Structural and functional analysis of somatic coding and UTR indels in breast and lung cancer genomes

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Insertions and deletions (Indels) represent one of the major variation types in the human genome and have been implicated in diseases including cancer. To study the features of somatic indels in different cancer genomes, we investigated the indels from two large samples of cancer types: invasive breast carcinoma (BRCA) and lung adenocarcinoma (LUAD). Besides mapping somatic indels in both coding and untranslated regions (UTRs) from the cancer whole exome sequences, we investigated the overlap between these indels and transcription factor binding sites (TFBSs), the key elements for regulation of gene expression that have been found in both coding and non-coding sequences. Compared to the germline indels in healthy genomes, somatic indels contain more coding indels with higher than expected frame-shift (FS) indels in cancer genomes. LUAD has a higher ratio of deletions and higher coding and FS indel rates than BRCA. More importantly, these somatic indels in cancer genomes tend to locate in sequences with important functions, which can affect the core secondary structures of proteins and have a bigger overlap with predicted TFBSs in coding regions than the germline indels. The somatic CDS indels are also enriched in highly conserved nucleotides when compared with germline CDS indels.

Insertion and deletion (indel) is an important variation type in the human genome, second only to the single nucleotide variations (SNVs)1–4. Previous studies have estimated that indels contribute to 16% to 25% of sequence polymorphisms in human populations2,9–11. Like other types of variations, indels can alter human traits and lead to diseases including cancer12–15. Indel analyses have been carried out in both healthy and cancer genomes. In 2011, about 1.96 million small indels were identified in 79 human genomes, which was reported to have more than 97% validation rate16. The 1000 Genomes Project reported 1.48 million indels in 2010. There are 463,377 common indels between the above two studies, a result that reflects a combination of indel diversity and inaccurate indel annotations1,16,17. In addition to indels in healthy human genomes, efforts have been carried out to investigate indels in different cancer types. Recent pan-cancer analyses indicated the substantial variations among different cancer types18–20. Similar to indel annotation in healthy genomes, different methods and algorithms may lead to different somatic indel annotations17.

In coding regions, an indel can be frameshift (FS) or non-frameshift (NFS) depending on the length of an insertion or a deletion2,16. If the length of an indel is a multiple of three nucleotides, it is an NFS indel as it only affects the amino acid(s) of the indel while other coding indels that change the open reading frame are considered FS indels. For germline indels in healthy human genomes, the number of FS indels is much lower than expected, suggesting FS indels are potentially deleterious and less tolerated during evolution21. Several programs have been developed to predict the potential disease-causing NFS and FS indels22–27. To better understand the role of somatic indels in cancer genomes, studies have been done at both domain and protein level. Pagel et al. mapped somatic NFS indels from COSMIC onto protein structures and found that pathogenic variants tend to be enriched in helical and stand regions of protein structures28. Niu et al. developed a tool to identify 3-dimensional (3D) variants clusters on protein structures that can be used in variant-drug interaction analysis in cancer genomes29. Among the mutation-mutation and mutation-drug clusters from more than 4,400 samples across 19 cancer types, more than 6000 clusters were identified at 3D structure level, including both intra-molecular and inter-molecular clusters. They reported that about 0.76% of the 553,496 somatic variants are indels29.

Mutations in non-coding regions can also cause diseases30–37. Most analyses on non-coding variants in the regulatory regions in cancer genomes either focused on SNVs or did not differentiate SNVs from indels with relatively small sample sizes or a single cancer type38,39. Sakthikumar et al. investigated non-coding variants in Glioblastoma (GBM) genomes and demonstrated that the GBM somatic variants are enriched in non-coding regions40.
regions of 78 GBM key genes. Imieliński et al. showed that somatic non-coding indels in 79 lung adenocarcinoma genomes are exclusively enriched in surfactant protein genes. Nakagomi et al. further analyzed 113 lung cancer samples and reported that other cancer types in lung also harbour non-coding indels and demonstrated the important role of those indels in lung cancer research.

While eukaryotic genomes generally are considered to have two major types of sequences: (1) coding-sequences (CDSs) that encode proteins or RNAs, and (2) non-coding sequences that include regulatory regions such as promoters and enhancers for regulation of gene expression, a number of studies have shown that sequences in CDSs and the untranslated regions (UTRs) can also function as enhancers. Mutations in coding and UTR enhancers can cause diseases by changing their enhancer activities. Recent large-scale studies have shown that transcription factor binding sites (TFBSs) exist in coding regions in both human and mouse genomes based on ChIP-seq data analyses. About 15% of codons in the human genome were hypersensitive to DNase I treatment, suggesting the existence of likely dual-use sequences for both amino acid coding and transcriptional regulation. These dual function sequences, termed as duons, were considered to be more conserved than non-duon coding sequences and mutations in these duons could lead to diseases.

