congo-A ancestry
279 observed at K=4 and 5, to the east of Nigeria and Niger-Congo-B (Bantu) in Nigeria
280 and to the West. This ancestral cluster could represent a secondary movement out of
281 Nigeria of migrants who adopted their host's language. One possible driver for such a
282 migration, if it occurred, was the development of iron smelting which may have
283 originated in Nigeria about 2,500 years ago [47]. Irrespective of the true number of
284 ancestral allele clusters there is evidence of back migration of people with Bantu
285 ancestral alleles into West Africa as has been observed before [44]. Interestingly this
286 migration to the west was not accompanied by language expansion as it was to the
287 east.
288 Regarding population history, the estimates of current N_e obtained from our data with
289 MCMS (Figure 3A) of around 200,000 in West and Central Africa and 57,000-
290 125,000 in East Africa (Supplementary Table S6) was consistent with previous
291 observations on other African samples using the same method [48] [12], but our larger
292 dataset provides higher resolution at recent time points than a previous analysis [12].
293 The faster growth in the Niger-Congo A and B than the Nilotic populations appears to
294 predate the Bantu expansion. The Niger-Congo A population was believed to be
295 expanding through West Africa as the climate became wetter after 10kya, consistent
296 with the separation times between the Guinea and Ivory Coast populations observed
297 on the Cross-Coalescence Plot (Figure 3B). The Nilo-Saharan population developed a
298 pastoralist economy probably after 6kya but their expansion into the tsetse belt may
299 have been delayed by trypanosomiasis and other diseases until the cattle developed
300 tolerance [49-51]. The effective population size did not grow as fast as that of the
301 Niger-Congo-A populations. The brief population decline dated at ~1340CE by

302 MSMC coincides with the timing of the Black Death (1343-1353), however time
303 resolution is low and the decrease was only observed at a single time point. There is
304 evidence of abandonment of multiple large settlements throughout West Africa
305 around the time of the Black Death and there is speculation that this was caused by
306 the plague [52]. The decrease at this time appears to have impacted the West and
307 Central African Niger-Congo but not the East African populations. Both Bantu and
308 Nilotic populations in East Africa were cattle keepers and pastoralists to varying
309 degrees [51] and the concomitant lower population density and mobile lifestyle may
310 have made them less vulnerable to plague than the more settled and urbanised West
311 Africans. The more recent decline in the Nilotic Lugbara effective population size is
312 unexplained, but the catastrophic Rinderpest outbreak in the 1880's and 1890's that
313 killed up 90% of indigenous cattle, which lead to the depopulation of the East African
314 savannahs and may have ended the dominance of the Nilotic speaking Maasai over
315 the Bantu Kikuyu could have been a contributory factor [53].
316 The Cross-Coalescence plots for comparison between populations other than the
317 Guinea and Ivory Coast Niger-Congo-A show long periods of separation (not shown).
318 This is not consistent with previous observations [12] or with the Ugandan Bantu
319 populations having separated from Niger-Congo-A populations even more recently
320 than the separation between Guinea and Ivory Coast populations, and is presumably
321 due to the extensive admixture with the Nilotics observed in this population. The
322 Central African cross-coalescence data also indicated older separation times than
323 linguistic evidence suggests (not shown) and although there was less evidence of
324 admixture in this population, these data should be treated with caution.
325 We identified evidence of selection in genes that have previously been associated
326 with HIV/AIDS, Tuberculosis and Malaria. Given the high prevalence of lethal

327 infections on the continent [26], the finding of positive natural selection at disease
328 susceptibility loci is not surprising. However not all these genes occurred in all the
329 populations, demonstrating spatially varying selection probably due to differing
330 environmental pressures [54, 55]. We looked for signatures of selection in genes and
331 pathways that are implicated in trypanosome infection, including the calcium
332 signalling pathway [29, 30], the MAPK pathway [31-34], and *HPR*, *APOL1, IL6,*
333 *HLAG,* genes [56-61] (Additional file 12: Figure S8, Additional file 13: Table S6).
334 However we only found evidence for selection for the calcium signalling and MAPK
335 pathway genes. This suggests that Trypanosomiasis may have had a selective force in
336 these populations.
337 In order to determine signatures of selection unique to the Nilo-Saharan Lugbara
338 population, we used a combination of linkage disequilibrium-based method (iHS and
339 Rsb) and population differentiation based method (F_{ST}) [62]. Using this approach we
340 identified 12 loci associated with coding genes, which are unique to the Nilo-Saharan
341 Lugbara and highly differentiated from the Niger-Congo population. Among these
342 was the variant associated with *APOBEC3G* that demonstrated significant positive
343 selection in the Nilo-Saharan Lugbara population. This protein is involved in viral
344 innate immunity [63], by inducing a high rate of dC to dU mutations in the nascent
345 reverse transcripts leading to the degradation of the viral genome [36, 37]. The
346 Lugbara have relatively low prevalence of HIV (4%) in comparison to the Basoga
347 (6.4%) and Baganda (10.7%) Bantu groups of Uganda but relatively high prevalence
348 of Hepattis B suggesting that either *APOBEC3G* has different effects on each of these
349 viruses [64-69].
350 We also identified the missense variant rs10930046 (T/C) located in the *IFIH1* CDS,
351 which was unique to the Nilo-Saharan Lugbara and highly selected (iHS –log p

352 3.264). This gene is associated with up regulation of type I interferon signalling
353 occurring in a spectrum of human diseases [39] and is believed to be involved in the
354 suppression of Hepatitis B viral replication [70]. Being a non-synonymous variant,
355 rs10930046 could alter the functioning of IFIH1 and thus increase susceptibility to
356 HBV in the Lugbara population, something that could be tested by a candidate gene
357 study for DNA virus infections. Northern Uganda is considered to have one of the
358 highest prevalence of Hepatitis B virus in the world [71] which has perhaps resulted
359 in a unique adaption of the Nilo-Saharan Lugbara population to infection.
360

