Article

Pan-cancer analysis of whole genomes

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The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium

Cancer is driven by genetic change, and the advent of massively parallel sequencing has enabled systematic documentation of this variation at the whole-genome scale[1–3]. Here we report the integrative analysis of 2,658 whole-cancer genomes and their matching normal tissues across 38 tumour types from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA). We describe the generation of the PCAWG resource, facilitated by international data sharing using compute clouds. On average, cancer genomes contained 4–5 driver mutations when combining coding and non-coding genomic elements; however, in around 5% of cases no drivers were identified, suggesting that cancer driver discovery is not yet complete. Chromothripsis, in which many clustered structural variants arise in a single catastrophic event, is frequently an early event in tumour evolution; in acral melanoma, for example, these events precede most somatic point mutations and affect several cancer-associated genes simultaneously. Cancers with abnormal telomere maintenance often originate from tissues with low replicative activity and show several mechanisms of preventing telomere attrition to critical levels. Common and rare germline variants affect patterns of somatic mutation, including point mutations, structural variants and somatic retrotransposition. A collection of papers from the PCAWG Consortium describes non-coding mutations that drive cancer beyond those in the *TERT* promoter[4]; identifies new signatures of mutational processes that cause base substitutions, small insertions and deletions and structural variation[5,6]; analyses timings and patterns of tumour evolution[7]; describes the diverse transcriptional consequences of somatic mutation on splicing, expression levels, fusion genes and promoter activity[8,9]; and evaluates a range of more-specialized features of cancer genomes[8,10–18].

Cancer is the second most-frequent cause of death worldwide, killing more than 8 million people every year; the incidence of cancer is expected to increase by more than 50% over the coming decades[19,20]. 'Cancer' is a catch-all term used to denote a set of diseases characterized by autonomous expansion and spread of a somatic clone. To achieve this behaviour, the cancer clone must co-opt multiple cellular pathways that enable it to disregard the normal constraints on cell growth, modify the local microenvironment to favour its own proliferation, invade through tissue barriers, spread to other organs and evade immune surveillance[21]. No single cellular program directs these behaviours. Rather, there is a large pool of potential pathogenic abnormalities from which individual cancers draw their own combinations: the commonalities of macroscopic features across tumours belie a vastly heterogeneous landscape of cellular abnormalities.

This heterogeneity arises from the stochastic nature of Darwinian evolution. There are three preconditions for Darwinian evolution: characteristics must vary within a population; this variation must be heritable from parent to offspring; and there must be competition for survival within the population. In the context of somatic cells, heritable variation arises from mutations acquired stochastically throughout life, notwithstanding additional contributions from germline and epigenetic variation. A subset of these mutations alter the cellular phenotype, and a small subset of those variants confer an advantage on clones during the competition to escape the tight physiological controls wired into somatic cells. Mutations that provide a selective advantage to the clone are termed driver mutations, as opposed to selectively neutral passenger mutations.

Initial studies using massively parallel sequencing demonstrated the feasibility of identifying every somatic point mutation, copy-number change and structural variant (SV) in a given cancer[1–3]. In 2008, recognizing the opportunity that this advance in technology provided, the global cancer genomics community established the ICGC with the goal of systematically documenting the somatic mutations that drive common tumour types[22].

The pan-cancer analysis of whole genomes

The expansion of whole-genome sequencing studies from individual ICGC and TCGA working groups presented the opportunity to undertake a meta-analysis of genomic features across tumour types. To achieve this, the PCAWG Consortium was established. A Technical Working Group implemented the informatics analyses by aggregating the raw sequencing data from different working groups that studied individual tumour types, aligning the sequences to the human genome and delivering a set of high-quality somatic mutation calls for downstream analysis (Extended Data Fig. 1). Given the recent meta-analysis

A list of members and their affiliations appears in the online version of the paper and lists of working groups appear in the Supplementary Information.

Box 1

Online resources for data access, visualization and analysis

The PCAWG landing page (http://docs.icgc.org/pcawg) provides links to several data resources for interactive online browsing, analysis and download of PCAWG data and results (Supplementary Table 4).

Direct download of PCAWG data

Aligned PCAWG read data in BAM format are also available at the European Genome Phenome Archive (EGA; https://www.ebi.ac.uk/ega/search/site/pcawg under accession number EGAS00001001692). In addition, all open-tier PCAWG genomics data, as well as reference datasets used for analysis, can be downloaded from the ICGC Data Portal at http://docs.icgc.org/pcawg/data/. Controlled-tier genomic data, including SNVs and indels that originated from TCGA projects (in VCF format) and aligned reads (in BAM format) can be downloaded using the Score (https://www.overture.bio/) software package, which has accelerated and secure file transfer, as well as BAM slicing facilities to selectively download defined regions of genomic alignments.

PCAWG computational pipelines

The core alignment, somatic variant-calling, quality-control and variant consensus-generation pipelines used by PCAWG have each been packaged into portable cross-platform images using the Dockstore system[84] and released under an Open Source licence that enables unrestricted use and redistribution. All PCAWG Dockstore images are available to the public at https://dockstore.org/organizations/PCAWG/collections/PCAWG.

ICGC Data Portal

The ICGC Data Portal[85] (https://dcc.icgc.org) serves as the main entry point for accessing PCAWG datasets with a single uniform web interface and a high-performance data-download client. This uniform interface provides users with easy access to the myriad of PCAWG sequencing data and variant calls that reside in many repositories and compute clouds worldwide. Streaming technology[86] provides users with high-level visualizations in real time of BAM and VCF files stored remotely on the Cancer Genome Collaboratory.

UCSC Xena

UCSC Xena[87] (https://pcawg.xenahubs.net) visualizes all PCAWG primary results, including copy-number, gene-expression, gene-fusion and promoter-usage alterations, simple somatic mutations, large somatic structural variations, mutational signatures and phenotypic data. These open-access data are available through a public Xena hub, and consensus simple somatic mutations can be loaded to the local computer of a user via a private Xena hub. Kaplan–Meier plots, histograms, box plots, scatter plots and transcript-specific views offer additional visualization options and statistical analyses.

The Expression Atlas

The Expression Atlas (https://www.ebi.ac.uk/gxa/home) contains RNA-sequencing and expression microarray data for querying gene expression across tissues, cell types, developmental stages and/or experimental conditions[88]. Two different views of the data are provided: summarized expression levels for each tumour type and gene expression at the level of individual samples, including reference-gene expression datasets for matching normal tissues.

