Somatic evolution and global expansion of an ancient transmissible cancer lineage

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Structured Abstract

INTRODUCTION

The canine transmissible venereal tumour (CTVT) is a sexually transmitted cancer that manifests as genital tumours in dogs. This cancer first arose in an individual 'founder dog' several thousand years ago, and has since survived by transfer of living cancer cells to new hosts during coitus. Today, CTVT affects dogs around the world and is the oldest and most prolific known cancer lineage. CTVT thus provides a unique opportunity to explore the evolution of cancer over the long-term, and to track the unusual biological transition from multicellular organism to obligate conspecific asexual parasite. Furthermore, the CTVT genome, acting as a living biomarker, has recorded the changing mutagenic environments experienced by this cancer throughout millennia and across continents.

RATIONALE

To capture the genetic diversity of the CTVT lineage, we analysed somatic mutations extracted from the protein-coding genomes (exomes) of 546 globally distributed CTVT tumours. We inferred a time-resolved phylogenetic tree for the clone and used this to trace the worldwide spread of the disease and to select subsets of mutations acquired at known geographical locations and time-periods. Computational methods were applied to extract mutational signatures and to measure their exposures across time and space. In addition, we assessed the activity of selection using ratios of non-synonymous and synonymous variants.

RESULTS

The CTVT phylogeny reveals that the lineage first arose from its founder dog 4,000–8,500 years ago, likely in Asia, with the most recent common ancestor of modern globally distributed tumours occurring ~1,900 years ago. CTVT underwent a rapid global expansion within the last 500 years, likely aided by intensification of human maritime travel. We identify a highly specific mutational signature dominated by C>T mutations at GTCCA pentanucleotide contexts which operated in CTVT up until ~1,000 years ago. The number of mutations caused by ultraviolet light exposure is correlated with latitude of tumour collection, and we identify CTVTs with heritable hyperactivity of an endogenous mutational process. Several 'driver' mutation candidates are identified in the basal trunk of the CTVT tree, but there is little evidence for ongoing positive selection. Although negative selection is detectable, its effect is largely confined to genes with known essential functions, thus implying that CTVT predominantly evolves via neutral processes.

CONCLUSION

We have traced the evolution of a transmissible cancer over several thousand years, tracking its spread across continents and contrasting the mutational processes and selective forces that moulded its genome with those described in human cancers. The identification of a highly context-specific mutational process that operated in the past but subsequently vanished, as

well as correlation of ultraviolet light-induced DNA damage with latitude, highlight the potential for long-lived, widespread clonal organisms to act as biomarkers for mutagenic exposures. Our results suggest that neutral genetic drift is the dominant evolutionary force operating on cancer over the long-term, in contrast to the ongoing positive selection which is often observed in short-lived human cancers. The weakness of negative selection in this asexual lineage may be expected to lead to the progressive accumulation of deleterious mutations, invoking Muller's Ratchet and raising the possibility that CTVT may be declining in fitness despite its global success.

Abstract

The canine transmissible venereal tumour (CTVT) is a cancer lineage that arose several millennia ago and survives by 'metastasising' between hosts via cell transfer. The somatic mutations in this cancer record its phylogeography and evolutionary history. We constructed a time-resolved phylogeny from 546 CTVT exomes and describe the lineage's worldwide expansion. Examining variation in mutational exposure, we identify a highly context-specific mutational process that operated early but subsequently vanished, correlate ultraviolet-light mutagenesis with tumour latitude, and describe tumours with heritable hyperactivity of an endogenous mutational process. CTVT displays little evidence of ongoing positive selection, and negative selection is detectable only in essential genes. We illustrate how long-lived clonal organisms capture changing mutagenic environments, and reveal that neutral drift is the dominant feature of long-term cancer evolution.

Introduction

Transmissible cancers are malignant somatic cell clones that spread between individuals via direct transfer of living cancer cells. Analogous to the metastasis of cancer to distant tissues within a single body, transmissible cancers 'metastasise' as allogeneic grafts between individuals within a population (*1*). Such clones have been observed only eight times in nature, suggesting that they arise rarely; however, once established, transmissible cancers can spread rapidly and widely and persist through time (*1, 2*). Such cancers provide a unique opportunity to explore the evolution of cancer over the long-term, and to track the unusual biological transition from multicellular organism to obligate conspecific asexual parasite.

The canine transmissible venereal tumour (CTVT) is the oldest and most prolific known contagious cancer (*2, 3*). It is a sexually transmitted clone that manifests as genital tumours in dogs. This cancer first arose from the somatic cells of an individual 'founder dog' that lived several thousand years ago (*2*). The cancer survived beyond the death of this original host by transfer of cancer cells to new hosts. Subsequently, this cancer has spread around the world,

and is a common disease in dog populations globally, although it declined and largely disappeared from many Western countries during the twentieth century due to the management and removal of free-roaming dogs (*4*).