361 **Conclusion**

362 We have incorporated a Nilo-Saharan population into the analysis of genomic
363 sequences of Niger-Congo populations for the first time and show extensive
364 admixture between Nilo-Saharan ancestry and Niger-Congo B (Bantu) populations.
365 We show evidence for signatures of selection within the Nilo-Saharan population in
366 genes associated with infectious diseases that have different prevalence from
367 surrounding Bantu (Niger-Congo B) populations.
368

369 **Methods**

370 **Sample collection**

371 The samples used for this study are part of the TrypanoGEN biobank [72], which
372 describes ethics approval, recruitment, sample processing and the meta data collected.
373 The ethical approval for the study was provided by the national ethics councils of the
374 TrypanoGEN consortium countries involved in the sample collection.
375 The sampled populations were assigned a 3 letter code with the following criteria:
376 Country name first letter, was used as the first letter of the code; For countries where

377 more than one linguistic group was sampled, we used abbreviations of the country
378 name, ZAM-Zambia, CAM-Cameroon, CIV-Ivory Coast, DRC-Democratic republic
379 of Congo; For countries where a homogeneous linguistic group was sampled, we used
380 the Country, language group and language, UNL-Uganda Nilotic Lugbara, UBB-
381 Uganda, Niger-congo B (Bantu), Basoga, GAS-Guinea, Niger-Congo A, Soussou.
382 Peripheral blood was collected from the participants at the field sites, frozen, and
383 transported to reference laboratories from where DNA extraction was carried out
384 using the Whole blood MidiKit (Qiagen). The DNA was quantified using the Qubit
385 (Qiagen) and approximately 1μg was shipped from each country for sequencing at the
386 University of Liverpool, UK except for Cameroon and Zambia whose DNA was
387 shipped to Baylor College, USA.
388
389 **Sequencing and SNP calling**
390 The whole genome sequencing libraries were prepared using the Illumina Truseq
391 PCR-free kit and sequenced on the Illumina Hiseq2500. The samples from Guinea,
392 Cote D'Ivoire, Uganda and DRC were sequenced to 10x coverage at the Center for
393 Genomic Research at the University of Liverpool. The samples from Zambia and
394 Cameroon were sequenced to 30X at the Baylor College of Medicine Human Genome
395 Sequencing Center.
396 The sequenced reads were mapped onto the 1000 genomes project
397 human_g1k_v37_decoy reference genome using BWA. The SNP calling on all the
398 samples was done using the genome analysis tool kit GATK v3.4. The SNPs were
399 then filtered by; a) removing loci with > 10% missing SNP, b) removing individuals
400 with > 10% missing SNP loci and c) removing loci with Hardy Weinberg P value <
401 0.01. In addition, loci with MAF < 0.05 were also removed for the population

402 stratification and Admixture analysis. Variant annotation was done using snpEff
403 (www.snpeff.sourceforge.net).
404
405 **MDS analysis**
406 Population stratification was done using Multi dimensional scaling tool in Plink 1.9
407 and R v 3.2.1 tools. For this analysis, in addition to the filtering mentioned above,
408 SNP loci less than 2000bp apart were removed in order to reduce the linkage
409 disequilibrium (LD) between adjacent SNP. MDS analysis was carried out for (i) all
410 TrypanoGEN data , (ii) all TrypanoGEN data plus African 1000 genome data, (iii) all
411 TrypanoGEN data including 50 European and all African 1000 genome data
412 excluding African Caribbean in Barbados (ACB) and African Southwest USA (ASW)
413 populations.
414
415 **Population Admixture**
416 The population ancestry of each individual was obtained using Admixture 1.23 [16]
417 on the filtered PLINK .bed files on the same TrypanoGEN, one thousand genome
418 African and European population data sets analysed by MDS. Admixture was run on
419 K1 to K8 with three replicates for each run. The Admixture plots were drawn using
420 the R tool 'strplot' [73].
421
422 **Genetic diversity: F_{ST}**
423 The genetic diversity due to difference in allele frequency among populations was
424 analysed by the inter-population Wright's F_{ST} [17] in PLINKv1.9. The F_{ST} estimates
425 were made between TrypanoGEN (UNL, UBB, DRC, CIV, GAS) and one thousand
426 genome African (LWK, YRI, ESN, MSL, GWD) populations. The F_{ST} dendrogram

427 was generated using Fitch in Phylip3.685 [74]. The geographic distance matrix
428 between populations was calculated based on their global position system (GPS)
429 coordinates [75].
430
431 **Population History.**
432 Population sizes and divergence times were calculated using MSMC [48] on data
433 phased with Beagle [76]. Since PCA and Admixture analysis had indicated little
434 difference between linguistic groups in each country with the exception of the
435 Ugandan Bantu and Nilotic populations, samples from each country with highest
436 coverage were analysed together except for Uganda where Bantu and Nilotic samples
437 were analysed as separate populations. For population size estimates, output from 3
438 independent runs each using 8 different haplotypes were combined. Using 8
439 haplotypes rather than 4 gives higher resolution at more recent time points. For
440 estimates of relative cross coalescence rate, three replicate runs were done, each using
441 2 different samples (4 haplotypes) from each pairwise comparison between
442 populations. Results presented are the means of the replicates.
443
444 **Signatures of selection**
445 The estimation of haplotypes was carried out by first Phasing of the genotyped SNPs
446 using SHAPEIT v2.2 software [77]. The extended haplotype homozygosity (EHH)
447 was then analysed using the R software package *rehh* [78]. Two main EHH derived
448 statistics were calculated from the phased haplotype data, that is, intra-population
449 integrated haplotype Score (iHS) [19] and inter-population Rsb [79]. Bedtools v2.26.0
450 was used to identify the intersection of the iHS, Fst and Rsb loci.
451

Declarations

Ethical approval

The ethical approval for the study was provided by the national ethics councils of the TrypanoGEN consortium countries involved in the sample collection which are: Uganda (HS 1344), Zambia (011-09-13), Democratic Republic of Congo (No 1/2013), Cameroon (2013/364/L/CNERSH/SP), Cote d'Ivoˆıre (2014/No 38/MSLS/CNER-dkn), and Guinea (1-22/04/2013). All the participants in the study were guided through the consent forms, and written consent was obtained to collect biological specimens.