PCAWG Scout

PCAWG Scout (http://pcawgscout.bsc.es/) provides a framework for -omics workflow and website templating to generate on-demand, in-depth analyses of the PCAWG data that are openly available to the whole research community. Views of protected data are available that still safeguard sensitive data. Through the PCAWG Scout web interface, users can access an array of reports and visualizations that leverage on-demand bioinformatic computing infrastructure to produce results in real time, allowing users to discover trends as well as form and test hypotheses.

Chromothripsis Explorer

Chromothripsis Explorer (http://compbio.med.harvard.edu/chromothripsis/) is a portal that allows structural variation in the PCAWG dataset to be explored on an individual patient basis through the use of circos plots. Patterns of chromothripsis can also be explored in aggregated formats.

of exome data from the TCGA Pan-Cancer Atlas[23–25], scientific working groups concentrated their efforts on analyses best-informed by whole-genome sequencing data.

We collected genome data from 2,834 donors (Extended Data Table 1), of which 176 were excluded after quality assurance. A further 75 had minor issues that could affect some of the analyses (grey-listed donors) and 2,583 had data of optimal quality (white-listed donors) (Supplementary Table 1). Across the 2,658 white- and grey-listed donors, whole-genome sequencing data were available from 2,605 primary tumours and 173 metastases or local recurrences. Mean read coverage was 39× for normal samples, whereas tumours had a bimodal coverage distribution with modes at 38× and 60× (Supplementary Fig. 1). RNA-sequencing data were available for 1,222 donors. The final cohort comprised 1,469 men (55%) and 1,189 women (45%), with a mean age of 56 years (range, 1–90 years) across 38 tumour types (Extended Data Table 1 and Supplementary Table 1).

To identify somatic mutations, we analysed all 6,835 samples using a uniform set of algorithms for alignment, variant calling and quality control (Extended Data Fig. 1, Supplementary Fig. 2 and Supplementary Methods 2). We used three established pipelines to call somatic single-nucleotide variations (SNVs), small insertions and deletions (indels), copy-number alterations (CNAs) and SVs. Somatic retrotransposition events, mitochondrial DNA mutations and telomere lengths were also called by bespoke algorithms. RNA-sequencing data were uniformly processed to call transcriptomic alterations. Germline variants identified by the three separate pipelines included single-nucleotide polymorphisms, indels, SVs and mobile-element insertions (Supplementary Table 2).

The requirement to uniformly realign and call variants on approximately 5,800 whole genomes presented considerable computational challenges, and raised ethical issues owing to the use of data from different jurisdictions (Extended Data Table 2). We used cloud computing[26,27] to distribute alignment and variant calling across 13 data centres on 3 continents (Supplementary Table 3). Core pipelines were packaged into Docker containers[28] as reproducible, stand-alone packages, which we have made available for download. Data repositories for raw and derived datasets, together with portals for data visualization and exploration, have also been created (Box 1 and Supplementary Table 4).

Benchmarking of genetic variant calls

To benchmark mutation calling, we ran the 3 core pipelines, together with 10 additional pipelines, on 63 representative tumour–normal genome pairs (Supplementary Note 1). For 50 of these cases, we performed validation by hybridization of tumour and matched normal DNA to a custom bait set with deep sequencing[29]. The 3 core somatic variant-calling pipelines had individual estimates of sensitivity of 80–90% to detect a true somatic SNV called by any of the 13 pipelines; more

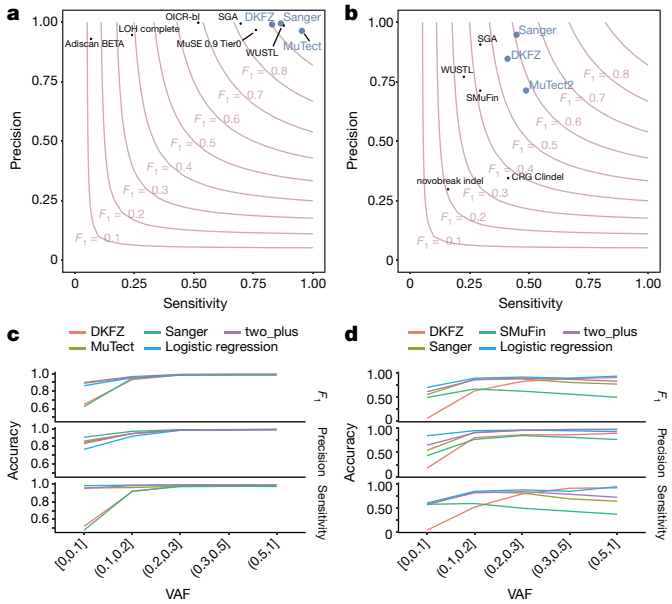

Fig. 1 | Validation of variant-calling pipelines in PCAWG. a, Scatter plot of estimated sensitivity and precision for somatic SNVs across individual algorithms assessed in the validation exercise across $n = 63$ PCAWG samples. Core algorithms included in the final PCAWG call set are shown in blue. **b**, Sensitivity and precision estimates across individual algorithms for somatic indels. **c**, Accuracy (precision, sensitivity and F_1 score, defined as 2 × sensitivity × precision/(sensitivity + precision)) of somatic SNV calls across variant allele fractions (VAFs) for the core algorithms. The accuracy of two methods of combining variant calls (two-plus, which was used in the final dataset, and logistic regression) is also shown. **d**, Accuracy of indel calls across variant allele fractions.

than 95% of SNV calls made by each of the core pipelines were genuine somatic variants (Fig. 1a). For indels—a more-challenging class of variants to identify with short-read sequencing—the 3 core algorithms had individual sensitivity estimates in the range of 40–50%, with precision of 70–95% (Fig. 1b). For individual SV algorithms, we estimated precision to be in the range 80–95% for samples in the 63-sample pilot dataset.

Next, we defined a strategy to merge results from the three pipelines into one final call-set to be used for downstream scientific analyses (Methods and Supplementary Note 2). Sensitivity and precision of consensus somatic variant calls were 95% (90% confidence interval, 88–98%) and 95% (90% confidence interval, 71–99%), respectively, for SNVs (Extended Data Fig. 2). For somatic indels, sensitivity and precision were 60% (34–72%) and 91% (73–96%), respectively (Extended Data Fig. 2). Regarding somatic SVs, we estimate the sensitivity of merged calls to be 90% for true calls generated by any one pipeline; precision was estimated as 97.5%. The improvement in calling accuracy from combining different pipelines was most noticeable in variants with low variant allele fractions, which probably originate from tumour subclones (Fig. 1c, d). Germline variant calls, phased using a haplotype-reference panel, displayed a precision of more than 99% and a sensitivity of 92–98% (Supplementary Note 2).