The goal of this study is to investigate the potential role of somatic indels and the overlap between somatic indels and TFBSs in two of the most analyzed cancer types, invasive breast carcinoma (BRCA) and lung adenocarcinoma (LUAD). BRCA has the second largest proportion of indels among 19 cancer types. LUAD has a high number of exonic somatic variants as reported in several studies. Since the BRCA and LUAD sequences in TCGA data portal are whole exome sequences, we focused our indel analysis on coding and the non-coding UTRs. In addition, while in principle the Whole Exome Sequencing (WXS) technology does not produce whole transcripts, studies have shown that 40–60% of the reads from exome sequencing are outside of the designed target regions including introns and these reads can be of high quality. Therefore, besides the coding regions and UTRs, we also compared the indels in other regions of the transcripts as a side analysis.

Since somatic indel calling programs also predict germline indels found in healthy genomes, we first identified these types of indels and removed them for downstream somatic indel analyses, including structural analysis of the effect of somatic NFS indels on protein secondary structure types, and gene enrichment and conservation analyses. We also mapped the somatic indels on the significantly mutated genes (SMGs) across major cancer types identified by Kandoth et al. More importantly, we investigated the somatic indels on the coding regions and UTRs that overlap with TFBSs, the dual-use sequences. To the best of our knowledge, this is the first large scale comparative study of mapping somatic coding indels to TFBSs.

Materials and methods

Sequence data and somatic indel calling. The 436 BRCA and 564 LUAD whole exome sequencing data, TCGA-BRCA and TCGA-LUAD, were downloaded from TCGA data portal at https://portal.gdc.cancer.gov (dbGaP Study Accession: phs000178.v11.p8). Both tumor and normal blood/tissue sequencing data were used to call somatic indels using the human genome reference GRCh38.p13 and Strelka. Previous studies have demonstrated that Strelka performed well for somatic variants calling. The indel set from the GATK Resource bundle with 1,267,008 germline indels was used as the reference of germline indel annotations in healthy human genomes (https://storage.cloud.google.com/genomics-public-data/resources/broad/hg38/v0/Mills_and_1000G_gold_standard.indels.hg38.vcf.gz). The transcript agreed by several references or the longest transcript for each gene was selected for annotating coding sequences and UTRs in both cancer exome sequences and germline sequences. In previous studies, a position \(i \pm 5\) has been used to determine whether two indels are the same, without concerning the indel types (insertion or deletion). Here we used a more stringent approach to identify the germline indels predicted as somatic indels by considering the indel types and insertion/deletion sequences in addition to the indel positions. Two indels are considered the same only if both have the same positions, indel types and sequences when comparing the predicted somatic cancer indels and the germline indels in the GATK Resource bundle. Since somatic indels are less conserved than the germline indels, we used the following two criteria: (1) the positions of two indels are within \(i \pm 5\); and (2) same insertion/deletion type to check the overlap of somatic indels between BRCA and LUAD cancer genomes.

Protein secondary structure type analysis of coding indels. To locate the positions of coding somatic indels, we downloaded all protein coding gene annotations from Ensembl. Each transcript with indel(s) was first searched against proteins with known structures in Protein Data Bank (PDB) using BLAST. If a protein has a known structure or highly homologous protein structure (with at least 50% coverage and 80% sequence identity), the secondary structure types of the protein or the template protein were used. The protein secondary structure types of the protein were assigned with DSSP. Of the eight secondary structure types from DSSP, H (α-helix), G (β-helix) and I (β-helix) states were grouped as helix conformations; E (extended strand) and B (residue in isolated β-bridge) states were grouped as strand conformations and all the remaining states were considered as loop conformations. If no known structures were found in PDB, RaptorX-Property was applied to predict secondary structure types with default settings. RaptorX-Property uses conditional neural fields method to predict secondary structure types and achieves close to 84% Q3 prediction accuracy based on five different datasets. The structural analysis of the germline indels from healthy genomes were performed with the 1370 coding indels annotated by the GATK Resource bundle.

Overlap of indels with TFBSs. To investigate the overlap between somatic/germline indels and TFBSs, we used the TFBS set predicted with dePCRM2, a recently developed program for genome scale TFBS prediction.
with a high sensitivity of more than 97%\cite{70}. A total of 25,297,119 non-overlapping TFBSs were predicted using dePCRM2 with a p-value cutoff of $5 \times 10^{-6}$.

**Gene enrichment analysis and assignment of conservation scores.** To investigate the functional categories of the genes affected by somatic coding indels in BRCA and LUAD, we applied DAVID 6.8 (the Database for Annotation, Visualization and Integrated Discovery) to perform functional enrichment analysis\cite{71}. A cutoff of 0.001 was set for the adjusted p-values with Bonferroni correction to identify the significantly enriched terms in biological process or molecular function.

The phyloP scores of each nucleotide position in the human genome were downloaded from the UCSC Genome Browser database\cite{72,73}. The two flanking nucleotides for each insertion site and the deletion sequences were collected for phyloP distribution analysis as well as for finding genes with high phyloP conservation scores.