Consent of publication

Informed consent was provided by the study participants, for sharing and publishing their anonymised data.

Availability of data and material

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. The sequenced data will be submitted to the EGA by H3ABionet under the study accession number EGAS00001002602.

Competing interests

The authors declare that they have no competing interests.

Funding

The study was under the TrypanoGEN project, funded by the Wellcome Trust (099310/Z/12/Z). The funders had no roles in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Author's contributions

EM, AM, IS, BB, CHZ conceived the study; HI, MK, DM, GS, JE, JC, MS, PA, collected samples; JM and HN analysed the data, interpreted output and wrote the manuscript; All authors read and approved the final manuscript.

Acknowledgements

The authors would like to acknowledge the study participants who donated their specimens, the personnel involved in the community engagement and coordinating sample collection and processing, the National sleeping sickness control programmes of the participating Countries. Z Lombard (University of Witwatersrand) and D Adeyemo (NHGRI) for facilitating sequencing of samples from Zambia and Cameroon at Baylor College of Medicine. The H3ABionet for training and support on data analysis. Also Fiona Marshall and Rebecca Grollemund for helpful discussions of African History.

Authors' Affiliations

1. Makerere University, Kampala, Uganda
2. Centre for Genomic Research, University of Liverpool, UK
3. Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso
4. Université Jean Lorougnon Guédé (UJLoG), Daloa, Côte d'Ivoire

5. Institut National de Recherche Biomedicale, Kinshasa, Democratic Republic of Congo
6. Faculty of Science, University of Dschang, Cameroon
7. University of Malawi, College of Medicine, Department of Basic Medical Sciences, Blantyre, Malawi
8. Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
9. Institut de Recherche pour le Développement (IRD), IRD-CIRAD 177, Montpellier, France
10. Wellcome Center for Molecular Parasitology, University Place, Glasgow, UK
11. Programme National de Lutte contre la Trypanosomose Humaine Africaine, Conakry, Guinea

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843 **Tables**

844

845 **Table 1.** Table showing the Ethnic groups and number of individual from each
846 Country that were used for Whole genome sequencing. The Ethnicity codes were
847 obtained from the Ethnologue languages of the World catalogue [80].

848

Country	Code	Ethnicity	Ethnologue code	No. of Samples
Uganda	UNL	Lugbara	lgg	50
	UBB	Basoga	xog	33
Zambia	ZAM	Soli/Chikunda	sby; kdn	28
		Tumbuka	tum	14
		Bemba	bem	8
Congo	DRC	Kimbala	mdp	20
		Kingongo	noq	30
Cameroon	CAM	Bamilike	fmp	6
		Mundani	mnf	8
		Ngoumba	nmg	12
Ivory Coast	CIV	Baoule	bci	11
		Gouro	goa	21
		More	Moa	12
		Senoufo	sef	4
		Malinke	loi	1
		Koyaka	kga	1
Guinea	GAS	Soussou	sus	50

849

850 **Table 2**. The number of SNPs and Indels obtained from the mapping and variant
851 calling pipeline. The SNPs were filtered for HWE, MAF and missing genotypes
852

Pop	Samples (WGS)	Samples after filtering	SNPs before filtering	SNPs after filtering	Indels before filtering	Indels after filtering
CIV	50	50	18,780,913	16,066,827	3,069,408	1,583,594
DRC	50	49	19,188,537	16,449,696	3,146,802	1,626,826
GAS	50	47	18,831,834	16,075,002	3,063,080	1,579,352
UBB	33	32	17,671,306	14,987,699	2,889,915	1,426,646
UNL	50	44	18,986,243	15,598,629	3,130,979	1,536,490
CAM*	26	26	17,183,994	14,579,603	3,283,543	1,539,459
ZAM*	41	41	18,232,386	15,548,110	3,448,501	1,651,467
Total	**300**	**289**	**34,116,333**	**30,591,165**	**5,336,622**	**3,166,196**

* Samples Sequenced at 30X coverage, rest of the samples were at 10X coverage. Identified 2,023,049 SNPS without rsIDs {Total SNPs (30,591,165) – SNPs with rsIDs (28,568,116)}

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854

855 **Table 3.** Extreme iHS loci that overlap with the UNL population
856

Pop	Extreme iHS SNPs (–log p > 3.0)	Extreme iHS SNPS associated with protein coding genes	Extreme iHS SNPs overlapping with UNL
UNL	8454	2613	2613
UBB	9617	3326	512
DRC	10037	3790	535
ZAM	5570	1990	86
CIV	10129	3541	534
CAM	7686	2597	82
GAS	11401	3741	382

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863 **Table 4**. DAVID [25] analysis of Genes that are highly selected within TrypanoGEN population and associated with HIV, Tuberculosis, and
864 Malaria. The Fisher's exact test *P*-values indicate significant gene enrichment in the associated disease
865