Analysis of PCAWG data

The uniformly generated, high-quality set of variant calls across more than 2,500 donors provided the springboard for a series of scientific working groups to explore the biology of cancer. A comprehensive suite of companion papers that describe the analyses and discoveries across these thematic areas is copublished with this paper[4–18] (Extended Data Table 3).

Pan-cancer burden of somatic mutations

Across the 2,583 white-listed PCAWG donors, we called 43,778,859 somatic SNVs, 410,123 somatic multinucleotide variants, 2,418,247 somatic indels, 288,416 somatic SVs, 19,166 somatic retrotransposition events and 8,185 de novo mitochondrial DNA mutations (Supplementary Table 1). There was considerable heterogeneity in the burden of somatic mutations across patients and tumour types, with a broad correlation in mutation burden among different classes of somatic variation (Extended Data Fig. 3). Analysed at a per-patient level, this correlation held, even when considering tumours with similar purity and ploidy (Supplementary Fig. 3). Why such correlation should apply on a pan-cancer basis is unclear. It is likely that age has some role, as we observe a correlation between most classes of somatic mutation and age at diagnosis (around 190 SNVs per year, $P = 0.02$; about 22 indels per year, $P = 5 \times 10^{-5}$; 1.5 SVs per year, $P < 2 \times 10^{-16}$; linear regression with likelihood ratio tests; Supplementary Fig. 4). Other factors are also likely to contribute to the correlations among classes of somatic mutation, as there is evidence that some DNA-repair defects can cause multiple types of somatic mutation[30], and a single carcinogen can cause a range of DNA lesions[31].

Panorama of driver mutations in cancer

We extracted the subset of somatic mutations in PCAWG tumours that have high confidence to be driver events on the basis of current knowledge. One challenge to pinpointing the specific driver mutations in an individual tumour is that not all point mutations in recurrently mutated cancer-associated genes are drivers[32]. For genomic elements significantly mutated in PCAWG data, we developed a 'rank-and-cut' approach to identify the probable drivers (Supplementary Methods 8.1). This approach works by ranking the observed mutations in a given genomic element based on recurrence, estimated functional consequence and expected pattern of drivers in that element. We then estimate the excess burden of somatic mutations in that genomic element above that expected for the background mutation rate, and cut the ranked mutations at this level. Mutations in each element with the highest driver ranking were then assigned as probable drivers; those below the threshold will probably have arisen through chance and were assigned as probable passengers. Improvements to features that are used to rank the mutations and the methods used to measure them will contribute to further development of the rank-and-cut approach.

We also needed to account for the fact that some bona fide cancer genomic elements were not rediscovered in PCAWG data because of low statistical power. We therefore added previously known cancer-associated genes to the discovery set, creating a 'compendium of mutational driver elements' (Supplementary Methods 8.2). Then, using stringent rules to nominate driver point mutations that affect these genomic elements on the basis of prior knowledge[33], we separated probable driver from passenger point mutations. To cover all classes of variant, we also created a compendium of known driver SVs, using analogous rules to identify which somatic CNAs and SVs are most likely to act as drivers in each tumour. For probable pathogenic germline variants, we identified all truncating germline point mutations and SVs that affect high-penetrance germline cancer-associated genes.

This analysis defined a set of mutations that we could confidently assert, based on current knowledge, drove tumorigenesis in the more than 2,500 tumours of PCAWG. We found that 91% of tumours had at least one identified driver mutation, with an average of 4.6 drivers per tumour identified, showing extensive variation across cancer types (Fig. 2a). For coding point mutations, the average was 2.6 drivers per tumour, similar to numbers estimated in known cancer-associated genes in tumours in the TCGA using analogous approaches[32].

To address the frequency of non-coding driver point mutations, we combined promoters and enhancers that are known targets of

