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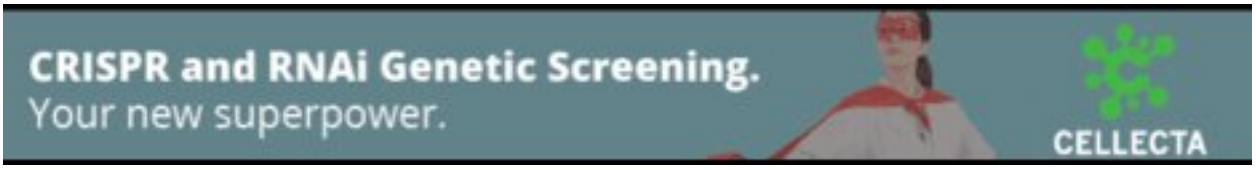

A calibrated human Y-chromosomal phylogeny based on resequencing

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Abstract

We have identified variants present in high-coverage complete sequences of 36 diverse human Y chromosomes from Africa, Europe, South Asia, East Asia and the Americas representing eight major haplogroups. After restricting our analysis to 8.97 Mb of unique male-specific Y sequence, we identified 6,662 high-confidence variants including SNPs, MNPs and indels. We constructed phylogenetic trees using these variants, or subsets of them, and recapitulated the known structure of the tree. Assuming a male mutation rate of $1x10^{-9}$ per bp per year, the time depth of the tree (haplogroups A3-R) was about 101-115 thousand years, and the lineages found outside Africa dated to 57-74 thousand years, both as expected. In addition, we dated a striking Paleolithic male lineage expansion to 41-52 thousand years ago and the node representing the major European Y lineage, R1b, to 4-13 thousand years ago, supporting a Neolithic origin for these modern European Y chromosomes. In all, we provide a nearly 10-fold increase in the number of Y markers with phylogenetic information, and novel historical insights derived from placing them on a calibrated phylogenetic tree.

Introduction

The human Y chromosome offers a unique perspective on human genetics because of its male-line inheritance and the very high resolution of its haplotype tree (Jobling and Tyler-Smith 2003). Insights it has provided include a recent African origin for paternal lineages (Cruciani et al. 2011, Hammer 1995), a single expansion of modern humans out of Africa (Underhill et al. 2000), evidence for Paleolithic movement back to Africa (Scozzari et al. 1999) and many examples of tracing events within historical times such as the Mongol (Zerjal et al. 2003) and Phoenician legacies (Zalloua et al. 2008) or the descendants of Thomas Jefferson (Foster et al. 1998). These conclusions have been reached after genotyping small numbers of known markers, or limited resequencing. Despite the valuable insights that have been obtained, there have been other areas of Y-chromosomal study where uncertainty or debate have persisted for decades, such as the understanding of the

causal mutations underlying Y-linked spermatogenic failure (Tyler-Smith and Krausz 2009), the role of Y-chromosomal variation in phenotypes such as coronary artery disease (Charchar et al. 2012), or the origins of European male lineages. In the last case, opinions have included a Paleolithic origin for the major lineage (Semino et al. 2000), a Neolithic origin for the equivalent lineage (Balaresque et al. 2010), and the view that reliable age estimates for this lineage are currently impossible (Busby et al. 2011).

Studies of mitochondrial DNA, often regarded as a female-line counterpart of the Y, have benefited substantially from developments in data acquisition, moving from early RFLP typing (Cann et al. 1987) and control-region resequencing (Vigilant et al. 1991) to large-scale complete sequencing of chosen lineages or populations (Behar et al. 2008, Gunnarsdottir et al. 2011). It seemed likely that complete sequencing of Y chromosomes might lead to similar or even greater benefits. Although its 3,000-fold greater length has made this more difficult, current technologies allow an enormous increase in sequence data collection. This is, however, accompanied by a cost: short reads are generated and these cannot be mapped accurately if they are derived from repetitive regions. Since the Y is richer in repeated sequences than any other chromosome, this is particularly problematic for sequencing the Y, but can be overcome by concentrating on unique regions. This approach has allowed the sequencing of a pair of related Y chromosomes with sufficient accuracy to provide a measurement of the mutation rate (Xue et al. 2009), and lower coverage sequencing of 77 Y chromosomes to discover 2,870 high-confidence Y-SNPs, 74% new (The 1000 Genomes Project Consortium 2010). Although low-coverage sequencing is an efficient way to discover variants, the interpretation of the resulting data is complicated by the incomplete ascertainment of variants in any single sample, and thus reliable information about some features, such as the time depth of the phylogeny, is difficult to extract. Sequencing technologies have now developed further and allow moderately-sized samples to be sequenced at high coverage from either the complete genome or targeted regions (Drmanac et al. 2010, Hu et al. 2012), which should permit more complete ascertainment of variants and simplify interpretation.