Gene	Chr	Populations affected	Associated Disease	p value	Reference
HLA-DRB1	6p21.32	ZAM, CAM, CIV, DRC, UBB	HIV/TB/Malaria	1.63E-09	[81, 82]
NLRP1	17p13.2	ZAM, CIV, DRC, GAS, UBB	HIV	2.42E-07	[83]
VPRBP	3p10.6	UBB, CIV, DRC, GAS	HIV	2.42E-07	[84]
TRIM5	11p15.4	UNL, CAM, GAS	HIV	7.30E-07	[85]
ANKRD30A	10p11.21	DRC, CIV	HIV	2.42E-07	[86]
HLA-A	6p22.1	ZAM, CAM	HIV/TB	4.70E-06	[87]
HLA-DQA1	6p21.32	UBB, DRC	HIV/TB	4.70E-06	[87]
HLA-DQB1	6p21.32	UBB, DRC	HIV/TB	4.70E-06	[87]
KIR3DL1	19q13.42	UNL, CIV	Malaria	1.63E-09	[88, 89]
CD36	7q21.11	UNL, CAM, CIV	Malaria	1.55E-06	[90]
DDC	7p12.2	UBB, DRC, GAS	Malaria	1.63E-09	[91]
HBE1	11p15.4	UBB, CAM, DRC	Malaria	5.48E-07	[92]
ADCY9	16p13.3	UNL, CIV	Malaria	5.48E-07	[93]

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872 **Table 5**. Genes that are highly differentiated between the Nilo-Saharan and Trypanogen Niger-Congo populations that contain SNPs
873 unique to UNL population
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875

Chr	Gene	Position of Unique SNP	UNL unique SNP	iHS pvalue	Differentiated loci	Mean Fst	Bonferroni pvalue	Rsb pvalue
22	*APOBEC3G*	22:39453783	rs112077004	4.002	rs5757467	0.110	8.17E-23	4.116
3	*TOP2B*	3:25670166	rs11712723	3.000	rs6786520	0.115	4.80E-11	4.151
1	*CAPN9*	1:230886378	rs113802713	3.362	rs16852681	0.105	1.89E-08	5.632
7	*LANCL2*	7:55476708	rs62457872	3.019	rs3807360	0.109	1.89E-08	3.269
3	*NEK4*	3:52762698	rs11130321	3.101	rs6445535	0.111	1.51E-04	6.871
20	*GDAP1L1*	20:42907542	rs1884607	3.187	rs4810417	0.114	0.003070493	3.931
2	*NBAS*	2:15527280	rs6723183	3.763	rs4668447	0.123	0.053555484	3.020
3	*PBRM1*	3:52698560	rs12488527	3.130	rs2878632	0.113	0.053555484	6.742
17	*ZPBP2*	17:38031164	rs11658278	3.115	rs9903250	0.113	0.053555484	3.384
12	*MGAT4C*	12:86435551	rs11513957	3.134	rs1502802	0.113	0.060467781	3.520
11	*FAT3*	11:92291634	rs675654	3.063	rs2852859	0.112	0.061504234	3.582
9	*MEGF9*	9:123462573	rs75959206	3.046	rs1530370	0.113	0.061504234	4.958

876 **Figures**

877 **Figure 1**. Multi dimensional scaling (MDS) analysis of the sequenced TrypanoGEN
878 samples, Guinea (GAS), Ivory Coast (CIV), Cameroon (CAM), Democratic Republic
879 of Congo (DRC), Uganda (Nilotics, UNL, Bantu, UGB) and Zambia (ZAM), (**A**); **B**,
880 TrypanoGEN and selected 1000 genomes African samples Nigeria (ESN, YRI),
881 Sierra Leone (MSL), Gambia (GWD), Kenya (LWK); The black circles indicate the
882 clustering of the samples into the West African Niger-Congo-A, Nigerian Niger-
883 Congo-A, Central African Niger-Congo-B, East African Niger-Congo-B and the Nilo-
884 Saharan Lugbara (NSL); **C,** 1000 genomes samples from Africa and the rest of the
885 world. AAM, African Americans; AMR, indigenous Americans; CAF, Central Africa;
886 EAF, East Africa; EAS, East Asia; EUR, Europe; NSA, Nilo-Saharan; SAS, South
887 Asia; WAF, West Africa;

888

889 **Figure 2**. Genetic admixture and diversity between TrypanoGEN and selected 1000
890 genome populations. **A**. Admixture plot of the K populations of the TrypanoGEN,
891 1000 genome African and European populations. **B**. Heatmap of mean Fst between
892 TrypanoGEN and 1000 genome African populations. **C**. Polynomial regression plot
893 of the mean Fst against the relative geographical distance of the African Niger-Congo
894 populations from the Uganda Nilotic population. **D**. Phylogeographic plot of the mean
895 Fst distances on the Trypanogen populations and selected 1000 genome African
896 populations; the pie charts represent the population sample size and admixture.

897

898 **Figure 3.** Population sizes and cross-coalescence rates compiled by MSMC. **A**
899 Effective population sizes for each population since 75kya. The Ugandan Bantu and
900 Nilotic populations have grown continuously but at a slower rate than the West and

901 Central African populations since 13kya. These latter populations experienced
902 declines of 6-23% between 1500 and 800 years ago. **B** Cross-coalescence rates for
903 pairs of populations. At 1.0 populations are panmictic and at 0.0 there is no gene flow.
904 The Guinea and Ivory Coast populations were panmictic until about 10 kya and then
905 became separated by 3kya. Other populations appear to have separated more
906 gradually but these may be confounded by admixture.

907

908 **Figure 4.** Illustration of signatures unique to the Uganda Nilotic population. Signal of
909 positive selection within the *APOBEC3G* gene on Chromosome 22 at the
910 rs112077004 loci of the Uganda Nilo-Saharan Lugbara population, in comparison
911 with the Niger-Congo B populations of Uganda (UGB) and Niger-Congo A
912 population of Guinea (GAS). **A**. The calculated site specific extended haplotype
913 homozygosity (EHH) within a population. **B**. Between population Fst analysis. **C.**
914 Across population (XPEHH) analysis.