**Results**

**Comparison of somatic indels between BRCA and LUAD.** We found 109,856 and 91,159 somatic indels from 436 BRCA samples and 564 LUAD samples respectively with 16,909 common indels between them (Table 1). As a reference, a total of 498,938 germline indels were mapped to transcripts in healthy genomes from the GATK Resource bundle. Since the predicted indels by somatic indel prediction algorithms include germline indels (false somatic indels), these germline indels need to be filtered out first for meaningful downstream analysis\cite{17}. As described in the Materials and Methods section, two indels are considered the same only if both have the same positions, indel types and sequences when comparing the germline indels from the GATK Resource bundle and the predicted somatic indels from BRCA and LUAD. We found that 16.74% and 19.64% of indels in BRCA and LUAD respectively are the same as the germline indels (Table 1). After removing the germline and non-transcript indels, 61,543 and 43,684 somatic transcript indels for BRCA and LUAD respectively were used for further analysis. Not surprisingly, the overlapped indels between BRCA and LUAD have a higher percentage of germline indels (23.16%) since germline indels are more conserved than the somatic indels within populations of different cancer types\cite{21}.

Similar to germline indels in healthy genomes, relatively more deletions than insertions were found in BRCA and LUAD. The percentages of deletions in both cancer types are slightly higher than those in the GATK germline indel set (Table 1). The distributions of insertion/deletion in both BRCA and LUAD are significantly different from germline indels (chi-squared test, p-value = $4.532 \times 10^{-16}$ for BRCA and p-value = $2.2 \times 10^{-16}$ for LUAD). The number of transcripts that have somatic indels are 14,519 and 13,593 in BRCA and LUAD respectively. It should be noted that while the whole exome sequences from the BRCA and LUAD contain all the coding and UTRs, they do not have the whole transcript sequences as the healthy genomes do. Therefore, at the transcript level, the somatic indels are undercounted.

**Somatic coding indels in BRCA and LUAD genomes.** As shown in Table 2, the number of transcripts with somatic coding indels and the number of coding indels in both BRCA and LUAD are much higher than those of germline coding indels in healthy genomes (Table 2). The proportions of somatic coding indels are 8.64% in BRCA and 17.89% in LUAD while it is only 0.62% for the germline coding indels in healthy genomes. In terms of the deletion/insertion ratio in coding regions, LUAD has more deletion types (~70%) than that in the germline indels from healthy genomes (64.6%) while about 57.86% of somatic coding indels from BRCA samples are deletions. For the overlapping indels between BRCA and LUAD, the deletion and insertion are about 43.6% and 56.4% respectively.

**Table 1.** Somatic transcript indels in BRCA and LUAD. *The numbers of somatic transcript indels in BRCA and LUAD are indels on transcripts after removing the ones that overlap with germline indels.*

| Cancer type | # of total indels | Overlap with germline indels | # of somatic transcript indels* | Deletions | Insertions | # of transcripts with indels |
|-------------|------------------|-----------------------------|-------------------------------|----------|-----------|------------------------------|
| BRCA        | 109,856          | 18,391 (16.74%)             | 61,543                        | 36,109   | 25,434    | 14,519                       |
| LUAD        | 91,159           | 17,900 (19.64%)             | 43,684                        | 27,148   | 16,536    | 13,593                       |
| BRCA∩LUAD   | 16,909           | 3916 (23.16%)               | 9988                          | 5330     | 4658      | 6600                         |
| Germline    | 1,267,008        | –                           | 498,938                       | 284,597  | 214,341   | 17,278                       |

**Table 2.** Somatic coding (CDS) indels in BRCA and LUAD. *The percentages are calculated against the number of transcript indels.*

| Cancer type | # of transcripts with CDS indels | CDS indels* | Deletions | Insertions | FS indels | NFS indels |
|-------------|---------------------------------|-------------|-----------|------------|-----------|------------|
| BRCA        | 3979                            | 5320 (8.64%)| 3078 (57.86%)| 2242 (42.14%)| 3947 (74.19%)| 1373 (25.81%)|
| LUAD        | 5458                            | 7813 (17.89%)| 5526 (70.73%)| 2287 (29.27%)| 6387 (81.75%)| 1426 (18.25%)|
| BRCA∩LUAD   | 798                             | 835 (8.36%)  | 364 (43.59%)| 471 (56.41%)| 496 (59.40%)| 359 (40.60%)|
| Germline    | 1180                            | 1370 (0.62%) | 885 (64.60%)| 485 (35.40%)| 679 (49.56%)| 691 (50.44%)|
SMGs while none of the top 10 genes with germline coding indels are in the 125 SMGs (Table 3). Some of these genes with multiple somatic coding indels in BRCA and LUAD respectively are in the list of 125 protein coding genes have served as targets for drug development, such as EGFR.

Functional enrichment analysis revealed that NFS indels. We also observed a similar pattern from germline coding indels in the GATK Resource bundle, genomes from the 1000 Genomes Project revealed that the number of germline FS indels is similar to that of NFS indels. Over eighty percent of the somatic coding indels in LUAD are FS indels (Table 2). The overlapped coding somatic indels between BRCA and LUAD genomes have a relatively lower ratio of FS indels (59.4%), but it is still much higher than that in the germline (49.56%).