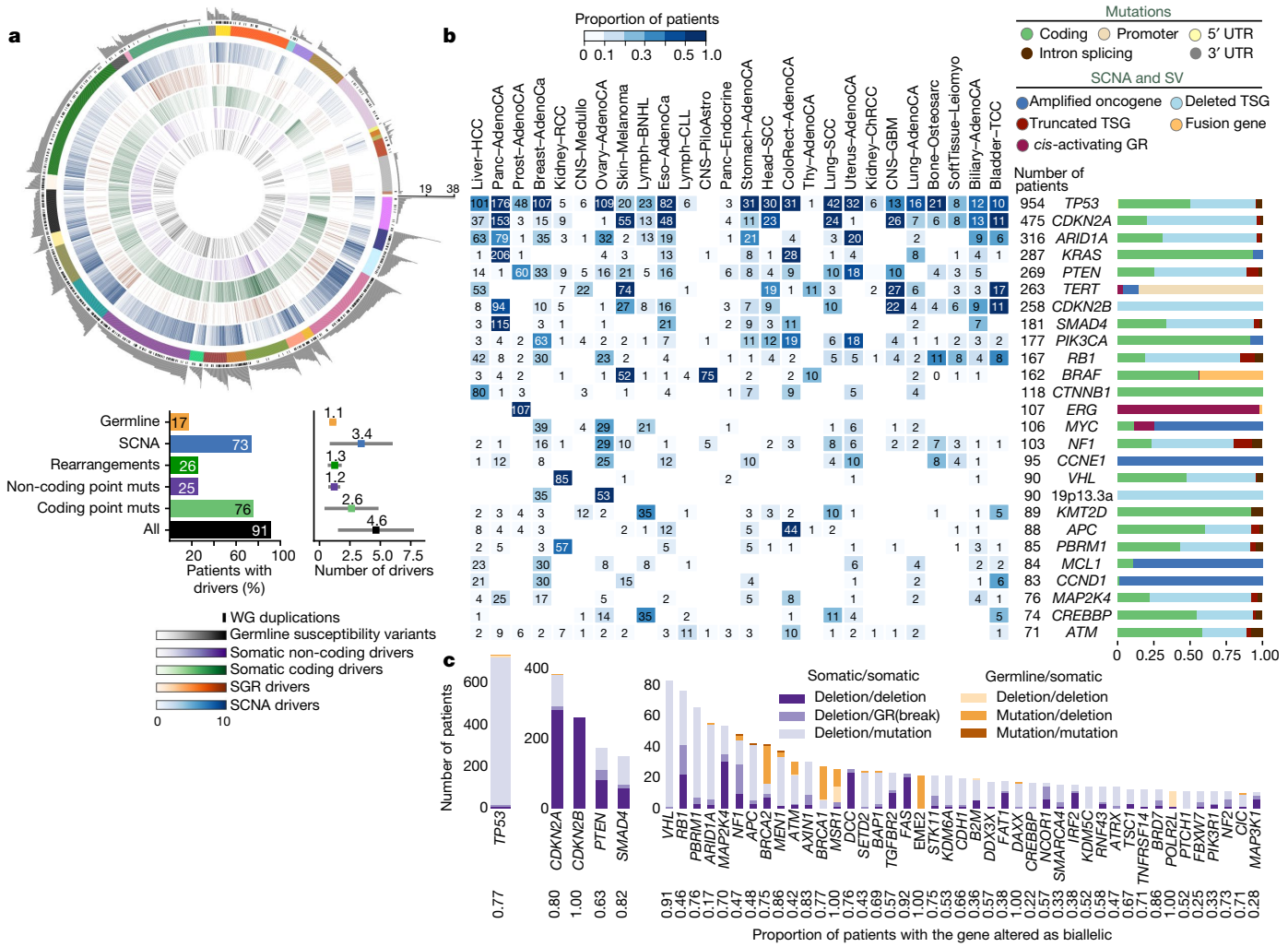

Fig. 2 | Panorama of driver mutations in PCAWG. a, Top, putative driver mutations in PCAWG, represented as a circos plot. Each sector represents a tumour in the cohort. From the periphery to the centre of the plot the concentric rings represent: (1) the total number of driver alterations; (2) the presence of whole-genome (WG) duplication; (3) the tumour type; (4) the number of driver CNAs; (5) the number of driver genomic rearrangements; (6) driver coding point mutations; (7) driver non-coding point mutations; and (8) pathogenic germline variants. Bottom, snapshots of the panorama of driver mutations. The horizontal bar plot (left) represents the proportion of patients with different types of drivers. The dot plot (right) represents the mean number of each type of driver mutation across tumours with at least one event (the square dot) and the standard deviation (grey whiskers), based on $n = 2{,}583$ patients. **b**, Genomic elements targeted by different types of mutations in the cohort altered in more than 65 tumours. Both germline and somatic variants are included. Left, the heat map shows the recurrence of alterations across cancer types. The colour indicates the proportion of mutated tumours and the number indicates the absolute count of mutated tumours. Right, the proportion of each type of alteration that affects each genomic element. **c**, Tumour-suppressor genes with biallelic inactivation in 10 or more patients. The values included under the gene labels represent the proportions of patients who have biallelic mutations in the gene out of all patients with a somatic mutation in that gene. GR, genomic rearrangement; SCNA, somatic copy-number alteration; SGR, somatic genome rearrangement; TSG, tumour suppressor gene; UTR, untranslated region.

non-coding drivers[34–37] with those newly discovered in PCAWG data; this is reported in a companion paper[4]. Using this approach, only 13% (785 out of 5,913) of driver point mutations were non-coding in PCAWG. Nonetheless, 25% of PCAWG tumours bear at least one putative non-coding driver point mutation, and one third (237 out of 785) affected the *TERT* promoter (9% of PCAWG tumours). Overall, non-coding driver point mutations are less frequent than coding driver mutations. With the exception of the *TERT* promoter, individual enhancers and promoters are only infrequent targets of driver mutations[4].

Across tumour types, SVs and point mutations have different relative contributions to tumorigenesis. Driver SVs are more prevalent in breast adenocarcinomas (6.4 ± 3.7 SVs (mean ± s.d.) compared with 2.2 ± 1.3 point mutations; $P < 1 \times 10^{-16}$, Mann–Whitney *U*-test) and ovary adenocarcinomas (5.8 ± 2.6 SVs compared with 1.9 ± 1.0 point mutations; $P < 1 \times 10^{-16}$), whereas driver point mutations have a larger contribution in colorectal adenocarcinomas (2.4 ± 1.4 SVs compared with 7.4 ± 7.0 point mutations; $P = 4 \times 10^{-10}$) and mature B cell lymphomas (2.2 ± 1.3 SVs compared with 6 ± 3.8 point mutations; $P < 1 \times 10^{-16}$), as previously shown[38]. Across tumour types, there are differences in which classes of mutation affect a given genomic element (Fig. 2b).

We confirmed that many driver mutations that affect tumour-suppressor genes are two-hit inactivation events (Fig. 2c). For example, of the 954 tumours in the cohort with driver mutations in *TP53*, 736 (77%) had both alleles mutated, 96% of which (707 out of 736) combined a somatic point mutation that affected one allele with somatic deletion of the other allele. Overall, 17% of patients had rare germline protein-truncating variants (PTVs) in cancer-predisposition genes[39], DNA-damage response genes[40] and somatic driver genes. Biallelic inactivation due to somatic alteration on top of a germline PTV was observed in 4.5% of patients overall, with 81% of