915

916 **Figure 5.** Migrations of Niger-Congo speakers. Map colours show vegetation
917 coverage approximately 10kya [94]. Colours for linguistic groups as for Figure 2.
918 Blue Nilo-Saharan; Yellow, Niger-Congo A; Red, Niger-Congo-B (Bantu); Green
919 putative expansion of an ancestral group out of modern Nigeria. Blue dotted arrow,
920 suspected route of proto-Niger-Congo-A speakers from Nuba mountains of Sudan to
921 Senegal across the Sahel [95] when it was much wetter than at present.

922

923 **Additional files**

924

925 **Additional file 1:** Table S0.xlsx. List of participant samples sequenced indicating
926 their sequencing ID, country of origin, language and sex of the individual.
927
928 **Additional file 2**: Figure S1-S3.pdf: Figure S1. Variant annotation of the population
929 whole genome sequence (WGS) data using snpEff. **a.** count of number of
930 substitutions for each base combination, **b.** count of number of Insertion-Deletions
931 with corresponding InDel length of affected loci **c.** Count of number of sites with
932 Transition-Tranversion ratios with the corresponding allele frequency, **d.** Percentage
933 count of the variant annotation classifications. Figure S2. Plot of the coefficient of
934 variation (CV) error verses the K population (number of clusters). The K population
935 with the lowest CV error is considered as the most appropriate K for the admixture.
936 Figure S3. Bar plot of the mean F_{ST} between TrypanoGEN populations.
937
938 **Additional file 3**: Table S1.xlsx. Table showing MSMC output of effective
939 populations sizes at different time points
940
941 **Additional file 4**: Figure S4.pdf. Extended haplotype homozygosity analysis of the
942 TrypanoGEN populations for signatures of natural selection. **A.** Manhattan plot of the
943 Intra-population integrated haplotype score (iHS) showing SNPs with extreme
944 positive (> 2.5) and extreme negative (< -2.5). **B.** Bar plot showing the number of
945 SNPs associated with protein coding with iHS > 3.0 in each population (black) and
946 those within each population that overlap with the UNL population (grey).
947 Distribution of the iHS scores compared by **C.** Histograms and **D.** QQ plots.
948
949 **Additional file 5**: Table S2.xlsx. List of SNPs with Ensembl annotation of the nearest

950 protein coding gene and iHS > -log p 3.0. It contains sheets for the TrypanoGEN
951 populations and combined sheet for all population genes and DAVID derived Genetic
952 associated diseases (GAD) and KEGG pathway affiliation
953
954 **Additional file 6**: Table S3.xlsx. List of signature SNPs associated with protein
955 coding genes, with _iHS > -log p 3.0 and unique to the Nilo-Saharan Lugbara
956 population.

957 **Additional file 7**: Figure S5.pdf. PANTHER Gene ontology database classification of
958 the Nilo-Saharan Lugbara unique genes based on the Biological process and protein
959 class they belong to. X-axis indicates the process and Y-axis the number of genes
960 corresponding to the process.

961 **Additional file 8**: Table S4.xlsx. List of SNPs that are highly differentiated between
962 the Niger-Congo and Nilo-Saharan Lugbara populations.

963 **Additional file 9**: Figure S6.pdf. Manhattan plot of the Rsb (**A**) and F_{ST} (**B**) against
964 the whole genome (chromosome 1 - 22) between the Nilo-Saharan Lugbara (UNL)
965 and TrypanoGEN Niger-Congo populations. **C.** Scatter plot of inter-population F_{ST}
966 against Rsb scores for the SNPs having a high F_{ST} (> 0.99 percentile) and high Rsb (-
967 log p-value > 3.0) between UGN and the Niger-Congo TrypanoGEN populations. **D**.
968 Venn diagram showing the number of intersecting protein coding SNP loci between
969 the high Rbs-Fst (UNL verses All populations (CAM, CIV, GAS, UBB, ZAM) and
970 high iHS (-log p-value > 3) protein coding loci unique to the UNL population only.

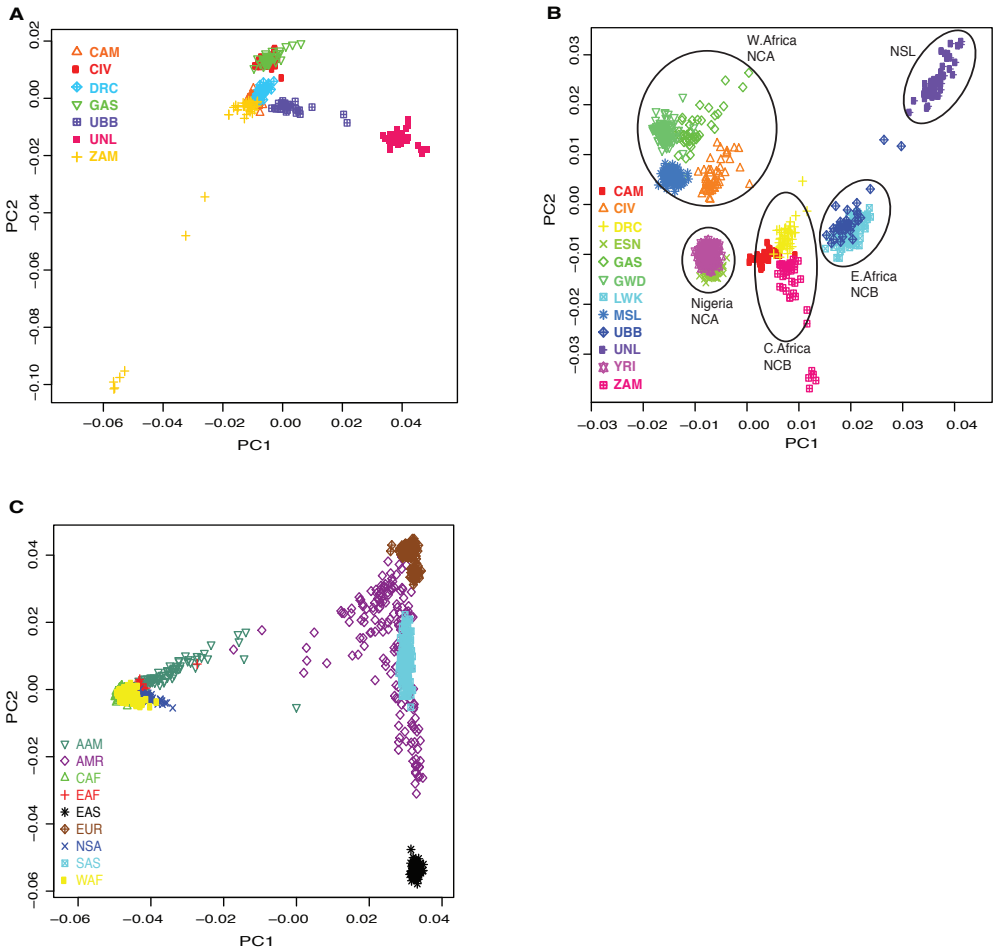
971 **Additional file 10**: Figure S7. Analysis of SNP loci in APOBEC3G gene
972 (Chr22:39480259-39485103). **A.** Plot of SNP minor allele frequency (MAF) against
973 chromosome position in the gene, the red dot is the position of rs112077004. **B.**

