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Research Article

Keywords: single nucleotide variation (SNV), copy number variation (CNV), PanCancer Analysis of Whole Genomes (PCAWG)

Posted Date: January 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-136297/v1>

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Annotations of Recurrent Structural Variant Events in Pan-Cancer Whole Genome Data for Precision Medicine

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Abstract

In personalized cancer genomic medicine, characterizing a patient's molecular profile based on comprehensive information is important for maximizing treatment benefits. However, current cancer genome analysis is centered on single nucleotide variation (SNV), gene expression, and copy number variation (CNV) but places little emphasis on structural variations (SV) beside fusions. To date, investigation of SVs has been limited because SV analysis entails a cumbersome annotation process. This study describes the design, development, and implementation of an annotation tool for SV, termed SVAnnotator. Detailed annotation was performed on the results of SV detection of 2,781 whole genome samples from the ICGC/TCGA PanCancer Analysis of Whole Genomes (PCAWG) with identifications of fusion, exon skipping, gene disruption, and tandem duplication SVs. These annotations of SVs will facilitate understanding of molecular events and further enhance utilities of precision medicine in stratifications, pathogenicity assessments and drug responses. Frequent novel SV events in *MACROD2*, *FHIT*, *WWOX* and *CCSER1* were observed across many cancers. Importantly, SV events were frequently identified in well-established tumor suppressor genes including *RB1*, *NF1*, *PTEN* and *TP53*. As such, it is plausible that potential therapeutic opportunities are overlooked when SV analysis is not appropriately performed. Given the frequency of SVs detected in our study, SV analysis with detailed annotation should be a routine part of comprehensive precision medicine analysis, and further studies are warranted to enhance clinical benefits as well as our understanding of uncharacterized SV events.

Introduction

Structural variations (SV) include deletions (DEL), duplications (DUP), inversions (INV), and translocations (TRA) with lengths typically 1,000 base-pairs (bp) or larger. They are a natural and key component of human variation¹ and affect a larger portion of the human genome than single nucleotide variants (SNV)². SV events cause not only copy number changes in the genes in a region but also gene disruptions, exon skipping, fusions, dysfunctional fusions, and internal tandem duplications (ITD).

SV events are quite common in cancer due to the inherent chromosomal instability associated with the disease³⁻⁸. In some cases, such as chromothripsis⁹⁻¹¹, chromoplexy¹², and other complex SVs¹³, a tumor may present with multiple SVs acquired as a single event resulting in complex genome rearrangements and genome instability.

In current cancer genomic analysis, copy number variations (CNV) and fusions are typically captured with traditional analytic methodologies; however, subtler events such as gene disruptions, exon skipping, and ITD are often neglected due to the laboriousness of current annotation methodologies for detected SVs. For instance, a typical CNV analysis will likely fail to find partial loss of a gene (defined as disruption in this study) and is also more likely to miss exon skipping and ITD events. It is known, however, that genes which are not prone to point mutations can be altered in SV-induced gene disruption and exon skipping. Common fragile sites (CFS) are an example of this phenomenon¹⁴. Besides fusions, the current knowledge of SV events in cancer has been primarily limited to EGFRvIII in glioblastoma multiforme (GBM), MET exon 14 skipping in lung adenocarcinoma, AR-V3 and AR-V7 in prostate cancer, and FLT3 ITD in acute myeloid leukemia.

No comprehensive list of recurrent SV events in cancer currently exists, partly due to the complexity of cancer genomes and variety of signals required for the comprehensive detection of SVs¹⁵⁻¹⁷. The generation of billions of short reads from whole genome sequencing presents challenges to develop algorithms and workflows to identify chromosomal break points¹⁸; conversely, long read sequencing

technologies have been developed, but their increased cost and high error rate is not conducive to accurate detection of SV events^{19,20}. Recent technological advances in sequencing and algorithmic detection of SVs, however, provide new opportunities for assessing the role of SVs in cancer^{18,21-24}. The purpose of this study was to design, develop, and implement a novel SV annotation tool, SVAnnotator, to discover recurrent SV events using International Cancer Genome Consortium (ICGC)/The Cancer Genome Atlas (TCGA) PanCancer Analysis of Whole Genomes (PCAWG) data in multiple cancer types. Identification of clinically targetable SV events will extend the current spectrum of actionable alterations beyond SNVs, CNVs, and fusions and will provide additional targeted treatment options at the point of care.