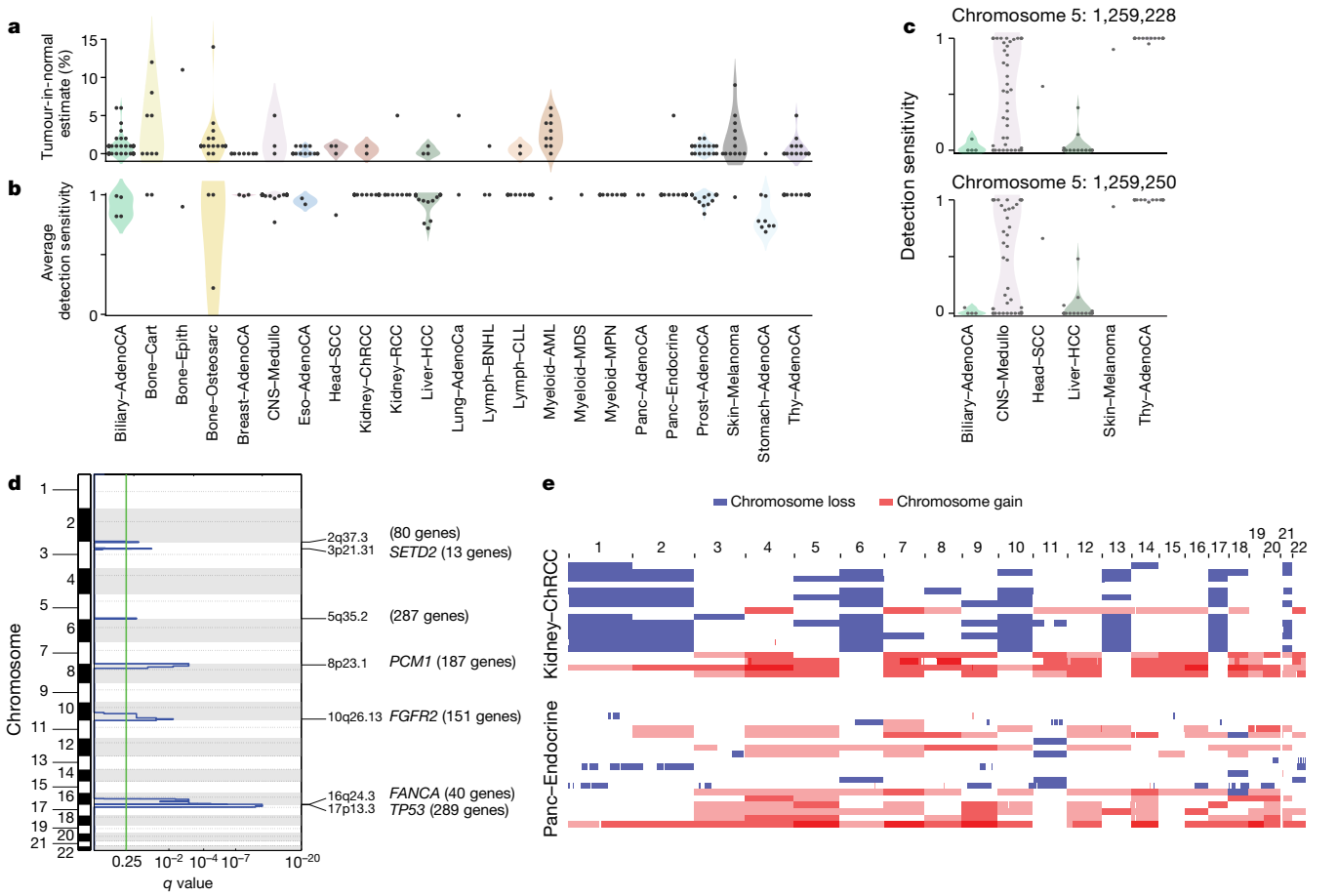

Fig. 3 | Analysis of patients with no detected driver mutations. a, Individual estimates of the percentage of tumour-in-normal contamination across patients with no driver mutations in PCAWG ($n = 181$). No data were available for myelodysplastic syndromes and acute myeloid leukaemia. Points represent estimates for individual patients, and the coloured areas are estimated density distributions (violin plots). Abbreviations of the tumour types are defined in Extended Data Table 1. **b**, Average detection sensitivity by tumour type for tumours without known drivers ($n = 181$). Each dot represents a given sample and is the average sensitivity of detecting clonal substitutions across the genome, taking into account purity and ploidy. Coloured areas are estimated density distributions, shown for cohorts with at least five cases. **c**, Detection sensitivity for *TERT* promoter hotspots in tumour types in which *TERT* is frequently mutated. Coloured areas are estimated density distributions. **d**, Significant copy-number losses identified by two-sided hypothesis testing using GISTIC2.0, corrected for multiple-hypothesis testing. Numbers in parentheses indicate the number of genes in significant regions when analysing medulloblastomas without known drivers ($n = 42$). Significant regions with known cancer-associated genes are labelled with the representative cancer-associated gene. **e**, Aneuploidy in chromophobe renal cell carcinomas and pancreatic neuroendocrine tumours without known drivers. Patients are ordered on the *y* axis by tumour type and then by presence of whole-genome duplication (bottom) or not (top).

these affecting known cancer-predisposition genes (such as *BRCA1*, *BRCA2* and *ATM*).

PCAWG tumours with no apparent drivers

Although more than 90% of PCAWG cases had identified drivers, we found none in 181 tumours (Extended Data Fig. 4a). Reasons for missing drivers have not yet been systematically evaluated in a pan-cancer cohort, and could arise from either technical or biological causes.

Technical explanations could include poor-quality samples, inadequate sequencing or failures in the bioinformatic algorithms used. We assessed the quality of the samples and found that 4 of the 181 cases with no known drivers had more than 5% tumour DNA contamination in their matched normal sample (Fig. 3a). Using an algorithm designed to correct for this contamination[41], we identified previously missed mutations in genes relevant to the respective cancer types. Similarly, if the fraction of tumour cells in the cancer sample is low through stromal contamination, the detection of driver mutations can be impaired. Most tumours with no known drivers had an average power to detect mutations close to 100%; however, a few had power in the 70–90% range (Fig. 3b and Extended Data Fig. 4b). Even in adequately sequenced genomes, lack of read depth at specific driver loci can impair mutation detection. For example, only around 50% of PCAWG tumours had sufficient coverage to call a mutation (≥90% power) at the two *TERT* promoter hotspots, probably because the high GC content of this region causes biased coverage (Fig. 3c). In fact, 6 hepatocellular carcinomas and 2 biliary cholangiocarcinomas among the 181 cases with no known drivers actually did contain *TERT* mutations, which were discovered after deep targeted sequencing[42].

Finally, technical reasons for missing driver mutations include failures in the bioinformatic algorithms. This affected 35 myeloproliferative neoplasms in PCAWG, in which the $JAK2^{V617F}$ driver mutation should have been called. Our somatic variant-calling algorithms rely on 'panels of normals', typically from blood samples, to remove recurrent sequencing artefacts. As 2–5% of healthy individuals carry occult haematopoietic clones[43], recurrent driver mutations in these clones can enter panels of normals.

With regard to biological causes, tumours may be driven by mutations in cancer-associated genes that are not yet described for that tumour type. Using driver discovery algorithms on tumours with no known drivers, no individual genes reached significance for point mutations. However, we identified a recurrent CNA that spanned *SETD2* in