Here, we have analysed a dataset of 36 Y chromosome sequences in order to explore how effectively complete sequence data from the Y can be used to construct and calibrate a phylogeny, and the insights that may result from such an analysis.

Results

High-coverage sequences from 36 males were used, 35 released by Complete Genomics and an additional sequence from a haplogroup A individual (the most basal haplogroup branch) generated for this study. In all, there were nine males from Africa, 15 from Europe (including a three-generation family containing eight males), three from South Asia, two from East Asia and seven from the Americas. SNP genotyping data available from the HapMap3 Project (Altshuler et al. 2010) revealed that haplogroups A, D, E, G, I, N, Q and R were represented, with many subdivisions of some of these, particularly E and R. Thus, despite the small number of individuals, there was good geographical representation of global populations and of the haplogroup tree. After QC and validation, we extracted 6,662 high-confidence variants (i.e. sites that differ from the Y chromosome reference sequence), including both

SNPs and indels, from 8.97 Mb of unique Y sequence (Table S1, S2). The variants were distributed evenly along the target regions of the chromosome (Figure 1), and increase the number of high-quality variants with phylogenetic information by almost 10-fold.

We could assign ancestral states to 6,271 of the variants, and then constructed a rooted parsimony-based phylogenetic tree containing all 6,662 variants (tree 1, Supplemental Figure 1), and additional trees containing subsets of these, consisting of SNPs only (tree 2, Supplemental Figure 2), or SNPs with ancestral state information, no recurrent mutations and high coverage in all individuals (tree 3, Figure 2). The branch leading to the haplogroup A individual was shorter than any other, most likely reflecting the low-coverage sequencing of parts of this chromosome and consequent under-calling of variants; this effect was largely eliminated in tree 3, where only high-coverage regions were used (Figure 2). The topology of all three trees was very similar, differing only by the lack of resolution of the haplogroup G and I branch order in the most highly-filtered tree, because the relevant SNPs lay outside the region used. The structure recapitulated the known phylogeny of the Y chromosome (Karafet et al. 2008) and identified new splits within the E1b1a8a and I1* branches. In addition, the new markers allowed all unrelated chromosomes to be distinguished.

5,865 (88.0%) of the variants identified were SNPs, 56 (0.8%) MNPs, and 741 (11.1%) indels. Based on the phylogeny, we found that 172 (2.9%) SNPs, 5 (9%) MNPs and 85 (11.5%) indels showed evidence of recurrent mutation, either reverting to the ancestral state, recurring in more than one location on the tree, or showing more than two alleles. Indels, most of which lay in mononucleotide runs or short tandem repeats, were highly enriched for this behavior ($P<0.001$, Chi-squared test). Among SNPs, 533 (9.1%) were present in CpG dinucleotides and these were also enriched for recurrent mutations, but not significantly (18/533 in CpGs compared with 154/5,332 outside, $P=0.30$, Fisher exact test).

It is in general difficult to identify functionally important variants from DNA sequence data alone, but variants that lead to loss-of-function or altered amino acid sequence in protein-coding genes can readily be identified and often influence function. No variants in our list were predicted to lead to loss-of-function, but there were six missense SNPs (Table S3), two of which had been reported in a previous study examining 16 Y genes in 105 men (Rozen et al. 2009). As in the earlier study, none lay within haplogroup I and so do not provide any functional insights into the coronary artery disease (Charchar et al. 2012) and HIV progression (Sezgin et al. 2009) associated with this haplogroup. Five of the six SNPs were predicted not to damage the protein, but one, in the gene *USP9Y* in the R1b family of European origin, was predicted to be highly damaging to the protein.