Results

Analysis of de-identified 2,781 samples of 31 cancer types from the PCAWG project with the novel SV annotation tool, SVAnnotator, annotated total 200,772 SV events and classified into seven SV types shown in Table 1. The most frequent SVs detected were gene disruptions, followed by dysfunctional fusions, fusions, exon skipping, ITDs, deletions, and deletion-insertion. Detailed results of SV annotations for each gene are provided in Table S1.

Table 2 lists the top recurrent fusion events identified by SVAnnotator with their frequency by cancer type. We compared the frequency of our most commonly observed fusion event, TMPRSS2-ERG in prostate cancer, with the frequency of the same fusion event in the COSMIC database²⁵. We identified 124 TMPRSS2-ERG fusions in 248 ICGC samples (44%) compared with a 38% frequency (2303/6145) in the COSMIC database. Additionally, we identified KIAA1549-BRAF fusion in 64% (57/89) of pilocytic astrocytoma cases which is consistent with a previously published study where this fusion was detected in 75% (24/32) of cases analyzed²⁶. The complete list of fusions obtained from our analysis can be found in Table S2.

Notably, gene disruptions in well-established cancer genes, including PTEN (68), were among the 49 genes most commonly affected by structural variant events in our analysis (Figure 1). Other cancer

census genes²⁷ such as CDK12 (38), RAD51B (37), RB1 (33), TP53 (31), NF1 (26), SMAD4 (23), ARID1A (21), and CDKN2A (20) were also frequently disrupted (Figure 2). Table S3 shows the complete list of dysfunctional fusions found in our analysis.

Exon skipping events were detected in a wide set of genes that have been previously associated with cancer including FHIT (235), MACROD2 (196), WWOX (147), LRP1B (139), PTPRD (122), NAALADL2 (114), CCSER1 (112), PDE4D (108), PARK2 (97), IMMP2L (71), CSMD1 (69), LSAMP (62), GMDS (61), PARD3B (56), RBFOX1 (55), ANKS1B (50), DLG2 (46), AUTS2 (45), and CNTNAP2 (45). Recurrent exon skipping genes in the cancer census list included FOXP1 (36), RUNX1 (32), PTEN (21), RB1 (21), EGFR (19), ARID1B (18), RAD51B (17), and NF1 (16). More details of which exons are skipped for each gene and their associated transcripts can be found in Table S4.

Although the prevalence of ITD events was less than that of exon skipping events, we identified recurrent hitherto unreported ITDs in LRP1B (29), RAD51B (29), NF1 (21), FHIT (18), and RB1 (16). Our evidence underscores the existence of cancer-associated ITD events beyond the well-characterized FLT3-ITD.

Dysfunctional fusions do not result in cohesive mRNAs and thus do not engender stable protein products; however, what's often overlooked is that these fusions lead to the loss of at least one copy of the genes involved leading to loss of heterozygosity. In this study we uncovered two novel dysfunctional fusions in ATAD1-PTEN (12) and PTEN-RNLS (13), rendering PTEN haploinsufficient.

Table 3 lists the most frequent SVs identified for each cancer type. The disease-specific events were selected using chi-square analysis with a threshold of p-value < 1.0e-25 (Table S5). Notable findings included 14 GBM cases (35%) with exon skipping events leading to constitutively active EGFRvIII and other oncogenic isoforms; 35 (11%) liver cancer and 15 (31%) lung squamous cell carcinoma cases with LRP1B exon skipping; 24 colorectal cancer cases (40%) with RBFOX1 exon skipping; over 20% of bone osteosarcoma cases with DLG2, CSAMP or CNTNAP2 exon skipping; both 5 prime and 3 prime

TENM4 fusions in melanoma; and 70 cases (80%) of esophageal adenocarcinoma with FHIT exon skipping located in the FRA3B locus.

Discussion

While advances in NGS technology have focused on SNVs, CNVs, and fusions as primary oncogenic drivers, structural variants have emerged as an under-appreciated yet integral component of the cancer genome. Limitations in analysis methodologies and availability of whole genome sequencing data have precluded the development of a comprehensive database of SVs in cancer. However, in this work, SVAnnotator was able to identify SV events spanning the entire genome. These included SVs in clinically actionable cancer-associated genes, currently non-targetable cancer-associated genes, and genes not yet associated with cancer but representing potentially novel targets for future investigation.