The somatic coding indels affect a total of 8,286 genes when BRCA and LUAD are combined. BRCA and LUAD have somatic coding indels in 3,979 and 5,458 genes respectively and 798 genes have somatic coding indels in both BRCA and LUAD. Among these genes, MAP3K1 has the most somatic coding indels in BRCA (45 indels), and TP53 has the most somatic coding indels in LUAD (37 indels) (Table 3). We compared the top genes with multiple somatic coding indels in our datasets to the 125 protein coding SMGs among the 127 total SMGs across 12 major cancer types (The other two are one lncRNA gene and one miRNA gene). Seven and five of the top 10 genes with multiple somatic coding indels in BRCA and LUAD respectively are in the list of 125 protein coding SMGs while none of the top 10 genes with germline coding indels are in the 125 SMGs (Table 3). Some of these genes have served as targets for drug development, such as EGFR. Functional enrichment analysis revealed that the genes with somatic coding indels in LUAD are highly enriched in biological processes involved in cell adhesion while the indels in BRCA affect more chromatin remodelling and transcription (Supplementary Table S1).

Since NFS somatic coding indels only affect part of the protein while keeping the remaining sequence unchanged, we compared the distributions of secondary structure types of these indels with the germline indels in healthy genomes. Among the proteins with NFS somatic coding indels, 181 and 106 proteins in BRCA and LUAD respectively were found to have known or homologous structures in PDB. For proteins having NFS somatic coding indels without known structures, we used RaptorX-Property to predict the secondary structure types for each amino acid of the indels, as described in the Materials and Methods section. While the distributions between the two cancer types are slightly different (chi-square test, p-value = 0.003), both are significantly different from that in germline indels (Fig. 1). Somatic coding NFS indels in cancer genomes have more helix and strand conformations with fewer loop types (chi-square test, p-values < 2.2 × 10−16), suggesting the NFS coding indels in both BRCA and LUAD cancer genomes affect more core secondary structures in the encoded proteins and are potentially more deleterious than the germline NFS coding indels in healthy genomes.

### Somatic non-coding UTR indels in BRCA and LUAD cancer genomes

For exonic non-coding somatic indels, we found 372 and 1940 indels in 5' UTR and 3' UTR respectively in BRCA, and 375 and 1187 indels in 5' UTR and 3' UTR respectively in LUAD (Supplementary Table S2). There are more somatic indels in 3' UTR than those in 5' UTR. In both BRCA and LUAD genomes, the indels are enriched in both 5' UTR (0.66% and 1.06% for BRCA and LUAD, respectively) and 3' UTR (3.45% and 3.31% for BRAC and LUAD, respectively) when compared with those in germline indels of healthy genomes, with 0.18% and 2.55% in the 5' UTR and 3' UTR respectively (Supplementary Table S2). The majority of transcript somatic indels are located in the non-CDS, non-UTR regions. Therefore, even though the goal of whole exome sequencing is to get the exonic sequences, exome sequencing can generate high quality data and cover large non-target regions. However, since the coverage of non-target regions in each cancer sample might be different from whole exome sequencing, it is difficult to draw conclusions when comparing the non-CDS, non-UTR noncoding transcript indels between two different cancer types and between cancer somatic indels and the germline indels.

### Conservation analysis of somatic CDS and UTR indels

It is interesting to see how conserved the somatic CDS and UTR indel sequences are in BRCA and LUAD when compared with germline CDS and UTR indels in healthy genomes. To this end, we compared the phyloP scores for nucleotides at the indel positions. Since the phyloP scores of nucleotides are based on the reference genome, we collected the nucleotides for

| Gene       | BRCA* CDS | LUAD* CDS | BRCA* UTR | LUAD* UTR | Germline CDS |
|------------|-----------|-----------|-----------|-----------|--------------|
| MAP3K1     | TP53      | TP53      | SSOP      |           |              |
| GATA3      | STK11     | PABPC3    | HLA-DRB1  |           |              |
| TP53       | TTN       | MUC5B     | TEKT4     |           |              |
| CDH1       | MUC16     | HAVERC1   | OR4C5     |           |              |
| DSPP       | KEAP1     | PABPC1    | SCYGR8    |           |              |
| KMT2C      | RBM10     | ACIN1     | MAML3     |           |              |
| PIK3R1     | RYR2      | ZFHX4     | ZFPM1     |           |              |
| SPEN       | CSSMD5    | EPHB6     | ABCA10    |           |              |
| TBX3       | NF1       | ZAN       | KRT14     |           |              |
| TTN        | EGFR      | FAM71D    | MYO15B    |           |              |