974 Haploview plot of Linkage Disequilibrium (LD) of haplotypes within the gene, **C.**
975 Zoomed LD plot of the SNPs upstream and downstream of the rs112077004 loci
976 which is unique to the UGN population. **D.** Ensemble gene analysis of *APOBEC3G*
977 gene locus on the GRCH37 build indicating the position of SNP rs112077004.

978 **Additional file 11**: Table S5.xlsx. Analysis of SNPs in the APOBEC3G gene
979 including the minor allele frequency (MAF) and linkage disequilibrium (LD)
980 statistics.

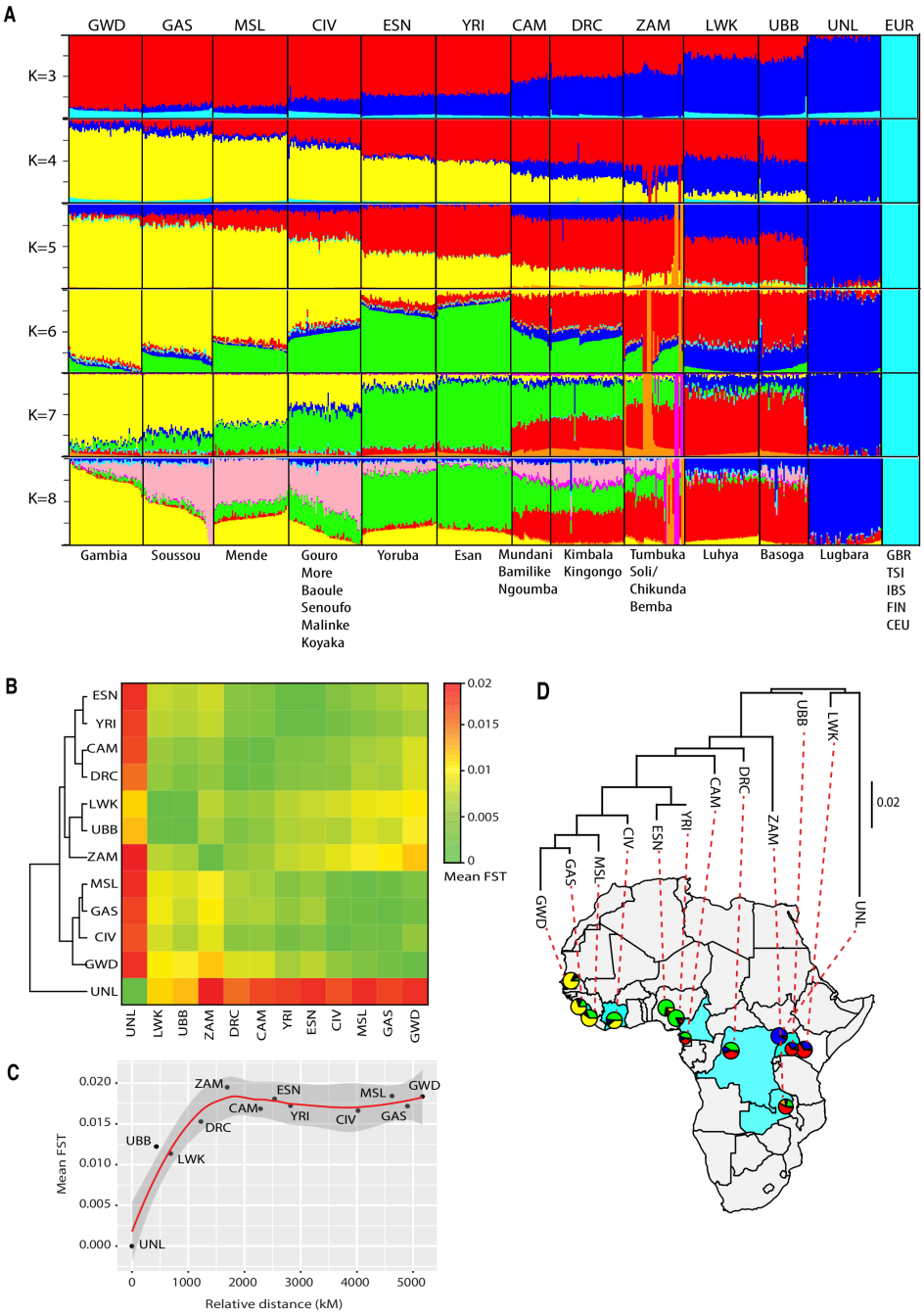
981 **Additional file 12**: Figure S8.pdf. Bar plots comparing the mean iHS *P*-values of the
982 SNP loci in *APOL1*, *HPR*, *HLAG*, and *IL6* genes within the TrypanoGEN
983 populations. These genes are associated with progression of Human African
984 Trypanosomiasis.