As expected, many of these top recurrent events were identified at CFS, including FRA3B, FRA2F, and FRA16D loci. Examples from our study include FHIT (localized within FRA3B) which positively regulates antigen presentation²⁸; thus, loss of FHIT might negatively affect immune checkpoint inhibitor response²⁹. On the contrary, loss of LRP1B (FRA2F) might be associated with positive immune checkpoint inhibitor response³⁰. Recently, MACROD2 (FRA20B) is reported to be related to chromosomal instability (CIN) in colorectal cancer³¹, and is mutually exclusive with microsatellite instability (MSI) which is known to respond to immune checkpoint inhibitors³². WWOX (FRA16D) has been reported to be responsible for metastasis in triple negative breast cancer³³. Notably, genes like PTPRD, EYS, TMPRSS2, and ERG do not belong to the currently known CFS. The aforementioned four CFS loci are associated with particular cancer types; however, SV events in PTPRD and EYS are more ubiquitous across many cancer types. Also, our work reiterates the importance of whole genome sequencing to capture these non-CFS loci events.

Detection of SVs in well-established cancer genes underscores the fact that clinically actionable alterations may be missed using traditional genomic screening approaches. Most notable in this class

of findings was the detection of SV events in NF1, RB1, CDKN2A, SMAD4, TP53, ARID1A/B and PTEN. NF1 is a known tumor suppressor gene which suppresses the MAPK pathway, and tandem duplication events of NF1 were specifically observed in ovarian cancer in our data set. NF1 loss of function mutations are detected in only 2.5% of the cases in the TCGA ovarian cancer dataset³⁴. However, we detected NF1 tandem duplication in 13% (15/112) of ovarian adenocarcinoma patients. As NF1 inactivation affects the mTOR pathway, disruption of NF1 through SV events represents an additional therapeutic opportunity to use inhibitors targeting the mTOR pathway. RB1 repeats were observed in many cancer types including breast, ovarian, pancreatic, and osteosarcoma. This study identified 4% (8/195) of breast cancer and 4.5% (5/112) of ovarian cancer samples had RB1 repeats; loss of function mutations in RB1 have been reported to confer resistance to CDK inhibitors³⁵. As such, SV events in RB1 should also be considered when CDK inhibitor treatment is deliberated. CDKN2A, a well-known tumor suppressor gene, shows many disruptions in liver cancer. CDKN2A gene disruptions were identified in 3% (7/238) of ICGC pancreatic adenocarcinoma cases. Loss of CDKN2A dysregulates the cell cycle and may represent a therapeutic opportunity with CDK inhibitors. SMAD4 copy loss or inactivation was reported in 38% of pancreatic cancer cases in the TCGA study and is indicative of poor prognosis in pancreatic cancer^{36,37}. We detected SMAD4 gene disruptions which would have been overlooked by gene panels and annotation services in 5% (12/238) of pancreatic tumors. TP53 disruptions were observed in 11% (4/37) of osteosarcoma and 7% (21/238) of prostate adenocarcinoma samples. While mutations causing loss of function are common in TP53 and PTEN, SVAnnotator detected gene disruptions and dysfunctional fusions which conventional NGS workflows would not have identified. PTEN loss impacts the PI3K pathway and may indicate a targeted therapy opportunity. CDK12 disruptions were mainly found in breast 7% (13/195), uterus 10% (5/49) and stomach 5% (4/76) cancers. It is reported that loss of CDK12 confers sensitivity to PARP1 inhibition³⁸ and checkpoint inhibitors³⁹.

Our study also identified SV events in many genes whose association to cancer is emerging. As such, these findings may not currently affect clinical decisions but present paths for future investigation.

Key examples (with references to their known associations with cancer) include: LRP1B⁴⁰, MACROD2^{31,41}, RUNX1⁸, FHIT⁴², SPREAD1⁴³, CCSER1⁴⁴, PTPRD⁴⁵, DLG2⁴⁶, SMYD3⁴⁷, and MSI2⁴⁸. SV events in genes that hitherto have not been widely associated with cancer were also frequently identified in our work, particularly novel fusions. These include PDE4D, NAALADL2, WWOX, EYS, IMMP2L, ANKS1B, RP11-145E5.5, RAD51B, and ZNF521 among others. High frequency of SV events in these genes warrant further investigation as to their role in cancer and may indicate opportunities for the development of novel therapeutics.