Similar to cancers that remain in a single individual, CTVT accumulates somatic mutations. These result from the activities of endogenous and exogenous mutational processes, and genetically imprint a cancer's history of mutagenic exposures (*5*). Thus, the CTVT genome can be considered a living biomarker that records the changing mutagenic environments experienced by this cancer throughout millennia and across continents. Although most somatic mutations in cancer have no functional effect and are considered neutral 'passenger' mutations, a subset of mutations are positively selected 'driver' mutations that confer the proliferation and survival advantages that spur cancer growth (*6*). Ordinary cancers, which remain in a single host, often acquire additional driver mutations during tumour progression (*7*); however, it is unknown whether transmissible cancers that survive for hundreds or thousands of years similarly continue to adapt. It seems possible that the evolution of long-lived cancers such as CTVT may instead be dominated by negative selection acting to remove deleterious mutations. Finally, in addition to recording a history of exposures and signatures of selection, somatic mutations provide a tool for tracing CTVT phylogeography, potentially revealing how dogs, together with humans, moved around the world over the last centuries. Here, we use somatic mutations extracted from the protein-coding genomes (exomes) of 546 globally distributed CTVT tumours to trace the history, spread, diversity, mutational exposures and evolution of the CTVT clone.

CTVT phylogeny

We sequenced the exomes (43.6 megabases, Mb; mean sequencing depth ~132×) of 546 CTVT tumours collected between 2003 and 2016 from 43 countries across all inhabited continents (Data sets S1 and S2). Candidate somatic mutations were defined as single nucleotide variants (SNVs) or short insertions and deletions (indels) identified in one or more CTVT tumours, but not found in 495 normal dog exomes from the CTVT tumours' matched hosts. This approach yielded 160,207 variants (148,030 SNVs, 3,392 per Mb; 12,177 indels, 279 per Mb; Table S1). The features of this set, including its variant allele fraction distribution, phylogenetic structure, comparison with the distribution of private germline variants in the dog population, mutational signature composition, and non-synonymous to synonymous mutation ratio (details in (*8*)), suggest that it is very highly enriched for somatic mutations. However, some minimal germline variation may remain, possibly including rare germline variants from the founder dog and residual contaminating alleles from matched hosts.

We identified the subset of the candidate somatic mutations belonging to a clock-like mutational process (specifically, cytosine-to-thymine (C>T) substitutions at CpG sites (*8, 9*)), and used these to construct a time-resolved phylogenetic tree for the CTVT lineage (Fig. 1A). The mutation rate was inferred by applying a Bayesian Poisson model to previously ascertained empirical observations (*10*), and was estimated as 6.87×10^{-7} C>T mutations per CpG site per year (*8*). The topology of the CTVT phylogenetic tree reveals a long basal trunk (Fig. 1A), representing the chain of CTVT transmissions from its origin ~6,220 years ago (95% highest posterior density interval, HPDI, 4,148–8,508 years ago) to the earliest detected node ~1,938 years ago (95% HPDI 993–3,055 years ago). This node splits a set of five tumours collected in India from the remaining population (groups labelled 57 and 58; Fig. 1A). The second and third most basal nodes (respectively ~1,004 years ago, 95% HPDI 497–1,570 years ago, and ~829 years ago, 95% HPDI 424–1,310 years ago) separate sixteen tumours from Eastern Europe and the Black Sea region, and three tumours from Northern India, from the remaining set, respectively (groups labelled 54–56 and 1; Fig. 1A). Together with evidence that the founder dog shared ancestry with ancient dog remains recovered in North-East Siberia and North America (*10*), the CTVT phylogeny supports a model whereby CTVT originated ~4,000–8,500 years ago in Central or Northern Asia, and remained within the area for the subsequent 2,000–6,000 years. Starting less than ~2,000 years ago, CTVT escaped from its founding population, perhaps due to contact between previously isolated dog groups, and spread to several locations in Asia and Europe (Fig. 1B).

The more recent history of CTVT is marked by rapid global expansion (*11*) (Figs. 1C and S1). CTVT was introduced to the Americas with early colonial contact (~500 years ago, 95% HPDI 284–888 years ago), probably initially to Central America, and further into North and South America (red sublineage 1; Fig. 1, A and C). About 300 years ago, this sublineage spread out of the Americas in an almost polytomous global sweep which brought CTVT into Africa at least five times and re-introduced the disease to Europe and Asia (black sublineage 1; Fig. 1, A and C). In parallel, a second tumour sublineage spread out of Asia or Europe into Australia and the Pacific (sublineage 2; Fig. 1, A and D). This second sublineage is also detected in North America, and its tumours were introduced to Africa on at least two occasions. By ~100 years ago, CTVT was present in dog populations worldwide, establishing local lineages that have since remained largely *in situ*. The CTVT phylogeny thus suggests that dogs, together with their neoplastic parasites, were extensively transported around the world in the fifteenth to early twentieth centuries, probably via sea travel.

Mutational processes in CTVT

The CTVT mutational spectrum, a representation of the six substitution types together with their immediate 5' and 3' base contexts, is dominated by C>T mutations, as previously described (*12, 13*) (Fig. 2A). Applying Markov chain Monte Carlo sampling on a Bayesian model of mutational signatures (*8, 14*), we extracted signatures of five mutational processes from the CTVT mutation load. These include three signatures that closely resemble COSMIC (*15*) signatures 1, 5 and 7 (Fig. 2B). These signatures, which have previously been described in CTVT (*12*), reflect endogenous mutational processes (signatures 1 and 5) and exposure to ultraviolet light (UV, signature 7) (*5*). A fourth signature displaying some similarity (cosine similarity 0.81) to COSMIC signature 2, which is associated with activity of APOBEC enzymes (*5*), was also detected (labelled signature 2*, Fig. 2B).