Table 3. Top ten genes with multiple somatic CDS indels in BRCA and LUAD. *The genes are ranked by the number of somatic CDS indels and the genes in bold are the ones in the 125 SMG list.
insertions and deletions differently. For insertion cases, the phyloP scores of the two flanking nucleotides at the indel site were collected while phyloP scores for the whole deletion sequences were considered (see Materials and Methods). The larger a positive phyloP score of a nucleotide position in the genome, the more conserved the position. Figure 2 shows the distributions of phyloP scores of insertions and deletions in CDS regions (Fig. 2a,b) and UTR (Fig. 2c,d) in BRCA (Fig. 2a,c) and LUAD (Fig. 2b,d) respectively. In CDS regions, the somatic insertions and deletions have more positions with higher phyloP scores when compared with the distribution of germline insertions and deletions. There seems no apparent differences between BRCA and LUAD as well as between insertion and deletion cases. As for UTR indels, there is a difference between the cancer indels and germline indels. However, the differences are very small, especially when compared with those in the CDS positions.

**Overlap between somatic indels and TFBSs.** The percent overlap between the somatic indels in cancer transcripts and TFBSs is larger than that between germline transcript indels and TFBSs (Table 4). The number
of somatic CDS indels that overlap with TFBSs is much higher in cancer genomes, 2367 and 3140 in BRAC and LUAD respectively while there are only 520 for germline coding indels, suggesting cancer coding indels are enriched in these dual-functional regions. Somatic non-CDS transcript indels in cancer genomes are also enriched in the predicted TFBS sequences (25.4% and 27.01% for BRCA and LUAD, respectively) when compared to 17.41% in germline non-CDS transcript indels in healthy genomes (Table 4). A detailed look at these non-CDS transcript indels shows that there is a smaller percentage of overlap between 5'UTR and TFBSs in BRCA and LUAD than that in healthy genomes while the 3'UTR is the opposite (Supplementary Table S3). While we also found that other non-CDS, non-UTR transcript indels have a larger percent overlap with TFBSs in cancer exomes, unlike the CDS cases, incomplete transcript sequences in the intron regions from exome sequencing makes it harder to make a fair comparison with the germline cases.

We also performed conservation enrichment analysis with a phyloP score cutoff of 5 for indel positions in CDS with TFBS overlap and indels positions in CDS without TFBS overlap. The genes were ranked by the number of indel positions with phyloP scores above the cutoff in each case. The top 10 genes in each case are listed in Table 5. More SMG genes were found in indels with CDS and TFBS overlap than those CDS indels without TFBS overlap in both cancer types (7 vs. 4 in BRAC and 4 vs. 1 in LUAD) (Table 5). Not surprisingly, none of the 125 SMG protein coding genes were found in the germline indels no matter if CDS overlaps TFBS or not.

Somatic indels in SMGs. We mapped the somatic indels to the annotated 125 protein coding SMGs and found that somatic indels in cancer genomes are enriched in SMGs, especially they are enriched in SMGs' coding regions in both BRCA and LUAD cancer genomes when compared with the germline indels from healthy genomes (Table 6). In healthy genomes, there are only 11 (0.23%) SMG indels in coding regions, but in BRCA and LUAD cancer genomes, 349 (33.82%) and 267 (38.98%) of SMG somatic indels are located in the coding

### Table 4. Somatic transcript indels overlapping with TFBSs.

| Cancer type | Transcript indels | Overlapping with TFBSs | CDS indels | CDS indels overlapping with TFBSs | Non-CDS transcript indels | Non-CDS indels overlapping with TFBSs |
|-------------|-------------------|------------------------|------------|----------------------------------|--------------------------|--------------------------------------|
| BRCA        | 61,543            | 16,646 (27.05%)        | 5320       | 2367 (44.49%)                    | 56,223                   | 14,279 (25.40%)                      |
| LUAD        | 43,684            | 12,830 (29.37%)        | 7813       | 3140 (40.19%)                    | 35,871                   | 9690 (27.01%)                       |
| BRCA/LUAD   | 9988              | 2977 (29.81%)          | 835        | 332 (39.76%)                     | 9153                     | 2645 (28.90%)                       |
| Germline    | 498,938           | 87,156 (17.47%)        | 1370       | 520 (37.96%)                     | 497,568                  | 86,636 (17.41%)                     |

### Table 5. Top ten genes with multiple high phyloP scores (> 5) in somatic CDS indels. *The genes in bold are among the 125 SMG list.