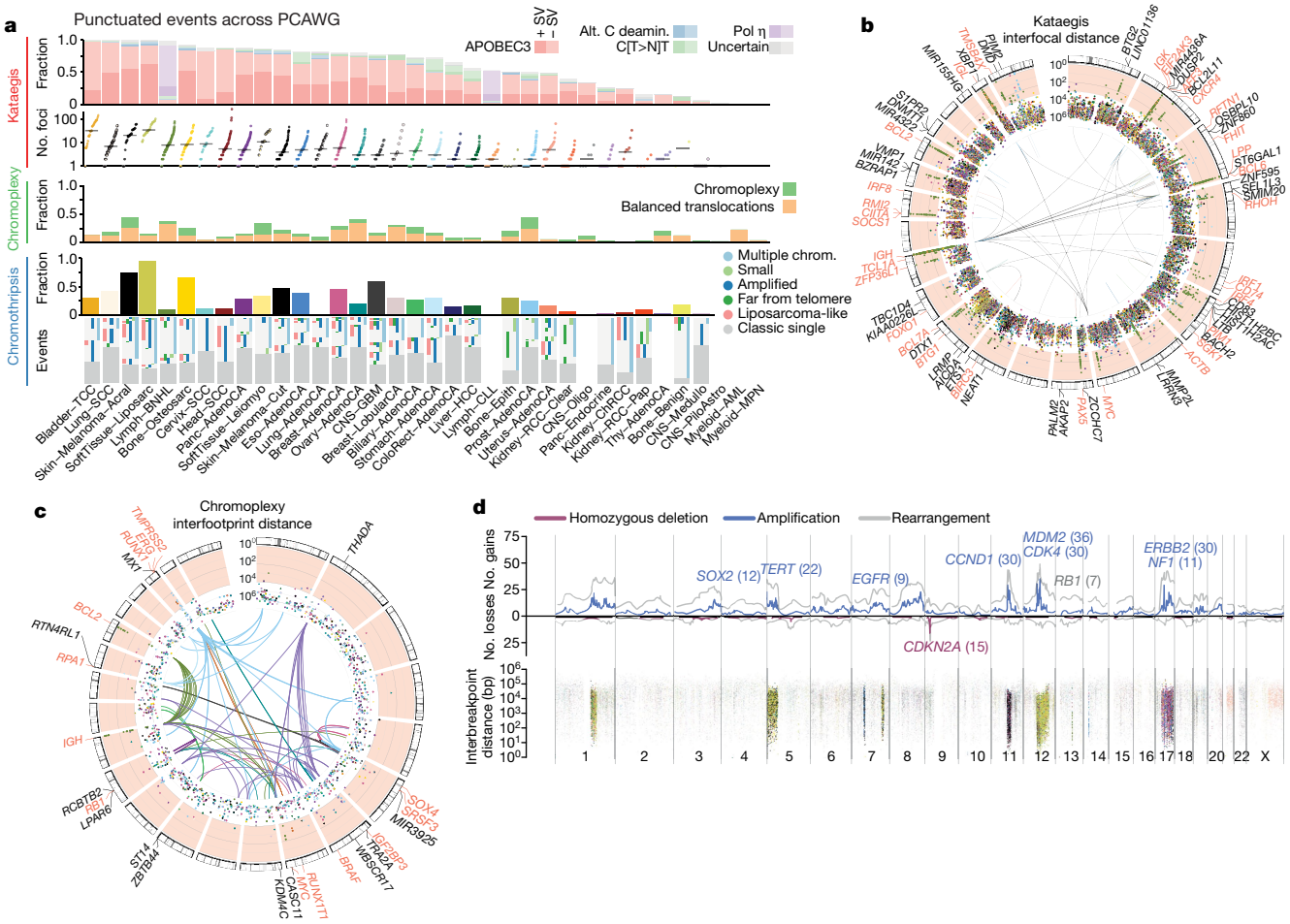

Fig. 4 | Patterns of clustered mutational processes in PCAWG. a, Kataegis. Top, prevalence of different types of kataegis and their association with SVs (≤1 kb from the focus). Bottom, the distribution of the number of foci of kataegis per sample. Chromoplexy. Prevalence of chromoplexy across cancer types, subdivided into balanced translocations and more complex events. Chromothripsis. Top, frequency of chromothripsis across cancer types. Bottom, for each cancer type a column is shown, in which each row is a chromothripsis region represented by five coloured rectangles relating to its categorization. **b**, Circos rainfall plot showing the distances between consecutive kataegis events across PCAWG compared with their genomic position. Lymphoid tumours (khaki, B cell non-Hodgkin's lymphoma; orange, chronic lymphocytic leukaemia) have hypermutation hot spots (≥3 foci with distance ≤1 kb; pale red zone), many of which are near known cancer-associated genes (red annotations) and have associated SVs (≤10 kb from the focus; shown as arcs in the centre). **c**, Circos rainfall plot as in **b** that shows the distance versus the position of consecutive chromoplexy and reciprocal translocation footprints across PCAWG. Lymphoid, prostate and thyroid cancers exhibit recurrent events (≥2 footprints with distance ≤10 kb; pale red zone) that are likely to be driver SVs and are annotated with nearby genes and associated SVs, which are shown as bold and thin arcs for chromoplexy and reciprocal translocations, respectively (colours as in **a**). **d**, Effect of chromothripsis along the genome and involvement of PCAWG driver genes. Top, number of chromothripsis-induced gains or losses (grey) and amplifications (blue) or deletions (red). Within the identified chromothripsis regions, selected recurrently rearranged (light grey), amplified (blue) and homozygously deleted (magenta) driver genes are indicated. Bottom, interbreakpoint distance between all subsequent breakpoints within chromothripsis regions across cancer types, coloured by cancer type. Regions with an average interbreakpoint distance <10 kb are highlighted. C[T>N]T, kataegis with a pattern of thymine mutations in a Cp **T**pT context.

medulloblastomas that lacked known drivers (Fig. 3d), indicating that restricting hypothesis testing to missing-driver cases can improve power if undiscovered genes are enriched in such tumours. Inactivation of *SETD2* in medulloblastoma significantly decreased gene expression ($P = 0.002$) (Extended Data Fig. 4c). Notably, *SETD2* mutations occurred exclusively in medulloblastoma group-4 tumours ($P < 1 \times 10^{-4}$). Group-4 medulloblastomas are known for frequent mutations in other chromatin-modifying genes[44], and our results suggest that *SETD2* loss of function is an additional driver that affects chromatin regulators in this subgroup.

Two tumour types had a surprisingly high fraction of patients without identified driver mutations: chromophobe renal cell carcinoma (44%; 19 out of 43) and pancreatic neuroendocrine cancers (22%; 18 out of 81) (Extended Data Fig. 4a). A notable feature of the missing-driver cases in both tumour types was a remarkably consistent profile of chromosomal aneuploidy—patterns that have previously been reported[45,46] (Fig. 3e). The absence of other identified driver mutations in these patients raises the possibility that certain combinations of whole-chromosome gains and losses may be sufficient to initiate a cancer in the absence of more-targeted driver events such as point mutations or fusion genes of focal CNAs.