In addition to carrying large numbers of new markers informative about tree topology, the trees based on sequence data have branch lengths that are informative about the times when lineages diverged. We made use of this information by estimating times for the entire tree, and divergences of particular interest corresponding to the out-of-Africa movement, a topologically-striking but poorly-understood Paleolithic expansion, and the expansion of R1b in Europe (Table 1). We made five estimates for each of the nodes of interest. Three of these used coalescent modelling either with a simple model of a constant-sized subdivided population (GENETREE-1), or with more complex models including variation in population

size and migration rate (GENETREE-2 and BEAST; details in Methods) (Bahlo and Griffiths 2000, Drummond et al. 2012). The other two estimates were based on the phylogeny (Rho-1 and Rho-2) (Forster et al. 1996, Saillard et al. 2000). These times were all broadly consistent and are relevant to debates about human expansions and migrations, so are discussed further below.

Discussion

Many aspects of the approach adopted in this study, including complete sequencing of Y chromosomes, using data from public sequence resources, generating additional data from lineages of particular interest, and filtering of the Y-chromosomal regions and variants, are likely to become standard in future analyses of this kind. The current study has provided insights into several relevant methodological topics, and also into the way that a calibrated Y-chromosomal phylogeny can provide insights into recent human evolution. We consider issues arising in these areas in this Discussion.

Next-generation sequence data are error-prone, with errors contributed by both base calling and mapping. The former can largely be overcome by high coverage, and so are minimized in this study; in order to exclude the latter, we stringently excluded repeated regions, which are most prone to mapping errors. This resulted in a high-quality dataset, but at the cost of not detecting and using all variants. Future studies should investigate the possibility of extracting reliable variant calls from additional regions of the chromosome, and this will be facilitated by the longer reads expected as sequencing technologies improve. An additional source of biological error is the mutations that occur somatically in the donor or during cell culture, relevant here since all sequences were derived from lymphoblastoid cell lines. We can estimate this number from the sequences of the three-generation family. The grandfather and father carry 13 and 11 specific variants respectively, two of which are absent from the grandfather, but present in the father and transmitted to all his sons, and thus likely to represent *in vivo de novo* mutations, while the remaining 22 are likely to be somatic (Supplementary Figure 1). This observation of two germline mutations in two transmissions of 8.97 Mb is consistent with the expectation of ~0.6 mutations in two transmissions (0.3 variants observed per meiosis in 10.5 Mb (Xue et al. 2009)). In addition, the sons carry from zero to 17 individual-specific variants each. If we assume that all the non-transmitted variants are somatic, we can estimate an upper limit to the number of somatic variants at 8/individual (3 SNPs/individual). These somatic variants are thus highly enriched for indels compared with total variants (42/67 compared with 699/6,595, $P<0.001$, Fisher exact test). 3 somatic SNPs/individual would have a negligible effect on the analyses presented.

This study identified 6,662 Y variants and placed them on a phylogenetic tree. 73% of the variants appear to be novel, in that they are not in dbSNP 135, which already includes the 1000 Genomes Pilot data (The 1000 Genomes Project Consortium 2010), and 97% have not been used in published phylogenetic studies. They thus represent a valuable resource for future studies. Variants on a branch represented by a single chromosome, such as A, D, G, N and Q will include everything from ancient haplogroup-defining variants to those private to the individual sequenced, and additional work is needed to refine their phylogenetic

positions. When multiple individuals within a haplogroup were sequenced, the shared variants already provide useful markers. For example, the geographical origin (south or west Asia) and time depth of haplogroup R1a are disputed and few useful markers within this haplogroup have previously been available (Underhill et al. 2010); typing the 173 shared variants identified here in additional geographically diverse R1a samples would be of great interest.

Although this study was not aimed at investigating Y gene function, it was striking that a variant predicted to be highly damaging to protein structure was discovered in the *USP9Y* gene. *USP9Y* loss of function has been associated with variable phenotypes ranging from azoospermia to oligoasthenoteratozoospermia and normal sperm production (Tyler-Smith and Krausz 2009). The transmission of this variant through three generations demonstrates that it is compatible with male fertility, providing further evidence for the phenotypic diversity linked to variation in this gene.