The fifth signature extracted from CTVT does not resemble any previously described mutational pattern. This signature, which we designate signature A, is characterised by C>T mutations at N<u>C</u>C contexts and shows striking pentanucleotide sequence preference for GT<u>C</u>CA (TG<u>G</u>AC on the complementary strand; Figs. 2, B and C, and S2). This extended sequence preference is markedly more pronounced than previously reported pentanucleotide context biases, such as those associated with UV light or DNA polymerase epsilon deficiency (Fig. 2C) (*16-18*), and is not explained by the sequence composition of the canine exome (Fig. S3). It is possible that signature A's causative mutagen is highly context-specific, or, alternatively, that this signature's associated repair processes are ineffective at certain sequence contexts ('repair shielding') (*19*). In addition, signature A displays strong transcriptional strand bias, with more mutations of guanine on the untranscribed compared to the transcribed strand of genes, indicating that its causative lesion is likely a guanine adduct subject to transcription-coupled repair (TCR). Interestingly, the guanine-directed transcriptional strand bias of signature A at T<u>C</u>C contexts counteracts the cytosine-directed transcriptional strand bias of signature 7 at T<u>C</u>C, such that no overall transcriptional strand bias is observed at this context in the CTVT mutational spectrum (Fig. 2A).

Using the CTVT phylogenetic tree to isolate subsets of mutations, we explored variation in mutational signature exposure across time and space (Figs. S4 and S5, and Data set S3). Remarkably, this revealed that signature A was highly active prior to ~2,000 years ago (causing ~35% of mutations in the basal trunk of the tree, branch A1), and persisted in parallel at lower levels in the two basal branches after the first node (~12% and ~9% of mutations in branches A2 and A3, respectively), but then abruptly vanished (Figs. 2C and S5). Importantly, signature A is not detectable within the germline of a global population of 495 dogs (Fig. S6). It is possible that signature A reflects the activity of an exogenous mutagen that was uniquely present in the environment that CTVT inhabited prior to its escape from its founding

population. Alternatively, it is plausible that signature A may result from an endogenous DNA-damaging agent that occurred in CTVT cells early during the lineage's history, but which ceased to accumulate from ~1,000 years ago, perhaps due to a cellular metabolic change. Although the nature of such a change is unknown, the replacement of possibly defective mitochondrial DNA by horizontal transfer, which likely occurred in parallel in branches A2 and A3 within the last ~1,690 years (*11*), may have altered the metabolic environment within CTVT cells.

Although CTVT usually occurs within the internal genital tract, it may sometimes protrude from the genital orifice or spread to perineal skin, resulting in sporadic exposure to solar UV radiation (*12, 13*). The amount of UV radiation reaching the Earth, however, varies significantly across global environments (*20*). We investigated whether latitude influenced the degree of UV exposure in CTVT tumours by estimating signature 7 contribution within subsets of mutations acquired at known latitudes. Indeed, qualitative assessment of mutational spectra of location-specific CTVT mutation subsets suggests substantial variation in UV exposure; for example, the mutational spectra of tumours collected in Mauritius show considerably more evidence of signature 7 compared with those of tumours collected in Russia (Fig. S4). Using CC>TT dinucleotide mutations (*21*) as a proxy for signature 7 (Fig. S7), we identified a non-linear association between latitude and UV exposure (Spearman's correlation –0.40, 95% HPDI [–0.65, –0.14]; Fig. 2D). By fitting CC>TT mutations observed in the basal trunk of the CTVT tree to this curve, we estimated the latitude of the CTVT founder population (Fig. 2, D and E) (*8*).

Examining the contribution of signature 5 across the CTVT lineage, we observed three independent phylogenetic groups of tumours that appear to have acquired signature 5-hyperactivity phenotypes (groups labelled 12–16, 20 and 40; Figs. 2, F and G, S4 and S5). In one case, involving tumours collected in several South and Central American countries (groups 12–16), the phenotype has been maintained for ~150 years. This phenotype is likely to result from signature 5, and not from the double-strand DNA repair deficiency-mediated COSMIC signature 3, which presents a similar mutational profile (*5, 22*), as we failed to observe the enrichment for indels which co-occurs with signature 3 (*22, 23*). It is, however, possible that these tumours were exposed to another, as yet undescribed, mutational process. Signature 5 is widespread in cancer and normal tissues and has unknown aetiology, although it may be partly associated with endogenously generated adducts subject to nucleotide excision repair (*5, 9, 18*). We annotated non-synonymous mutations occurring in the three groups' respective clonal ancestors, providing a catalogue of genes which may play a role in generation or suppression of signature 5 (Data set S4).

CTVT mutations and gene expression

The prevalence of substitution mutations in CTVT decreases with increasing gene expression, likely reflecting the activity of TCR operating on DNA damage associated with signatures 7 and A, as well as a signature 1 preference for genes with lower expression (*16, 24, 25*) (Fig. S8, A and B). We observed that exons have a higher substitution prevalence than introns, possibly due to sequence context (Figs. S8A and S9). The prevalence of indels is positively correlated with increasing gene expression, as has been observed in human cancers, and may reflect transcription-associated damage (*26*) (Fig. S8A).