| Cancer type | CDS overlap with TFBS | CDS not overlap with TFBS | CDS overlap with TFBS | CDS not overlap with TFBS | CDS overlap with TFBS | CDS not overlap with TFBS |
|-------------|-----------------------|---------------------------|-----------------------|---------------------------|-----------------------|---------------------------|
| BRCA        | GATA3                 | PIK3CA                    | STK11                 | ADGRL3                    | LZTR1                 | RFX7                      |
| LUAD        | MAP3K1                | PIK3K1                    | TP53                  | CDH8                      | ZEB2                  | CEL71L                    |
|              | TP53                  | MAP3K1                    | EGFR                  | LRFN5                     | CDK8                  | RBBP6                     |
|              | Pten                  | TAF2                     | ATP2B1                | GIB7                      | CHD9                  |                           |
|              | YTHDF2                | TTN                       | APC                   | RPL5                      | DBX1                  | NEDD4                     |
|              | CDH1                  | TM9SF2                    | PCDH9                 | KCNH7                     | GMNC                  | RERE                      |
|              | TM9SF4                | PLCE1                     | MEAF6                 | CNOT1                     | OR5A1U1                | CARD11                    |
|              | TBX3                  | EPHB3                     | TUBB8B                | DMX9                      | SRM3                  | HYDEN                     |
|              | TTN                   | ADCYAP1R1                 | DOCK5                 | PSEN2                     | ZNF730                | TMCC1                     |
|              | RUNX1                 | PDE11A                    | TAPT1                 | COG2                      | SPON1                 | DNAJC28                   |

### Table 6. Somatic transcript indels in 125 SMGs of BRCA and LUAD.

| Cancer type | Indels in SMGs | SMGs with indels in CDS regions | Indels in SMGs' CDS | SMG CDS indels overlapping with TFBSs | Indels in SMGs' non-CDS regions | SMG non-CDS indels overlap with TFBSs |
|-------------|----------------|---------------------------------|---------------------|--------------------------------------|---------------------------------|--------------------------------------|
| BRCA        | 1032           | 70 (56.00%)                     | 349 (33.82%)        | 172 (49.28%)                        | 683                             | 154 (22.55%)                        |
| LUAD        | 685            | 71 (56.80%)                     | 267 (38.98%)        | 132 (49.44%)                       | 418                             | 105 (25.12%)                        |
| BRCA/LUAD   | 129            | 12 (9.6%)                       | 19 (14.73%)         | 9 (47.37%)                         | 110                             | 35 (31.82%)                         |
| Germline    | 4818           | 9 (7.2%)                        | 11 (0.23%)          | 5 (45.45%)                         | 4807                            | 620 (12.90%)                        |
regions respectively (Table 6). Among the 125 SMGs, 70 and 71 of them have BRCA and LUAD somatic indels in CDS regions respectively while only 9 SMGs have germline coding indels. Twelve SMGs have somatic coding indels in both cancer types, suggesting different mutation/variant patterns in different cancer types while there are some commonalities between cancer types.

The overlap between SMG somatic coding indels with TFBSs is significantly more in BRCA and LUAD than that in germline indels in healthy genomes (Table 6). There are 172 (49.28%) and 132 (49.44%) somatic coding indels overlap with TFBSs in SMGs in BRCA and LUAD while there are only 5 (45.45%) such cases in healthy genomes (Table 6). The overlap between the non-CDS somatic indels and TFBSs in BRCA (22.55%) and LUAD (25.12%) is higher than that in healthy genomes (12.9%) as well.

Discussion
With the advancement of biotechnology, especially the NGS technology, a large number of genomes have been sequenced for a variety of cancer types. Somatic variations in cancer genomes have been one of the main focuses in cancer studies, including variants in both coding and non-coding regions\(^7\). However, most of the studies in cancer genomes focused on SNVs\(^9,40,52\). In this study, we carried out a comparative study of the somatic indels in two major cancer types, BRCA and LUAD with their whole exome sequences and compared some of the features with germline indels from healthy genomes.

There are several novel aspects from this study. First, we removed the germline indels predicted from the somatic indels calling program before performing downstream analyses. We demonstrated previously that some of the predicted somatic indels are exactly the same as the germline indels in healthy human genomes\(^17\). Therefore, these indels are considered as false somatic indels and represent "noise" when analyse features in cancer genomes, which need to be filtered out. Secondly, we investigated the overlap between cancer somatic indels, especially the coding indels with TFBSs. Previous case studies as well as large-scale analyses revealed the existence of the so-called duons that encode amino acids and also serve as TFBSs\(^43–47,50\). The percentage of such DNA sequences with dual functions varies in species and by different TFBSs annotations. Based on ChIP-seq data, Birnbaum et al. showed that there are 7% and 6% of binding peaks located in protein coding regions in human genomes and mouse genomes respectively\(^47\). Using DNase I footprinting method, Stergachis et al. found that at least 14% of coding regions in human genomes can bind transcription factors\(^50\). The prevalence of such sequences and their implication in diseases suggest their important roles in human genomes and diseases\(^47–49,51\). Third, we compared the conservation score distributions of CDS and UTR indels between cancer genomes and healthy genomes. Finally, we assessed the structural effects of the coding somatic NFS indels and investigated somatic indels in the 125 SMGs identified from different cancer types.