A novel aspect of using extensive sequence data is that it is possible to investigate the time depth of the entire Y-chromosomal phylogeny represented by the samples, or of any subset of lineages. Times are determined in mutational steps, and in order to convert these into a more useful unit such as years, a calibration metric has to be applied. We used a calibration based on direct measurement of the Y-chromosomal SNP mutation rate both in years and generations from a deep-rooting family (Xue et al. 2009) since this requires the minimum number of assumptions and has already been adopted in the literature (Cruciani et al. 2011). The measurement does, however, have wide confidence intervals since only a small number of mutations were observed, and these confidence intervals were not included in our consideration of times, which used the point estimate. Two lines of reasoning suggest that we may have more confidence in the point estimate than simple consideration of the number of mutations might suggest. First, it is consistent with other direct measurements of the human mutation rate, allowing for the expected higher mutation rate on the Y because of its permanent location in the mutation-prone male germ line (e.g. Roach et al. 2010). Second, it is consistent with the rate inferred from human-chimpanzee comparisons of the same sections of the Y chromosome: 1.3×10^{-9} mutations/nucleotide/year for a 6.5 million year Y-chromosomal divergence time (Scally et al. 2012, Xue et al. 2009). Nevertheless, additional measurements of mutation rate are urgently needed to improve calibration.

We based our time estimates solely on SNPs, because indel mutation rates are poorly known and likely to be complex. We used either the complete set of SNPs (with the rho estimator), or a reduced set where coverage was high in all samples, ancestral state was known and recurrent mutations were absent, and related individuals were excluded, required by GENETREE and also used with BEAST and rho. As a result, we obtained five estimates for each time, which were similar (Table 1). Point estimates of the TMRCA for the complete set of chromosomes examined were 101-115 KYA. This is consistent with the published estimate of 105 KYA for haplogroup A3 (Cruciani et al. 2011). We identified three additional nodes in the tree as being of particular interest. The first of these was DR, corresponding to the expansion of Y chromosomes following the out-of-Africa migration (Jobling and Tyler-Smith 2003, Underhill et al. 2000). The time of 57-74 KYA years is consistent with abundant non-genetic evidence, for example of the first colonization of Australia around 50 KYA (Roberts et al. 1990). The agreement of these two times with

previous work strengthens our confidence in the remaining estimates. The second internal node examined was the multifurcation of haplogroups I, G and NR (i.e. K), the representation in this reduced set of individuals of a larger multifurcation also involving several F sublineages, H and J (Karafet et al. 2008). The minimal resolution of the lineages, even in a phylogeny based on sequencing of 8.97 Mb, implies a rapid expansion, which we date here at 41-52 KYA. This could correspond to an expansion into the interior of Europe and Asia following adaptation to these novel environments soon after the initial rapid coastal migration. The third internal node was that of R1b, a well-documented expansion in Europe, but with a much-debated time depth. Here, we estimate a time of 4.3-13 KYA, the most uncertain of the dates. Despite the range of estimates, all these dates favor a Neolithic (Balaresque et al. 2010) more than a Paleolithic (Semino et al. 2000) or Mesolithic expansion of this lineage. The three haplogroup I individuals, representing the next most frequent haplogroup in Europe, show signs of an expansion at approximately the same time (Figure 2), although the number of individuals is too low to present any clear conclusion about whether or not this lineage was influenced by the same demographic events as R1b. Nevertheless, the rapid expansion of R1b (and possibly I1) in Europe contrasts with the less starlike expansion of E1b1a in Africa, which has been associated with the spread of farming, ironworking and Bantu languages in Africa over the last 5,000 years (Berniell-Lee et al. 2009). Both R1b and E1b1a samples are from a mixture of indigenous donors (from Europe and Africa, respectively) and admixed American donors, so sampling strategy does not provide an obvious explanation for the difference. Instead, the different phylogenetic structure, with far more resolution of the individual E1a1a branches, may reflect expansion starting from a larger and more diverse population, and thus retaining more ancestral diversity.