985 **Additional file 13**: Table S6.xlsx. Population iHS scores of SNPs in genes that play a
986 role in African trypanosomiasis pathology



987

988 Figure 1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GWD
GAS
MSL
CIV
ESN
YRI
CAM
DRC
ZAM
LWK
UBB
UNL
EUR
K=3
K=4
K=5
K=6
K=7
K=8
Gambia
Soussou
Mende
Gouro
More
Baoule
Senoufo
Malinke
Koyaka
Yoruba
Esan
Mundani
Bamilike
Ngoumba
Kimbala
Kingongo
Tumbuka
Soli/
Chikunda
Bemba
Luhya
Basoga
Lugbara
GBR
TSI
IBS
FIN
CEU
B
Mean FST
C
Mean FST
Relative distance (kM)
D
0.02

Figure 2

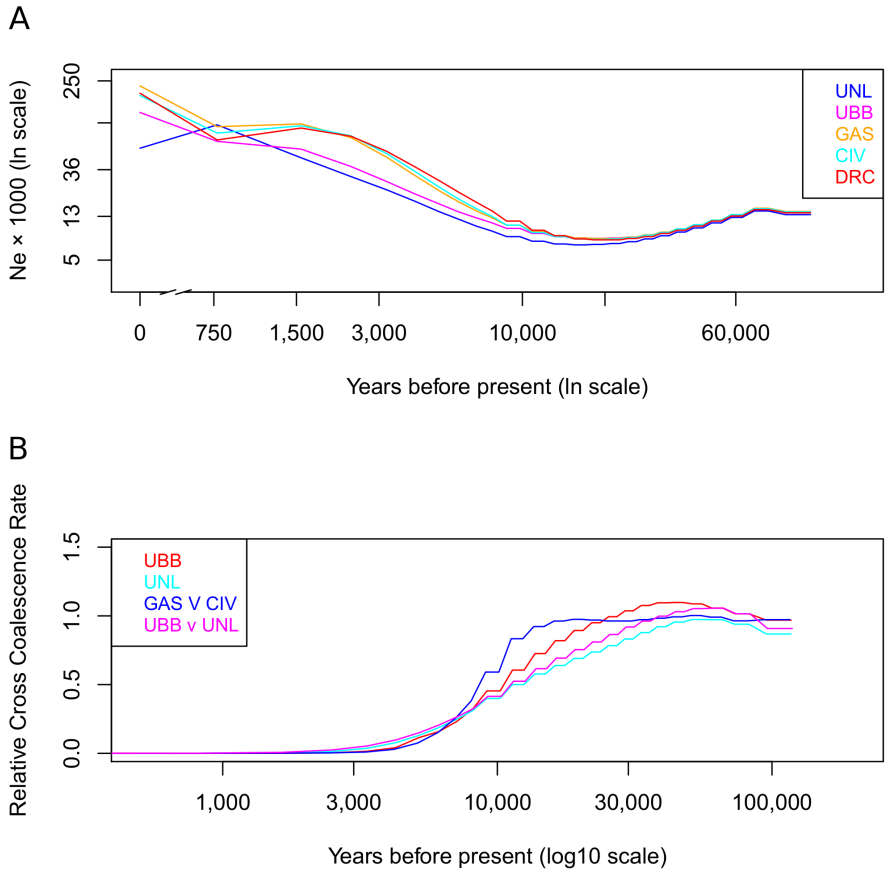
A
Ne × 1000 (ln scale)
250
36
13
5
UNL
UBB
GAS
CIV
DRC
0
750
1,500
3,000
10,000
60,000
Years before present (ln scale)
B
Relative Cross Coalescence Rate
1.5
1.0
0.5
0.0
UBB
UNL
GAS V CIV
UBB v UNL
1,000
3,000
10,000
30,000
100,000
Years before present (log10 scale)