The somatic indels from different cancer types vary greatly. As shown in Table 1, only 9988 somatic indels appear in both cancer types, which account for 16.23% of BRCA and 22.86% of LUAD somatic indels respectively. The somatic transcript indels in two cancer types have different proportion of indel types. LUAD has more deletions, more indels in coding regions and more FS indels, than the BRCA cancer type. In our datasets, we did not find any complex indels, which are formed by simultaneously deleting and inserting DNA fragments of different sizes at a common genomic location\(^7\). Our data on somatic coding indels revealed a number of top SMGs with the highest proportion of somatic coding indels in both cancer types, suggesting different mutation/variant patterns in different cancer types while there are some commonalities between cancer types.

Compared with germline transcript indels in healthy genomes, somatic transcript indels in cancer genomes have higher proportions involved in the CDS regions. Coding somatic indels also have a higher rate of FS types, especially in SMGs (Tables 2, 6). This phenomenon is not surprising since FS indels are prone to be deleterious\(^41–43,74,75\). More importantly, the somatic coding indels are more likely to be enriched in the structurally and functionally important regions of proteins than the germline indels in the healthy genomes. First of all, we found that the NFS somatic indels in BRCA and LUAD are enriched in helical and strand secondary structure types (Fig. 1). Helices and strands represent the core of protein structures. Changes in the core would more likely affect the stability of the protein and disrupt the structure, which in turn affect the function of the protein. Secondly, the somatic coding indels are enriched in coding regions that are also predicted as TFBSs, or duons (Tables 4, 6). Therefore, these indels not only affect the protein sequences, they can also change the regulation of gene expression. In addition, compared to germline indels, somatic CDS indels are enriched in positions that have high conservation score based on phyloP analyses, suggesting these indels are more deleterious (Fig. 2).

While the cancer whole exome sequencing data have all the coding and UTR sequences that can be compared directly with the germline coding and UTR sequences in healthy genomes, one of the limitations of the whole exome sequences is that they only have partial non-coding sequences for the transcripts. It would be interesting to see the differences in the non-coding regions among different cancer types and between germline indels and cancer somatic indels from a large-scale comparative analysis. More detailed analyses on structural and functional effect can be carried out in the future to investigate the structural basis for better understanding these somatic indels as previous work done on point mutations\(^79–82\) and if a somatic indel is deleterious\(^7\).

Data availability
The data used in this study were downloaded from the TCGA data portal at https://portal.gdc.cancer.gov (dbGaP Study Accession: phs000178.v11.p8).

Received: 15 July 2021; Accepted: 14 October 2021
Published online: 27 October 2021
References