Even after accounting for technical issues and novel drivers, 5.3% of PCAWG tumours still had no identifiable driver events. In a research setting, in which we are interested in drawing conclusions about populations of patients, the consequences of technical issues that affect occasional samples will be mitigated by sample size. In a clinical setting, in which we are interested in the driver mutations in a specific patient, these issues become substantially more important. Careful and critical appraisal of the whole pipeline—including sample acquisition, genome sequencing, mapping, variant calling and driver annotation, as done

As reported in a companion paper[13], 16% of tumours in the PCAWG dataset exhibited somatic mutations in at least one of *ATRX*, *DAXX* and *TERT*. *TERT* alterations were detected in 270 samples, whereas 128 tumours had alterations in *ATRX* or *DAXX*, of which 71 were protein-truncating. In the companion paper, which focused on describing patterns of ALT and *TERT*-mediated telomere maintenance[13], 12 features of telomeric sequence were measured in the PCAWG cohort. These included counts of nine variants of the core hexameric sequence, the number of ectopic telomere-like insertions within the genome, the number of genomic breakpoints and telomere length as a ratio between tumour and normal. Here we used the 12 features as an overview of telomere integrity across all tumours in the PCAWG dataset.

On the basis of these 12 features, tumour samples formed 4 distinct subclusters (Fig. 7a and Extended Data Fig. 13a), suggesting that telomere-maintenance mechanisms are more diverse than the well-established *TERT* and ALT dichotomy. Clusters C1 (47 tumours) and C2 (42 tumours) were enriched for traits of the ALT pathway—having longer telomeres, more genomic breakpoints, more ectopic telomere insertions and variant telomere sequence motifs (Supplementary Fig. 9). C1 and C2 were distinguished from one another by the latter having a considerable increase in the number of TTCGGG and TGAGGG variant motifs among the telomeric hexamers. Thyroid adenocarcinomas were markedly enriched among C3 samples (26 out of 33 C3 samples; $P < 10^{-16}$); the C1 cluster (ALT subtype 1) was common among sarcomas; and both pancreatic endocrine neoplasms and low-grade gliomas had a high proportion of samples in the C2 cluster (ALT subtype 2) (Fig. 7b). Notably, some of the thyroid adenocarcinomas and pancreatic neuroendocrine tumours that cluster together (cluster C3) had matched normal samples that also cluster together (normal cluster N3) (Extended Data Fig. 13a) and which share common properties. For example, the GTAGGG repeat was overrepresented among samples in this group (Supplementary Fig. 10).

Somatic driver mutations were also unevenly distributed across the four clusters (Fig. 7c). C1 tumours were enriched for *RB1* mutations or SVs ($P = 3 \times 10^{-5}$), as well as frequent SVs that affected *ATRX* ($P = 6 \times 10^{-14}$), but not *DAXX*. *RB1* and *ATRX* mutations were largely mutually exclusive (Extended Data Fig. 13b). By contrast, C2 tumours were enriched for somatic point mutations in *ATRX* and *DAXX* ($P = 6 \times 10^{-5}$), but not *RB1*. The enrichment of *RB1* mutations in C1 remained significant when only leiomyosarcomas and osteosarcomas were considered, confirming that this enrichment is not merely a consequence of the different distribution of tumour types across clusters. C3 samples had frequent *TERT* promoter mutations (30%; $P = 2 \times 10^{-6}$).

There was a marked predominance of *RB1* mutations in C1. Nearly a third of the samples in C1 contained an *RB1* alteration, which were evenly distributed across truncating SNVs, SVs and shallow deletions (Extended Data Fig. 13c). Previous research has shown that *RB1* mutations are associated with long telomeres in the absence of *TERT* mutations and *ATRX* inactivation[80], and studies using mouse models have shown that knockout of Rb-family proteins causes elongated telomeres[81]. The association with the C1 cluster here suggests that *RB1* mutations can represent another route to activating the ALT pathway, which has subtly different properties of telomeric sequence compared with the inactivation of *DAXX*—these fall almost exclusively in cluster C2.

Tumour types with the highest rates of abnormal telomere maintenance mechanisms often originate in tissues that have low endogenous replicative activity (Fig. 7d). In support of this, we found an inverse correlation between previously estimated rates of stem cell division across tissues[82] and the frequency of telomere maintenance abnormalities ($P = 0.01$, Poisson regression) (Extended Data Fig. 13d). This suggests that restriction of telomere maintenance is an important tumour-suppression mechanism, particularly in tissues with low steady-state cellular proliferation, in which a clone must overcome this constraint to achieve replicative immortality.

Conclusions and future perspectives

The resource reported in this paper and its companion papers has yielded insights into the nature and timing of the many mutational processes that shape large- and small-scale somatic variation in the cancer genome; the patterns of selection that act on these variations; the widespread effect of somatic variants on transcription; the complementary roles of the coding and non-coding genome for both germline and somatic mutations; the ubiquity of intratumoral heterogeneity; and the distinctive evolutionary trajectory of each cancer type. Many of these insights can be obtained only from an integrated analysis of all classes of somatic mutation on a whole-genome scale, and would not be accessible with, for example, targeted exome sequencing.

The promise of precision medicine is to match patients to targeted therapies using genomics. A major barrier to its evidence-based implementation is the daunting heterogeneity of cancer chronicled in these papers, from tumour type to tumour type, from patient to patient, from clone to clone and from cell to cell. Building meaningful clinical predictors from genomic data can be achieved, but will require knowledge banks comprising tens of thousands of patients with comprehensive clinical characterization[83]. As these sample sizes will be too large for any single funding agency, pharmaceutical company or health system, international collaboration and data sharing will be required. The next phase of ICGC, ICGC-ARGO (https:// www.icgc-argo.org/), will bring the cancer genomics community together with healthcare providers, pharmaceutical companies, data science and clinical trials groups to build comprehensive knowledge banks of clinical outcome and treatment data from patients with a wide variety of cancers, matched with detailed molecular profiling.

Extending the story begun by TCGA, ICGC and other cancer genomics projects, the PCAWG has brought us closer to a comprehensive narrative of the causal biological changes that drive cancer phenotypes. We must now translate this knowledge into sustainable, meaningful clinical treatments.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-1969-6.

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Methods

Samples

We compiled an inventory of matched tumour–normal whole-cancer genomes in the ICGC Data Coordinating Centre. Most samples came from treatment-naive, primary cancers, although a small number of donors had multiple samples of primary, metastatic and/or recurrent tumours. Our inclusion criteria were: (1) matched tumour and normal specimen pair; (2) a minimal set of clinical fields; and (3) characterization of tumour and normal whole genomes using Illumina HiSeq paired-end sequencing reads.