Figure 3

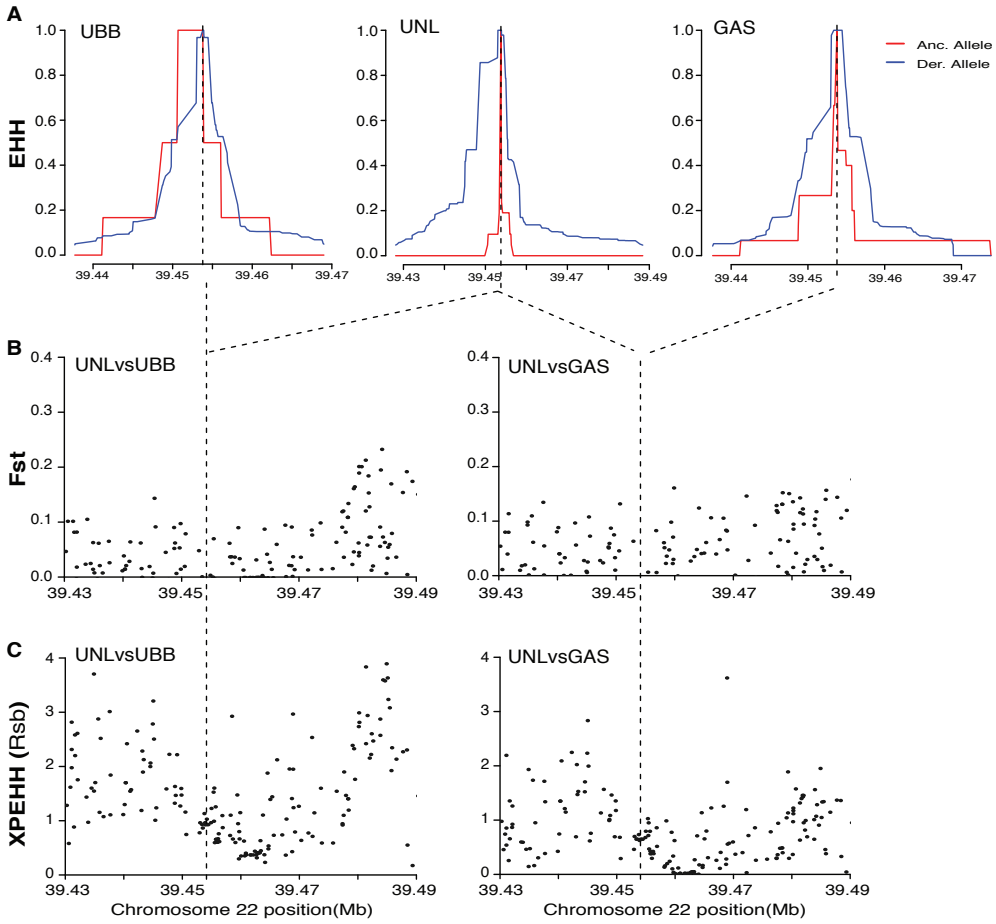
A
UBB
UNL
GAS
Anc. Allele
Der. Allele
EHH
B
UNLvsUBB
UNLvsGAS
Fst
C
UNLvsUBB
UNLvsGAS
XPEHH (Rsb)
Chromosome 22 position(Mb)
Chromosome 22 position(Mb)

Figure 4

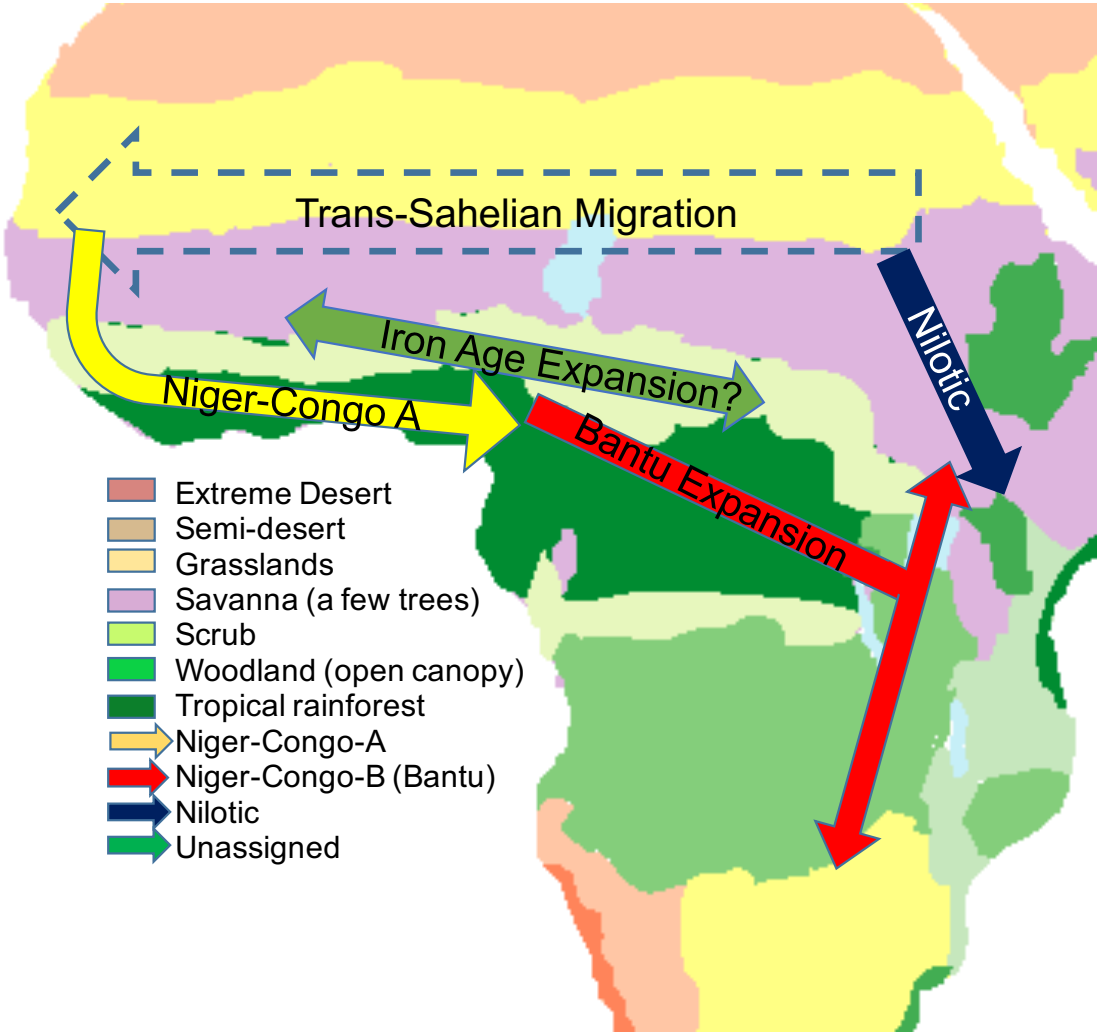
Trans-Sahelian Migration
Iron Age Expansion?
Niger-Congo A
Bantu Expansion
Nilotic
Extreme Desert
Semi-desert
Grasslands
Savanna (a few trees)
Scrub
Woodland (open canopy)
Tropical rainforest
Niger-Congo-A
Niger-Congo-B (Bantu)
Nilotic
Unassigned

Figure 5