We assessed the contribution of TCR in two temporally distinct subsets of mutations: those acquired prior to the earliest detectable node in the phylogenetic tree (~8,500–2,000 years ago; branch A1 in Fig. 1A), and those acquired subsequent to this node (~2,000 years ago to present). Interestingly, although C>T mutations acquired at T<u>C</u>C contexts in highly expressed genes in branch A1 have little strand bias, likely due to the opposing transcriptional strand preferences of signatures 7 and A at this context, those genes with very low expression predominantly show the transcriptional strand bias associated with signature A (Fig. S8C). Assuming that the transcriptional strand bias observed in these low-expressed genes reflects earlier expression and subsequent silencing of genes, this suggests that there may have been an early period in CTVT evolution when the lineage was exposed to signature A more intensely than it was to signature 7. This may reflect variation in the climate or environment to which CTVT was exposed early in its history.

Selection in CTVT

CTVT has a massive mutation burden, which exceeds that observed in even the most highly mutated human cancer types (Fig. 3A). Each CTVT tumour carries on average 37,800 SNVs across its predominantly diploid (*12*) exome (~2 million SNVs genome-wide; Table S2). Indeed, the tally of somatic mutations that have accumulated in CTVT since it departed its original host is comparable with the number of germline variants that distinguish some pairs of outbred dogs (Fig. S10). Within the set of 546 tumours, 14,412 (~73%) protein-coding genes carry at least one non-synonymous mutation, and 5,704 (~29%) have mutations predicted to cause protein truncation (Fig. 3B).

We searched for evidence of positive selection in CTVT. The driver mutations which initially caused CTVT, and which promoted its transmissible phenotype, will have occurred in the basal trunk of the CTVT tree. *SETD2*, *CDKN2A*, *MYC* (previously described (*12*)), *PTEN* and *RB1*, known cancer genes that frequently harbour driver mutations in human cancers (*15*),

carry biallelic loss-of-function or potential activating mutations in the trunk and may be early drivers of CTVT (Fig. 3C and Table S3). To search for late drivers, which may have been acquired in more recent parallel CTVT lineages, we identified independent mutations that occurred repeatedly across the tree, and measured the normalised ratio of non-synonymous to synonymous mutations (dN/dS) per gene after correcting for mutational biases and context effects (*8*). This approach only yielded two uncharacterised genes with dN/dS > 1 (q-value < 0.05), predicted to encode a neuroligin precursor and a roundabout homologue (Data set S5). The potential for these genes to act as late drivers in CTVT cannot be assessed, and it is possible that local sequence structures may result in higher than expected recurrent mutation rates at these loci (*27*). Overall, we find little evidence that CTVT is continuing to adapt to its environment.

Negative selection, which acts to remove deleterious mutations, is very weak in human cancers (*17, 28, 29*). Human cancers have short life-spans, and their evolution is dominated by sweeps of strong positive selection, thus reducing the potential for negative selection to act (*17*). Given its long life-span, high mutation burden and lack of ongoing positive selection, it is possible that negative selection may be a more dominant force in CTVT evolution. Further, unlike in ordinary cancers, in CTVT inter-tumour competition may offer more opportunities for negative selection to manifest, purging lineages less able to infect new hosts and spread through the host population. Indeed, negative selection has been detected operating on CTVT mitochondrial genomes (*11*). Our analysis of dN/dS in CTVT across all genes, however, yielded dN/dS ≈ 1 for both missense and nonsense mutations, indicating near-neutral evolution (Fig. 3D and Data set S5). Similarly, dN/dS did not differ from neutrality in genes categorised by expression level (Fig. 3D). Negative selection, acting both on missense and nonsense mutations, could be detected, however, in sets of genes with known essential functions (Fig. 3D), and was particularly pronounced for nonsense mutations in essential genes occurring in haploid regions (dN/dS = 0.33, p-value < 10^{-4}). A slight signal of negative selection acting on nonsense mutations in haploid regions (dN/dS = 0.88, p-value = 0.027) is explained by 269 essential genes, as negative selection was not detected after removal of these genes (Fig. 3D and Data set S5). These results imply that CTVT largely evolves via neutral genetic drift. This may partly reflect functional obsolescence of many mammalian genes in this relatively simple parasitic cancer, as well as the buffering effect of CTVT's largely diploid genome (*12*). However, it is also likely that transmission bottlenecks between hosts render weak selection inefficient. This may be expected to lead to the progressive accumulation of deleterious mutations in the population (Muller's ratchet) (*30*), raising the possibility that CTVT may be declining in fitness despite its global success.

Discussion

Studies of cancer evolution typically focus on how malignant clones alter during the first years, or perhaps decades, of their existence. We have tracked the evolution of a cancer over several thousand years, and compared the mutational processes and selective forces that moulded its genome with those described in short-lived human cancers.

Our results suggest that neutral genetic drift may be the dominant evolutionary force operating on cancer over the long-term, in contrast to the ongoing positive selection which is often observed in human cancers (*7, 17*). Thus, our results suggest that CTVT may have optimised its adaptation to the transmissible cancer niche early. Subsequently acquired advantageous mutations may have offered incremental change of minimal benefit, such that they were insufficient to overcome the neutral effects of drift. Importantly, since the 1980s, CTVT has been routinely treated with vincristine, a cytotoxic microtubule inhibitor (*31*). Despite the strong selection pressure imposed by vincristine treatment, we find no evidence of convergent evolution of vincristine resistance mechanisms in CTVT at the level of point mutations or indels.