In conclusion, our study identifies the methodological steps necessary to obtain reliable biological insights from current next-generation sequence data, and the novel information that is available. It reveals, for example, how rapid some expansions of the Y phylogeny were, so that even extensive sequence data do not resolve all multifurcations. During the expansion of the six unrelated R1b lineages examined, only one mutation has arisen, despite a mutation rate of 0.3/generation/10 Mb. The study also poses challenges for the field, among which are (1) integrating sequence data generated by different technologies with different error modes on different samples; (2) the question of whether an inferred ancestral reference sequence would be more useful than the current hybrid of modern sequences; (3) the need to develop a compact and useful nomenclature system that can accommodate extensive sequence information, for example based on major haplogroups and significant sub-clusters that provide a memorable but not complete indication of the location in the phylogeny; and (4) the difficulty of understanding the archaeological events associated with the male expansions detected, and the standards of evidence that should be required in order to accept links.

Methods

Definition of Y-specific unique regions

We identified unique regions within the male-specific part of the Y chromosome reference sequence (GRCh37 http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/) where we expected read mapping and variant detection to escape complications introduced by repeated sequences. This was achieved by excluding the pseudoautosomal, heterochromatic, X-transposed and ampliconic segments (Skaletsky et al. 2003), leaving nine separate regions (Figure 1, Table S1). Together, these spanned 8.97 Mb. All analyses in this study were restricted to these regions, or subsets of them.

Y-chromosomal sequence data sources

High-coverage Y-chromosomal sequence data from 35 males were downloaded from the Complete Genomics database (ftp://ftp2.completegenomics.com/vcf_files/Build37_2.0.0/). Coverage of the Y unique regions ranged from 19x to 35x (Table S4). These males did not include any individual belonging to the most basal haplogroup, haplogroup A. We therefore generated additional sequence data from a haplogroup A individual, NA21313. This chromosome was derived for the markers M32, M190, M220, M144, M202, M305 and M219, and ancestral for P97, placing it in haplogroup A3b2*. Sequencing included both low coverage (mean 5x) whole-genome data, and high coverage data from long-PCR products between chrY:13,798,579-19,720,738 (GRCh37).

NA21313 Low coverage sequencing
Low coverage reads were mapped and variants called in combination with 1000 Genomes samples using samtools and bcftools (Li et al. 2009), then filtered (StrandBias 1e-5; EndDistBias 1e-7; MaxDP 10000; MinDP 2; Qual 3; SnpCluster 5,10; MinAltBases 2; MinMQ 10; SnpGap 3).

NA21313 High coverage sequencing
High coverage sequence information was generated by amplifying 5-6 kb overlapping fragments by long-PCR. Approximately equimolar amounts of PCR fragments were pooled and used for library preparation and paired end sequencing (54 bp) on an Illumina GAII Genome Analyzer to obtain 475x median coverage (ENA Sample Accession Number: ERS006694; www.ebi.ac.uk/ena/). MAQ (Li et al. 2008) was used for mapping and SNP calling in the high coverage data. Read depth coverage (>1/2 mean depth) and mapping (consensus >30, Mapping > 63) filters reduced the raw variant calls from a total of 5,684 to 2,233. Subsequent filters included removal of heterozygous calls and sites that were within 4 bp of each other to create a high confidence filtered list of 615 SNPs, none of which were discordant with the established Y phylogeny.

Ancestral states
We extracted the ancestral allele for each position that was variable in humans (assumed to be the allele present in chimpanzee) using the Ensembl-Compara pipeline (Vilella et al. 2009) release 66 and obtained calls for 6,271 of the total number of 6,662 variable sites (Figure 1, Table S2).

Data QC and filtering

We extracted the variants from the Y-specific unique regions present in the 35 male individuals from the database of Complete Genomics, including both SNPs and indels. The number of differences from the reference sequence ranged from 210 to 1,257. Examination of the distribution of variants along the chromosome revealed an excess in one sample, GS19649, in the region 28,670,244 – 28,735,914 compared with the other individuals; this was associated with low coverage of this region, so we hypothesised that the excess arose from a combination of deletion of the region in this individual and mismapping of reads originating from other parts of the chromosome. We excluded from further consideration all variable sites from all individuals that fell into this region.