1. The 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. Nature 467, 1061 (2010).
2. Mills, R. E. et al. An initial map of insertion and deletion (INDEL) variation in the human genome. Genome Res. 16, 1182–1190. https://doi.org/10.1101/gr.4565806 (2006).
3. Sachidanandam, R. et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 409, 928–934 (2001).
4. Sun, H. & Yu, G. New insights into the pathogenicity of non-synonymous variants through multi-level analysis. Sci. Rep. 9(1), 1–11 (2019).
5. The International HapMap Consortium. A haplotype map of the human genome. Nature 437, 1299 (2005).
6. Weber, J. L. et al. Human diallelic insertion/deletion polymorphisms. Am. J. Hum. Genet. 71, 854–862 (2002).
7. Bhagale, T. R., Rieder, M. J., Livingston, R. J. & Nickerson, D. A. Comprehensive identification and characterization of diallelic insertion–deletion polymorphisms in 330 human candidate genes. Hum. Mol. Genet. 14, 59–69 (2005).
8. Iafrate, A. J. et al. Detection of large-scale variation in the human genome. Nat. Genet. 36, 949–951 (2004).
9. Berger, J. et al. Genetic mapping with SNP markers in Drosophila. Nat. Genet. 29, 475–481 (2001).
10. Wicks, S. R., Yeh, R. T., Gish, W. R., Waterston, R. H. & Plasterk, R. H. Rapid gene mapping in Caenorhabditis elegans using a high density polymorphism map. Nat. Genet. 28, 160–164 (2001).
11. Dawson, E. et al. A SNP resource for human chromosome 22: Extracting dense clusters of SNPs from the genomic sequence. Genome Res. 11, 170–178 (2001).
12. Mullaney, J. M., Mills, R. E., Pittard, W. S. & Devine, S. E. Small insertions and deletions (INDELS) in human genomes. Hum. Mol. Genet. 19, R131–R136 (2010).
13. Montgomery, S. B. et al. The origin, evolution, and functional impact of short insertion–deletion variants identified in 179 human genomes. Genome Res. 23, 749–761 (2013).
14. Chuzhanova, N. A., Anassis, E. J., Ball, E. V., Krawczak, M. & Cooper, D. N. Meta-analysis of indels causing human genetic disease: Mechanisms of mutagenesis and the role of DNA sequence complexity. Hum. Mutat. 21, 28–44 (2003).
15. Collins, F. S. et al. Construction of a general human chromosome jumping library, with application to cystic fibrosis. Science 235, 1046–1049 (1987).
16. Mills, R. E. et al. Natural genetic variation caused by small insertions and deletions in the human genome. Genome Res. 21, 830–839 (2011).
17. Chen, J. & Guo, J.-T. Comparative assessments of indel annotations in healthy and cancer genomes with next-generation sequencing data. BMC Med. Genomics 13, 1–11 (2020).
18. Turajlic, S. et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: A pan-cancer analysis. Lancet Oncol. 18, 1009–1021 (2017).
19. Alexandrov, L. B. et al. The repertoire of mutational signatures in human cancer. Nature 578, 94–101. https://doi.org/10.1038/s41586-020-1943-3 (2020).
20. The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. Nature 578, 82–93. https://doi.org/10.1038/s41586-020-1969-6 (2020).
21. Lin, M. et al. Effects of short indels on protein structure and function in human genomes. Sci. Rep. 7, 1–9 (2017).
22. Folkman, L. et al. DDIG-in: Detecting disease-causing genetic variations due to frameshifting indels and nonsense mutations employing sequence and structural properties at nucleotide and protein levels. Bioinformatics 31, 1599–1606. https://doi.org/10.1093/bioinformatics/btu862 (2015).
23. Zhao, H. H. et al. DDIG-in: discriminating between disease-associated and neutral non-frameshifting micro-indels. Genome Biol. 14, R23. https://doi.org/10.1186/gb-2013-14-3-r23 (2013).
24. Hu, J. & Ng, P. C. Predicting the effects of frameshifting indels. Genome Biol. 13, R9. https://doi.org/10.1186/gb-2012-13-2-r9 (2012).
25. Kircher, M. et al. A general framework for estimating the relative pathogenicity of human genetic variants. Nat. Genet. 46, 310–315. https://doi.org/10.1038/ng.2892 (2014).
26. Yue, Z., Zhao, L., Cheng, N., Yan, H. & Xia, J. dbCID: A manually curated resource for exploring the driver indels in human cancer. Brief Bioinform. 20, 1925–1933. https://doi.org/10.1093/bib/bby059 (2019).
27. Yue, Z., Chu, X. & Xia, J. PredCID: Prediction of driver frameshift indels in human cancer. Brief Bioinform. https://doi.org/10.1093/bib/bbaa119 (2021).
28. Pagel, K. A. et al. Pathogenicity and functional impact of non-frameshifting insertion/deletion variation in the human genome. PLoS Comput. Biol. 15, e1007112 (2019).
29. Niu, B. et al. Protein-structure-guided discovery of functional mutations across 19 cancer types. Nat. Genet. 48, 827–837 (2016).
30. Zhang, F. & Lupski, J. R. Non-coding genetic variants in human disease. Hum. Mol. Genet. 24, R102–R110 (2015).
31. Arking, D. E. et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. Nat. Genet. 38, 644–651 (2006).
32. Kapoor, A. et al. An enhancer polymorphism at the cardiomyocyte intercalated disc protein NOS1AP locus is a major regulator of the QT interval. Am. J. Hum. Genet. 94, 854–869 (2014).
33. Spieler, D. et al. Restless legs syndrome-associated intronic common variant in Meis1 alters enhancer function in the developing telencephalon. Genome Res. 24, 592–603 (2014).
34. Bauer, D. E. et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science 342, 253–257 (2013).
35. Stadhouders, B. et al. HBS1L-MYB intergenic variants modulate fetal hemoglobin via long-range MYB enhancers. J. Clin. Investig. 124, 1699–1710 (2014).
36. Khurana, E. et al. Role of non-coding sequence variants in cancer. Nat. Rev. Genet. 17, 93–108. https://doi.org/10.1038/nrg.2015.17 (2016).
37. Fujimoto, A. et al. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. Nat. Genet. 48, 500–509. https://doi.org/10.1038/ng.3547 (2016).
38. Melton, C., Reuter, J. A., Spacek, D. V. & Snyder, M. Recurrent somatic mutations in regulatory regions of human cancer genomes. Nat. Genet. 47, 710–716. https://doi.org/10.1038/ng.3332 (2015).
39. Capasso, M. et al. Transcription Factors involved in tumorigenesis are over-represented in mutated active DNA-binding sites in neuroblastoma. Cancer Res. 80, 382–393. https://doi.org/10.1158/0008-5472.CAN-19-2883 (2020).
40. Sakhikumar, S. et al. Whole-genome sequencing of glioblastoma reveals enrichment of non-coding constraint mutations in known and novel genes. Genome Biol. 21, 1–22 (2020).
41. Imielinski, M., Guo, G. & Meyerson, M. Insertions and deletions target lineage-defining genes in human cancers. Cell 168, 460–472 (2017).
42. Nakagomi, T. et al. Clinical implications of noncoding indels in the surfactant-coding genes in lung cancer. Cancers 11, 552 (2019).
Author contributions
J.G. conceived the study and designed the experiments. J.C. carried out the experiments and performed data analysis. J.G. and J.C. wrote the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by the National Science Foundation [DBI-2051491 to J.G]; and National Institutes of Health [R15GM132846 to J.G]. Funding for open access charge: National Science Foundation.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-00583-1.

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