The mechanisms whereby CTVT is tolerated by the host immune system, despite its status as an allogeneic graft, are poorly understood (*32, 33*). The weakness of negative selection beyond genes essential for cell viability implies that there are negligible selective pressures imposed via immunoediting of somatic neoepitopes at a genome-wide level. This is perhaps unsurprising, given the massive antigenic burden already presented by allogeneic epitopes. These findings support evidence that CTVT largely circumvents the adaptive immune system, at least during its initial stages of progressive tumour growth, perhaps in part via down-regulation of major histocompatibility complex molecules (*13, 33-35*).

Our analyses reveal a mutational signature, signature A, which occurred in the past, but ceased to be active from about 1,000 years ago. Interestingly, a recent study (*36*) detected evidence for an excess of C>T mutations at T<u>C</u>C contexts, the mutation type most prevalent in signature A, accumulating in the human germline between 15,000 and 2,000 years ago. If this human mutation pulse is due to signature A, it could indicate a shared environmental exposure which was once widespread, but which has now disappeared. However, we find no evidence of an excess of C>T mutations at GT<u>C</u>CA pentanucleotides in the dog germline, suggesting that dogs as a whole were not systemically exposed to signature A in their past. Further research will be required to elucidate the biological origin of signature A and the mechanism of its striking pentanucleotide sequence bias; however, this study highlights the

potential for long-lived, widespread clonal organisms to act as biomarkers for the activity of mutational processes.

Genomic instability and ongoing positive selection are often considered key hallmarks of carcinogenesis (*37*). CTVT does not have an intrinsically high point mutation rate ('genomic instability'), at least at the level of SNVs, and its vast mutation burden simply reflects the lineage's age. We find no clear evidence for continued positive selection beyond initial truncal events. Thus, CTVT illustrates that, once spawned and sufficiently well-adapted to its niche, neither hallmark is necessary to sustain cancer over the long term.

CTVT is a remarkable biological entity. It is the oldest, most prolific and most divergent cancer lineage known in nature; it has spread throughout the globe and has seeded its tumours in many thousands of dogs. Here, we have traced this cancer's route through the steppes of Asia and Europe and as an unwelcome stowaway on global voyages. We have observed the patterns in its mutational profiles reflecting the dynamics of its exogenous and endogenous environment. Further, we have shown that CTVT largely evolves via neutral processes, and that the mutations that it continues to acquire may pose a threat, rather than an advantage, to its long-term fitness.

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10.17863/CAM.24962. Custom algorithms employed for data processing and analysis are available in GitHub (https://github.com/baezortega/TCG2019).

SUPPLEMENTARY MATERIALS

Materials and Methods

Figs. S1 to S16

Tables S1 to S4

Data sets S1 to S6

References (*38*–*68*)