We collected genome data from 2,834 donors, representing all ICGC and TCGA donors that met these criteria at the time of the final data freeze in autumn 2014 (Extended Data Table 1). After quality assurance (Supplementary Methods 2.5), data from 176 donors were excluded as unusable, 75 had minor issues that could affect some analyses (grey-listed donors) and 2,583 had data of optimal quality (white-listed donors) (Supplementary Table 1). Across the 2,658 white- and grey-listed donors, whole-genome sequences were available from 2,605 primary tumours and 173 metastases or local recurrences. Matching normal samples were obtained from blood (2,064 donors), tissue adjacent to the primary tumour (87 donors) or from distant sites (507 donors). Whole-genome sequencing data were available for tumour and normal DNA for the entire cohort. The mean read coverage was 39× for normal samples, whereas tumours had a bimodal coverage distribution with modes at 38× and 60× (Supplementary Fig. 1). The majority of specimens (65.3%) were sequenced using 101-bp paired-end reads. An additional 28% were sequenced with 100-bp paired-end reads. Of the remaining specimens, 4.7% were sequenced with read lengths longer than 101 bp, and 1.9% with read lengths shorter than 100 bp. The distribution of read lengths by tumour cohort is shown in Supplementary Fig. 11. Median read length for whole-genome sequencing paired-end reads was 101 bp (mean = 106.2, s.d. = 16.7; minimum–maximum = 50–151). RNA-sequencing data were collected and re-analysed centrally for 1,222 donors, including 1,178 primary tumours, 67 metastases or local recurrences and 153 matched normal tissue samples adjacent to the primary tumour.

Demographically, the cohort included 1,469 men (55%) and 1,189 women (45%), with a mean age of 56 years (range, 1–90 years) (Supplementary Table 1). Using population ancestry-differentiated single nucleotide polymorphisms, the ancestry distribution was heavily weighted towards donors of European descent (77% of total) followed by East Asians (16%), as expected for large contributions from European, North American and Australian projects (Supplementary Table 1).

We consolidated histopathology descriptions of the tumour samples, using the ICD-0-3 tumour site controlled vocabulary[89]. Overall, the PCAWG dataset comprises 38 distinct tumour types (Extended Data Table 1 and Supplementary Table 1). Although the most common tumour types are included in the dataset, their distribution does not match the relative population incidences, largely owing to differences among contributing ICGC/TCGA groups in the numbers of sequenced samples.

Uniform processing and somatic variant calling

To generate a consistent set of somatic mutation calls that could be used for cross-tumour analyses, we analysed all 6,835 samples using a uniform set of algorithms for alignment, variant calling and quality control (Extended Data Fig. 1, Supplementary Fig. 2, Supplementary Table 3 and Supplementary Methods 2). We used the BWA-MEM algorithm[90] to align each tumour and normal sample to human reference build hs37d5 (as used in the 1000 Genomes Project[91]). Somatic mutations were identified in the aligned data using three established pipelines, which were run independently on each tumour–normal pair. Each of the three pipelines—labelled 'Sanger'[92–95], 'EMBL/DKFZ'[96,97] and 'Broad'[98–101] after the computational biology groups that created or assembled them—consisted of multiple software packages for calling somatic SNVs, small indels, CNAs and somatic SVs (with intrachromosomal SVs defined as those >100 bp). Two additional variant algorithms[102,103] were included to further improve accuracy across a broad range of clonal and subclonal mutations. We tested different merging strategies using validation data, and choses the optimal method for each variant type to generate a final consensus set of mutation calls (Supplementary Methods S2.4).

Somatic retrotransposition events, including Alu and LINE-1 insertions[72], L1-mediated transductions[73] and pseudogene formation[104], were called using a dedicated pipeline[73]. We removed these retrotransposition events from the somatic SV call-set. Mitochondrial DNA mutations were called using a published algorithm[105]. RNA-sequencing data were uniformly processed to quantify normalized gene-level expression, splicing variation and allele-specific expression, and to identify fusion transcripts, alternative promoter usage and sites of RNA editing[8].

Integration, phasing and validation of germline variant call-sets

Calls of common (≥1% frequency in PCAWG) and rare (<1%) germline variants including single-nucleotide polymorphisms, indels, SVs and mobile-element insertions (MEIs) were generated using a population-scale genetic polymorphism-detection approach[91,106]. The uniform germline data-processing workflow comprised variant identification using six different variant-calling algorithms[96,107,108] and was orchestrated using the Butler workflow system[109].

We performed call-set benchmarking, merging, variant genotyping and statistical haplotype-block phasing[91] (Supplementary Methods 3.4). Using this strategy, we identified 80.1 million germline single-nucleotide polymorphisms, 5.9 million germline indels, 1.8 million multi-allelic short (<50 bp) germline variants, as well as germline SVs ≥ 50 bp in size including 29,492 biallelic deletions and 27,254 MEIs (Supplementary Table 2). We statistically phased this germline variant set using haplotypes from the 1000 Genomes Project[91] as a reference panel, yielding an N50-phased block length of 265 kb based on haploid chromosomes from donor-matched tumour genomes. Precision estimates for germline SNVs and indels were >99% for the phased merged call-set, and sensitivity estimates ranged from 92% to 98%.

Core alignment and variant calling by cloud computing

The requirement to uniformly realign and call variants on nearly 5,800 whole genomes (tumour plus normal) presented considerable computational challenges, and raised ethical issues owing to the use of data from different jurisdictions (Extended Data Table 2). To process the data, we adopted a cloud-computing architecture[26] in which the alignment and variant calling was spread across 13 data centres on 3 continents, representing a mixture of commercial, infrastructure-as-a-service, academic cloud compute and traditional academic high-performance computer clusters (Supplementary Table 3). Together, the effort used 10 million CPU-core hours.

To generate reproducible variant calling across the 13 data centres, we built the core pipelines into Docker containers[28], in which the workflow description, required code and all associated dependencies were packaged together in stand-alone packages. These heavily tested, extensively validated workflows are available for download (Box 1).

Validation, benchmarking and merging of somatic variant calls

To evaluate the performance of each of the mutation-calling pipelines and determine an integration strategy, we performed a large-scale deep-sequencing validation experiment (Supplementary Notes 1). We selected a pilot set of 63 representative tumour–normal pairs, on which we ran the 3 core pipelines, together with a set of 10 additional somatic variant-calling pipelines contributed by members of the PCAWG SNV Calling Methods Working Group. Sufficient DNA remained for 50 of the 63 cases for validation, which was performed by hybridization of tumour and matched normal DNA to a custom RNA bait set, followed