We identified 631 variable sites mapping within the 8.79 Mb region that had been described in the literature and placed on the 2008 Y Chromosome Consortium phylogenetic tree (Karafet et al. 2008) and its subsequent partial updates (Chiaroni et al. 2009, Cruciani et al. 2011, Cruciani et al. 2008, Cruciani et al. 2010, Debnath et al. 2011, Jota et al. 2011, Mendez et al. 2011, Sims et al. 2009, Trombetta et al. 2011, Trombetta et al. 2010). These were used for assigning a standard haplogroup to each sample and in validation.

From all 36 individuals, we identified a total of 6,662 variable sites. Missing calls ranged from 31 to 165 per individual; call rates were on average 98.9%. We imputed the missing sites based on the tree structure and the assumption of parsimony; the only errors introduced by this procedure will be missed reversions or recurrent mutations, both very rare.

Validation

SNP validation

We performed *in silico* validation of the SNP calls using three approaches. First, calls from 101 SNPs based on the intersection of the Affymetrix Human SNP array 6.0 array and the Illumina Human 1M beadchip were available for 11 individuals from the HapMap3 genotyping of the same samples (Altshuler et al. 2010). We observed 100% concordance at these 1,122 positions (Table S5). Second, high-coverage Illumina GA2 sequence data were available for two individuals, NA19239 and NA12891, the trio fathers in the 1000 Genomes Pilot Project (The 1000 Genomes Project Consortium 2010). There was 85.1% (553/650) and 96.5% (694/719) concordance between the two datasets. In the light of the other validation results, we ascribe the relatively low concordance in this test to the early sequencing technologies used in this part of the 1000 Genomes Pilot project, much of it single-ended and particularly susceptible to mapping problems. Third, using the literature SNP (see Data QC and filtering above) with its derived allele furthest from the root in each individual to assign a haplogroup, we could predict the allelic states expected for the remaining 630 literature sites. Here, after correcting some typographical errors in site positions (Table S6) there was 99.8% concordance between prediction and observation (22,680 genotypes); discrepancies are listed at the top of Table S6 and several may even represent mistakes in the locations of published makers on the phylogeny or recurrent mutations not previously recorded, rather than genotyping errors in the present dataset. Validation rates in the two reliable tests are thus very high.

Indel validation

We used two approaches to assess the quality of the indel calls. First, using a logic similar to the third SNP validation method above, we identified 40 reported indel sites within the unique regions of the Y chromosome (Karafet et al. 2008). Genotype calls were made at 28 of these in our dataset, and we observed 99.7% concordance between the observed calls and those expected from the phylogeny (974/977). The false negative call rate was therefore low. Second, most indel mutations occur in simple sequences such as mononucleotide runs or short tandem repeats. We therefore examined the sequences flanking the calls, and observed a preponderance of these motifs: 471 poly A or poly T, 17 poly C or poly G, 87 short tandem repeats, 166 other (Table S7). This shows that, although these variants have not been experimentally tested, at least 575/741 of the indels (78%) are highly plausible candidates. In all, we can have reasonable confidence in this indel callset.

Constructing the Y-chromosomal haplogroup tree

FASTA formatted sequence files used to generate haplogroup trees. Sequence alignments were built using CLUSTALW2 (http://www.clustal.org/), and a maximum parsimony (MP) phylogenetic tree was created using the PHYLIP software (http://evolution.gs.washington.edu/phylip.html). We generated three trees. First, we used all the 6,662 sites in 36 individuals to construct a haplogroup tree, which was rooted using the chimpanzee Y sequence. The resulting tree (Tree 1) is shown in Supplemental Figure 1. Second, we excluded all indels and MNPs to generate a second haplogroup tree based only on SNPs (Tree 2, Supplemental Figure 2). Third, we removed all sites that were recurrent in this set of 36 males or lacked ancestral information, and restricted the region considered to the 3.2 Mb with high coverage in the haplogroup A individual, NA21313 (Tree 3, Figure 2). Stringent sites of this kind are required by GENETREE (Bahlo and Griffiths 2000), one of the approaches used to estimate times on the tree.