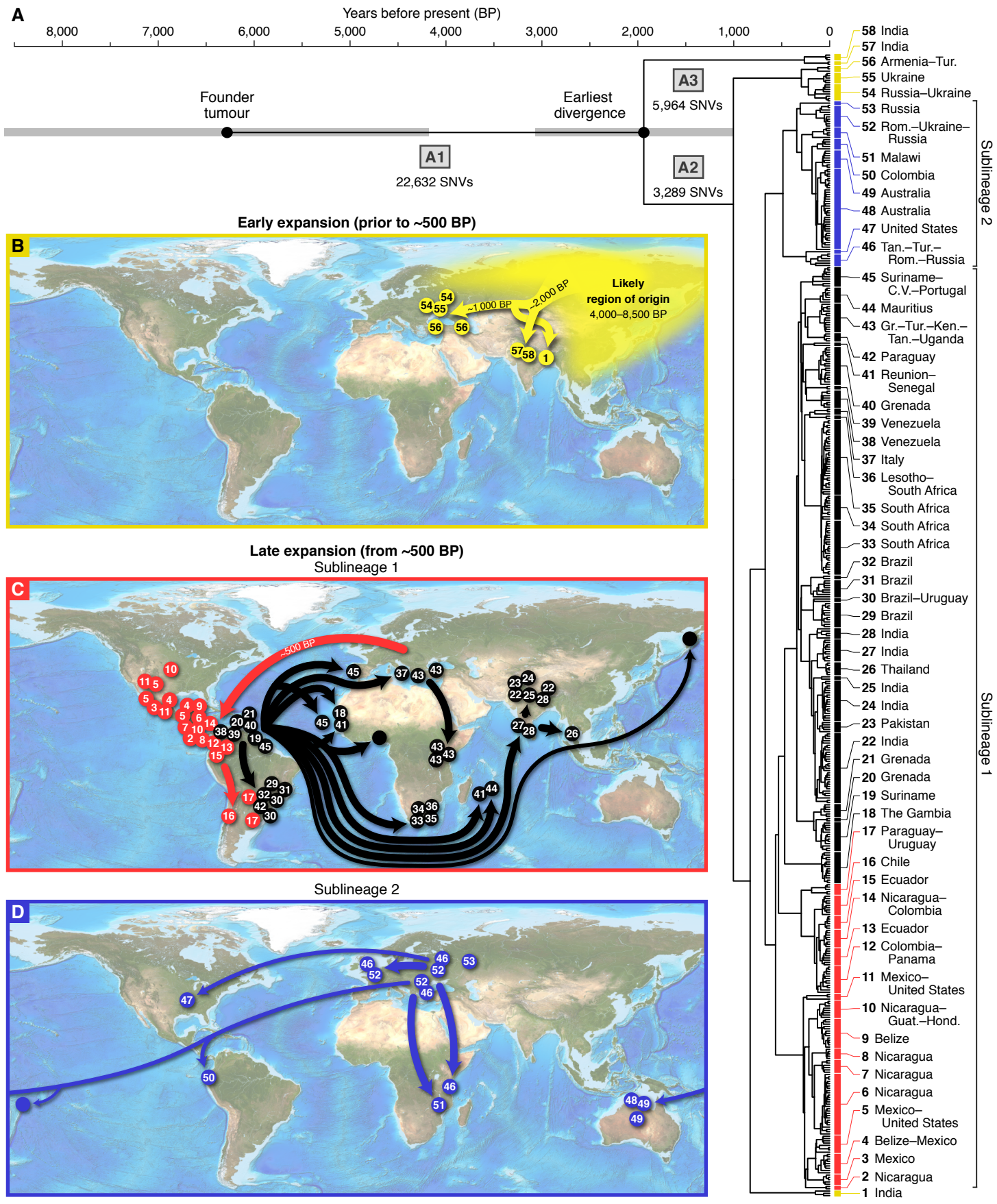
Main figure legends

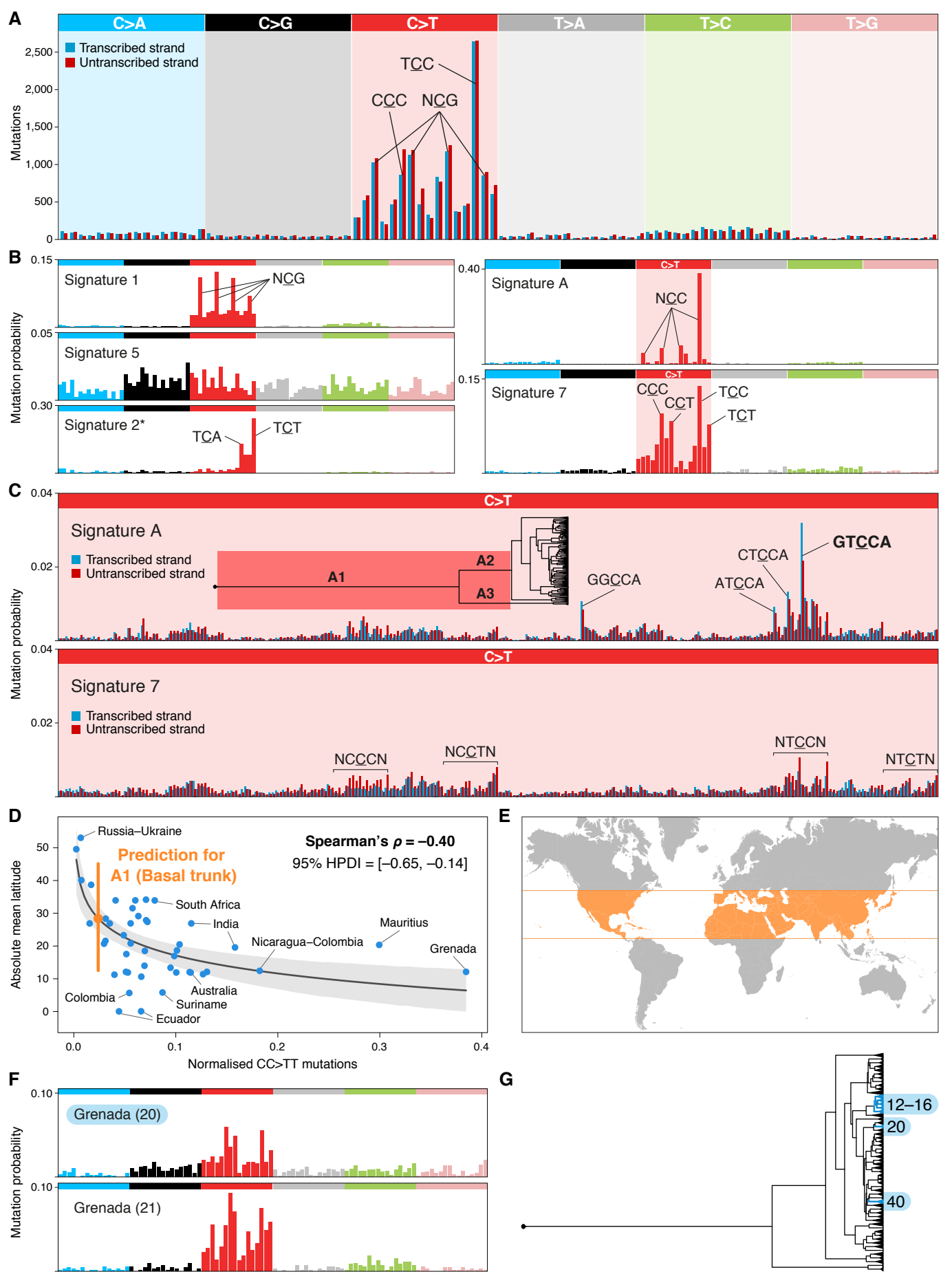
Fig. 1. Phylogeny and geographical expansion of CTVT. (**A**) Time-resolved phylogenetic tree inferred from clock-like exonic somatic variation in CTVT. Each tip is a tumour and sampling locations are labelled. Numbers refer to phylogenetic groups displayed on maps in **B**–**D**. Sublineages 1 and 2, referred to in **C** and **D** respectively, are marked. Three groups of ancestral somatic variation (A1, A2, A3) and their respective numbers of single nucleotide variants (SNVs) are indicated. The estimated age of the CTVT founder tumour and the earliest detected node are indicated in years before present (BP), with grey error bars depicting Bayesian 95% HPDI. (**B** to **D**) Maps presenting likely routes of early (prior to ~500 years BP) and late (from ~500 years BP) expansion of CTVT. Numbered circles indicate the geographical locations of phylogenetic groups labelled in **A**; arrows represent inferred geographical movements. Circle and arrow colours indicate different sets of geographical movements, as labelled in **A**. Thin arrows indicate expansion routes for which there is limited phylogenetic evidence; dots without numbers denote tumours that are not represented in the tree. C.V., Cape Verde; Gr., Greece; Guat., Guatemala; Hond., Honduras; Ken., Kenya; Rom., Romania; Tan., Tanzania; Tur., Turkey.