It is difficult to identify break points at single bp resolution and the results of various SV detection tools do not align with one another. This resolution issue and the capricious emergence of pseudo acceptors and donors make in-frame and out-of-frame prediction of resulting gene products even harder. Similarly, the ramifications of some SVs are not easily predicted and multiple complex SV event in a locus cannot be annotated by the SVAnnotator. Also, it should be noted that not all SVs identified, even in tumor suppressor and cancer-related genes, are necessarily associated with cancer risk and development. For example, when oncogenes are amplified, incomplete copies of genes that are snipped can sometimes leave traces observed as gene disruptions, as seen in EGFR disruptions. Dysfunctional fusions as observed in PTEN can lead to lack of heterozygosity in tumor suppressor genes. Thus, SV events, regardless of the type, should be carefully examined using polymerase chain reaction or RNA-seq analysis. Detailed studies should be carried out to confirm the role of SV events in cancer development and pathogenesis.

In summary, the SVAnnotator tool was able to identify various genes that are frequently subjected to SV events in cancer patients. These findings included SV events in well-established cancer genes that are clinically actionable as well as genes with limited association to cancer. Further work is required to elucidate the role of these SVs in tumorigenesis and progression. In conclusion, this study suggests that identification of SVs in cancer can provide additional clinically pertinent insights that may be

overlooked by conventional genomic analysis workflows and as such should be integrated as a routine practice of comprehensive precision medicine analysis.

Materials and methods

Samples

All samples used in this study were derived from the Pan-Cancer Analysis of Whole Genomes (PCAWG) study subset of the ICGC final consensus genome data (2,781 total samples across 31 cancer types). We used the results of somatic structural variant detection analysis performed in the PCAWG project. For 67.3% of samples, tumors were freshly frozen solid samples whereas controls were blood samples. In contrast, for 20.2% of samples, controls were derived from tumor-adjacent normal tissues or other non-blood tissues, particularly in hematological cancers⁴⁹. 93.8% of the tumor samples came from treatment-free primary cancers, but 6.2% originated from donors with multiple samples of primary, metastatic, and/or recurrent tumors. Due to large contributions from European, North American, and Australian genomic projects, the continental ancestry distribution of the sample was heavily weighted towards Europeans (77%) and East Asians (16%)^{49,50}.

Sequencing

Sequencing was performed by each data provider of the PCAWG project and Illumina whole-genome paired-end sequencing, which reads lengths of 100-150 bp from tumor and normal samples was used. The mean whole genome sequencing coverage in this set was 30 reads per bp for control samples, whereas tumors had a bimodal coverage distribution with maximums at 38 and 60 reads per bp^{49,50}. The BWA-MEM v0.7.17 algorithm⁵¹ was used to align each tumor and normal sample to Human Genome Reference Build GRCh37⁵², version hs37d5.

Structural variant calling

In order to generate a consistent call set to be used for cross-tumor type analysis, all samples were analyzed using a uniform set of algorithms for alignment, variant calling, and quality control. The SV

call set was generated by one of the working groups of the PCAWG study^{50,53,54}. The call set was generated by merging the SV calls from independent calling pipelines, and the merged SV calls were further required to have a consistent copy number change. The data of analysis results are publicly available at ICGC Data Portal, <https://dcc.icgc.org/releases/PCAWG>.

Structural variant annotation; SVAnnotator

We developed an annotation tool named SVAnnotator to automate the cumbersome task of transforming structural variant data into human comprehensible events such as fusions, exon skipping, ITD, and gene disruptions. SVAnnotator takes 2 break points' coordinates, their connection orientations (5'-5', 5'-3', 3'-5', 3'-3'), and types (INV, DEL, DUP, TRA) as inputs; SVAnnotator uses tab separated text files with coordinates, strand orientations, and types of event as inputs. BEDPE files generated by an SV caller can be easily transformed into tab separated text files by a simple script or a spreadsheet. Variant Call Format (VCF) and BND format can be transformed by Bioconductor⁵⁵ or other freely available tools. The headers must start with '#' if any. Most SV callers provide ranges rather than chromosomal position for break points; however, SVAnnotator cannot accept ranges so users need to pick a position. Also, it is important to remove false positives which originate from alignment issues in low complexity regions or other causes⁵⁶. The overall program flow of SVAnnotator is illustrated in Figure S1. First, the program checks the location of break points using gene definitions as defined in a General Transfer Format (GTF) file. If both break points fall in the same transcript, SVAnnotator classifies it into subtypes (1-9) as shown in Figure S2, considering break point orientations, gene orientation, and connection directions before translation into an event like exon skipping and ITD. SV events with a transcript and an intergenic locus are considered gene disruptions. SV events with a transcript and an intergenic locus are considered gene disruptions. SVAnnotator defines a disruption as either end of a transcript being connected to an intergenic region and the gene effectively considered as a copy loss; whereas a deletion is defined as an event in which internal transcript parts are missing. SV events where both break points fall in two distinct transcripts are