Estimating the TMRCA and ages of nodes of the haplogroup tree

To estimate the TMRCA of the complete tree and the ages of nodes of particular interest, DR, FR and R1b, we applied two broad approaches that provided five individual estimates for each timepoint. The first was to use coalescent modelling implemented either in GENETREE (Bahlo and Griffiths 2000) or BEAST (Drummond et al. 2012) using the sites in Tree 3. To avoid the influence of related individuals, we removed seven out of the eight individuals who were part of the same three-generation pedigree. This resulted in 2,004 SNPs from 29 individuals.

GENETREE

We were unable to successfully run GENETREE using all 2,004 sites. We therefore divided the data into non-overlapping sets of 90-99 SNPs according to their position on the chromosome, resulting in 21 sets and thus 21 GENETREE runs. GENETREE version 8.3 was run in two ways. The first used a demographic model of a constant-sized but subdivided population with fixed migration rates. Run details: an initial theta value was calculated using the formula: $\theta = Ne \times \mu \times$ length of region spanning 90-99 SNPs ($Ne = 2{,}000$; $\mu = 3 \times 10^{-8}$ mutations/nucleotide/generation); 1,000,000 coalescent simulations and 101 surface points were used to find the optimal value. The migration rate between Africa and Europe was set at 3.2×10^{-5}/generation and between Africa and Asia at 0.8×10^{-5}/generation (Schaffner et

al. 2005) to generate the migration file. From this calculation, a best estimate of theta was obtained and used to calculate the TMRCA and other times (GENETREE-1, Table 1, Figure S3.1-3.21). The second used a demographic model of exponential growth and a subdivided population, with growth and migration rates derived from the data. Run details: an initial theta value was calculated as before and used to generate the migration file; initial exponential growth rates for Europe (0.20), Asia (0.41) and Africa (0.74) were used (Shi et al. 2010). 1,000,000 coalescent simulations and 101 surface points were used to find the optimal value for theta, the migration rate and the growth rate. From these, a best estimate of theta, migration rate and exponential growth rate were obtained and used to calculate the TMRCA and other times (GENETREE-2, Table 1)

BEAST

We also used BEAST v1.7.2 (http://beast.bio.ed.ac.uk) to estimate the TMRCA and divergence time for each branch of interest. The NEXUS formatted file was used to generate the BEAST XML input file for the BEAST v1.7.2 program. To obtain the TMRCA and the time for the branches, four taxon subsets were set up (AR, DR, FR and R1b). We chose GTR as the substitution model, Gamma as the site heterogeneity model, lognormal relaxed clock as the clock model, exponential growth as the tree prior, $\mu = 10^{-9}$ mutations/nucleotide/year, and 100,000,000 as the MCMC chain length. The log output files were obtained by running the BEAST software. We carried out two independent BEAST runs from the same XML input file and combined the two log output files using LogCombiner, as recommended to increase the ESS (effective sample size) of the analysis and also allow us to determine whether or not the two independent runs were converging on the same distribution (Table S8). Tracer v1.5 (http://beast.bio.ed.ac.uk/Tracer) was used to analyze the output file and times (BEAST in Table 1), and TreeAnnotator v1.7.2 to obtain an estimate of the phylogenetic tree. Finally, we viewed the tree in FigTree (http://beast.bio.ed.ac.uk/FigTree) (Supplemental Figure 4).

Rho

In the second broad approach, we used the phylogeny-based rho statistic (Forster et al. 1996, Saillard et al. 2000), applied both to the same set of 2,004 SNPs (Tree 3, Rho-1 in Table 1), and also to the complete set of 5,865 SNPs, including recurrent SNPs (Tree 2, Rho-2 in Table 1).

In all five estimates (two GENETREE, one BEAST, two rho), calibration was achieved using the directly-measured SNP mutation rate of 1.0×10^{-9} mutations/nucleotide/year or 3.0×10^{-8} mutations/nucleotide/generation (Xue et al. 2009). Where necessary, we converted estimates in generations to estimates in years using 30 years/generation.

Annotation

Each variable site was annotated (where relevant) with its type (SNP, MNP or indel), location in hg36 and hg37, the reference, ancestral and derived alleles, dbSNP ID, name in the existing phylogenetic tree, haplogroup in which it is variable, presence as part of a CpG dinucleotide, location in a gene and consequences for protein structure (Table S2). The predicted consequences of missense variants for protein function were taken from the modified Condel scores (Gonzalez-Perez and Lopez-Bigas 2011) in Ensembl.