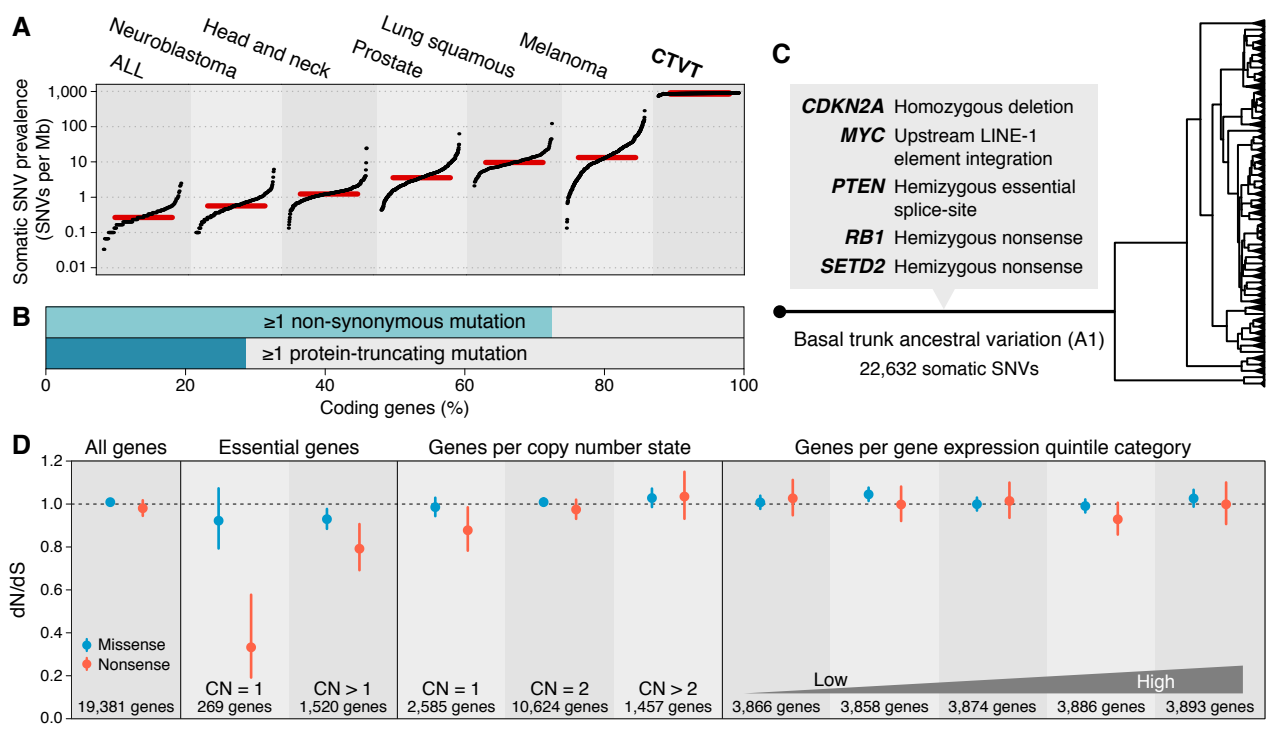
Fig. 2. Mutational processes in CTVT. (**A**) Trinucleotide-context mutational spectrum of somatic SNVs in a single CTVT tumour. Horizontal axis presents 96 mutation types displayed in pyrimidine context. Relevant trinucleotide mutation contexts are indicated. (**B**) Trinucleotide-context mutational spectra of extracted mutational signatures 1, 5, 2*, A and 7, with relevant trinucleotide mutation contexts indicated. (**C**) Pentanucleotide-context mutational spectra of signature A (top) and signature 7 (bottom). Horizontal axis presents 256 C>T mutation types with relevant mutation contexts indicated. The inset tree shows the phylogenetic branches with exposure to signature A. (**D**) Bayesian logarithmic regression and Spearman's correlation between absolute mean latitude and normalised CC>TT mutations in phylogenetic groups shown in Fig. 1A. Normalised CC>TT mutations represent the ratio between group-unique

CC>TT mutations and group-unique C>T changes at CpG dinucleotides. The black line and shadowed area indicate the regression curve and associated 95% HPDI. The orange dot and bars represent predicted absolute mean latitude and associated 90% prediction interval for the basal trunk ancestral variation (group A1). Posterior median and 95% HPDI of the correlation coefficient are shown. (**E**) Map showing the latitude range corresponding to the 90% prediction interval for group A1, presented in **D**, in the northern hemisphere. (**F**) Trinucleotide-context mutational spectra of a phylogenetic tumour group showing evidence of signature 5 hyperactivity (top) and a closely related group without signature 5 hyperactivity (bottom). (**G**) Diagram indicating the phylogenetic situation of the tumour groups displaying signature 5 hyperactivity.