classified as either fusions identified by 5/3 prime gene or dysfunctional fusions, after considering strand directions and connection orientations, as illustrated in Figure S3. SVAnnotator identifies loss of an acceptor or a donor side of an exon as exon skipping. However, these losses can be indicative of non-exon skipping events⁵⁷; it is strongly encouraged that users check actual transcripts to confirm.

Identifying tumor-type-specific SV events

To identify SV events that are specifically enriched in some tumor types, a Chi-Square goodness-of-fit test was employed for each SV event that was characterized by an affected gene name and an SV type. The test evaluates how a set of observed counts of a specific SV event across multiple tumor types differs from that of expected ones, which are proportional to the sample sizes of the tumor types.

We restricted our analysis to tumor types with greater than 20 samples and focused on the following 27 cancer subtypes: Biliary.AdenoCA (34), Bladder.TCC (23), Bone.Osteosarc (37), Breast.AdenoCA (195), CNS.GBM (40), CNS.Medullo (147), CNS.PiloAstro (89), ColoRect.AdenoCA (60), Eso.AdenoCA (88), Head.SCC (57), Kidney.ChRCC (45), Kidney.RCC (144), Liver.HCC (323), Lung.AdenoCA (37), Lung.SCC (48), Lymph.BNHL (107), Lymph.CLL (96), Myeloid.AML (29), Myeloid.MPN (58), Ovary.AdenoCA (112), Panc.AdenoCA (238), Panc.Endocrine (85), Prost.AdenoCA (284), Skin.Melanoma (106), Stomach.AdenoCA (76), Thy.AdenoCA (48), and Uterus.AdenoCA (49), where the number in parentheses after each tumor type is the sample size, and the total number of samples is 2,655. We removed 126 samples belonging to 11 other cancer types of smaller cohorts from the analysis to avoid statistical skewing for cancer type specific event extraction.

For each SV event that was characterized by an affected gene name and an SV type, the Chi-Square goodness-of-fit test was applied to the observed counts of the SV across the 27 tumor types. The total number of distinct SV events considered was 99,498. The result, i.e., p-values and the observed counts of SV events, are shown in Table S5.

Software Availability

SVAnnotator is available from <https://github.ibm.com/ComputationalGenomics/>

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Acknowledgements

We would like to thank the PCAWG Technical Working group and The PCAWG Network for providing the whole genome data for this study. Super-computing resources were provided by Human Genome Center, the Institute of Medical Science, the University of Tokyo (<http://sc.hgc.jp/shirokane.html>).

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Contributions

TK and SI conceived and designed the study. TK and LP created software used in this work. TK, TH, RK, ES, DW, VW, JS, RY and SI conducted the data analysis. RT, KaY, KiY, SM and LP helped with data analysis. TK, TH, RT, KaY, KiY, AT, YF, HN, SM, LP, RY and SI conducted data interpretation. RT, DW, VW, JS and HN helped with manuscript preparation. TK, TH, DW, VW, JS, RY and SI wrote and edited the manuscript.

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Ethics declarations

Competing Interests

TK, RT, DW, VW, JS, and LP were all employed by IBM at the time this work was conducted. The other authors (TH, RK, ES, KaY, KiY, AT, YF, HN, SM, RY and SI) declare no competing interests.

Legends (Tables and Figures)

Table 1. Number of events by SV type in total and distinct gene and event combinations. SV = structural variant, ITD = internal tandem duplication, DEL = deletion, DELINS = deletion-insertion

Table 2. Top recurrent fusion events and their frequency in each cancer type.

Table 3. Most frequent SV events by cancer type. ES = exon skipping, DF=Dysfunctional Fusion, DIS=disruption, F3=fusion 3 prime, F5=fusion 5 prime, ITD = internal tandem duplication, N/A = not available

Figure 1. Heatmap of the top recurrent SV events. Color represents frequency of the SV for the given cancer type.

Figure 2. Heatmap of the SV events among cancer census genes. Color represents frequency of the SV for the given cancer type.

Figures

Figure 2

Heatmap of the SV events among cancer census genes. Color represents frequency of the SV for the given cancer type.

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