Data access

NA21313 low coverage: www.ebi.ac.uk/ena/ Accession Number ERS037274
NA21313 high coverage: www.ebi.ac.uk/ena/ Accession Number ERS006694

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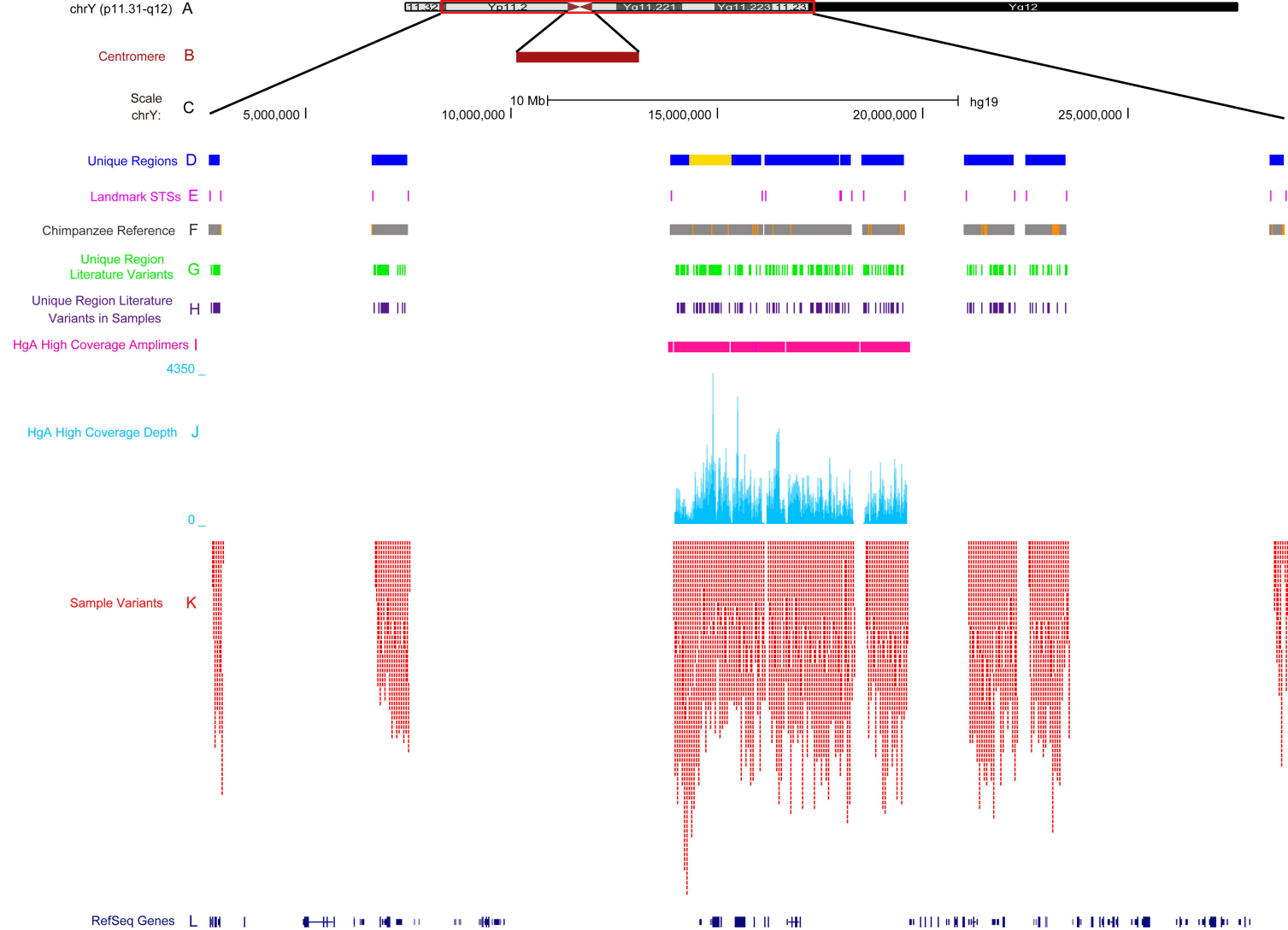
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