Fig. 3. Selection in CTVT. (**A**) Somatic SNV prevalence across six human cancer types and CTVT. Dots represent individual tumours; red lines indicate median SNV prevalence. ALL, acute lymphoblastic leukaemia. (**B**) Bars showing the percentage of protein-coding genes in the CTVT genome harbouring ≥1 non-synonymous somatic mutation (SNV or indel; 14,412 genes) and ≥1 somatic protein-truncating somatic mutation (5,704 genes). (**C**) Diagram presenting the putative driver events found in the set of basal trunk ancestral variants (group A1, Fig. 1A). A description of each somatic alteration is shown next to the corresponding gene symbol. (**D**) Exome-wide dN/dS ratios estimated for somatic SNVs in all protein-coding genes (left) and in sets of genes defined according to gene essentiality, copy number state and expression level. Estimates of dN/dS are presented for missense (blue) and nonsense (orange) mutations in each gene group. The dashed line indicates dN/dS = 1 (neutrality); error bars indicate 95% confidence intervals.


A
Years before present (BP)
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
Founder tumour
Earliest divergence
A1
22,632 SNVs
A2
3,289 SNVs
A3
5,964 SNVs
Early expansion (prior to ~500 BP)
B
~1,000 BP
~2,000 BP
Likely region of origin
4,000–8,500 BP
Late expansion (from ~500 BP)
Sublineage 1
C
~500 BP
Sublineage 2
D
58 India
57 India
56 Armenia–Tur.
55 Ukraine
54 Russia–Ukraine
53 Russia
52 Rom.–Ukraine–Russia
51 Malawi
50 Colombia
49 Australia
48 Australia
47 United States
46 Tan.–Tur.–Rom.–Russia
45 Suriname–C.V.–Portugal
44 Mauritius
43 Gr.–Tur.–Ken.–Tan.–Uganda
42 Paraguay
41 Reunion–Senegal
40 Grenada
39 Venezuela
38 Venezuela
37 Italy
36 Lesotho–South Africa
35 South Africa
34 South Africa
33 South Africa
32 Brazil
31 Brazil
30 Brazil–Uruguay
29 Brazil
28 India
27 India
26 Thailand
25 India
24 India
23 Pakistan
22 India
21 Grenada
20 Grenada
19 Suriname
18 The Gambia
17 Paraguay–Uruguay
16 Chile
15 Ecuador
14 Nicaragua–Colombia
13 Ecuador
12 Colombia–Panama
11 Mexico–United States
10 Nicaragua–Guat.–Hond.
9 Belize
8 Nicaragua
7 Nicaragua
6 Nicaragua
5 Mexico–United States
4 Belize–Mexico
3 Mexico
2 Nicaragua
1 India
Sublineage 2
Sublineage 1


A
C>A
C>G
C>T
T>A
T>C
T>G
Transcribed strand
Untranscribed strand
Mutations
TCC
CCC
NCG
B
Mutation probability
Signature 1
NCG
Signature 5
Signature 2*
TCA
TCT
Signature A
NCC
Signature 7
CCC
CCT
TCC
TCT
C
Signature A
A1
A2
A3
GGCCA
CTCCA
ATCCA
GTCCA
Signature 7
NCCCN
NCCTN
NTCCN
NTCTN
D
Absolute mean latitude
Normalised CC>TT mutations
Spearman's ρ = −0.40
95% HPDI = [−0.65, −0.14]
Prediction for
A1 (Basal trunk)
Russia–Ukraine
South Africa
India
Mauritius
Nicaragua–Colombia
Grenada
Colombia
Australia
Suriname
Ecuador
E
F
Grenada (20)
Grenada (21)
G
12–16
20
40


A
ALL
Neuroblastoma
Head and neck
Prostate
Lung squamous
Melanoma
CTVT
Somatic SNV prevalence (SNVs per Mb)
1,000
100
10
1
0.1
0.01
B
≥1 non-synonymous mutation
≥1 protein-truncating mutation
0
20
40
60
80
100
Coding genes (%)
C
CDKN2A Homozygous deletion
MYC Upstream LINE-1 element integration
PTEN Hemizygous essential splice-site
RB1 Hemizygous nonsense
SETD2 Hemizygous nonsense
Basal trunk ancestral variation (A1)
22,632 somatic SNVs
D
All genes
Essential genes
Genes per copy number state
Genes per gene expression quintile category
dN/dS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Missense
Nonsense
19,381 genes
CN = 1
269 genes
CN > 1
1,520 genes
CN = 1
2,585 genes
CN = 2
10,624 genes
CN > 2
1,457 genes
Low
High
3,866 genes
3,858 genes
3,874 genes
3,886 genes
3,893 genes