Distinct sequence features underlie microdeletions and gross deletions in the human genome

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Abstract

Microdeletions and gross deletions are important causes (˜20%) of human inherited disease. Their genomic locations are strongly influenced by the local DNA sequence environment. Yet no systematic study has examined the generative mechanisms. Here, we obtained 42,098 pathogenic microdeletions and gross deletions from the Human Gene Mutation Database (HGMD) that together form a continuum of germline deletions ranging in size from 1 bp to 28,394,429 bp. We analyzed the sequence within 1-kb of the breakpoint junctions and found the frequencies of non-B DNA-forming repeats, GC content, and the presence of seven of 78 specific sequence motifs in the vicinity of pathogenic deletions correlated with deletion length for deletions of length [?]30 bp. Furthermore, we found the repeats of DR, GQ, and STR appear to be important for the formation of longer deletions (>30 bp) but not for the formation of shorter deletions ([?]30 bp) and significantly (Chi-square test P-value < 2E-16) more microhomologies were identified in flanking short deletions than long deletions (length >30 bp). We provide evidence to support a functional distinction between microdeletions and gross deletions. A deletion length cut-off of 25-30 bp may serve as an objective means to functionally distinguish microdeletions from gross deletions.

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Abstract

Microdeletions and gross deletions are important causes (˜20%) of human inherited disease. Their genomic locations are strongly influenced by the local DNA sequence environment. Yet no systematic study has examined the generative mechanisms. Here, we obtained 42,098 pathogenic microdeletions and gross deletions from the Human Gene Mutation Database (HGMD) that together form a continuum of germline deletions ranging in size from 1 bp to 28,394,429 bp. We analyzed the sequence within 1-kb of the breakpoint junctions and found the frequencies of non-B DNA-forming repeats, GC content, and the presence of seven of 78 specific sequence motifs in the vicinity of pathogenic deletions correlated with deletion length for deletions of length [?]30 bp. Furthermore, we found the repeats of DR, GQ, and STR appear to be important for the formation of longer deletions (>30 bp) but not for the formation of shorter deletions ([?]30 bp) and significantly (Chi-square test P-value < 2E-16) more microhomologies were identified in flanking short deletions than long deletions (length >30 bp). We provide evidence to support a functional distinction between microdeletions and gross deletions. A deletion length cut-off of 25-30 bp may serve as an objective means to functionally distinguish microdeletions from gross deletions.

Keywords: DNA structure; microdeletions; gross deletions; non-B DNA-forming repeats; GC content; DNA sequence motifs

Background

Deletions are responsible for many human genetic diseases and together constitute about 20% of all mutations known to cause human inherited disease(Stenson et al., 2020). Deletions are associated not only with common disorders, such as Alzheimer's disease(Cukier et al., 2016; Prihar et al., 1999), Parkinson's disease(Tan, 2016), intellectual disability(Sharp et al., 2006), autistic spectrum disorders(Sato et al., 2012; Vaags et al., 2012), and heritable cancers(Guo et al., 2018; Xu et al., 2012) but also rare or low-frequency diseases(Nambot et al., 2018). Disease-associated deletions in humans may range in length between 1 bp up to many thousands or even millions of base-pairs (bp). Historically, the Human Gene Mutation Database (HGMD) has subdivided genomic deletions into microdeletions (1-20 bp) and gross deletions (>20 bp)(Stenson et al., 2020), but this distinction was originally made fairly arbitrarily for reasons of practical utility rather than for any cogent biological reason. Many studies(Claudia MB Carvalho & James R Lupski, 2016; Keute et al., 2020; Maranchie et al., 2004; Sahoo et al., 2006) have suggested the involvement of different mechanisms in the formation of microdeletions and gross deletions including non-homologous end-joining (NHEJ), microhomology-mediated end-joining (MMEJ), non-allelic homologous recombination (NAHR), retrotransposon-mediated mechanisms, and replication-based errors including fork stalling and template switching (FoSTeS) and microhomology-mediated break-induced replication (MMBIR)(Abelleyro et al., 2020; Bauters et al., 2008; Carvalho et al., 2009; Férec et al., 2006; Gadgil et al., 2020; P. Hastings, Ira, & Lupski, 2009; P. J. Hastings, Lupski, Rosenberg, & Ira, 2009; Hu et al., 2019; Lee, Carvalho, & Lupski, 2007; Marey et al., 2016; Summerer et al., 2018; J. Vogt et al., 2014; Zhang et al., 2009; Zhang et al., 2010). Jahic et al.(Jahic et al., 2017) have presented doublet-mediated DNA rearrangements as a mechanism for the formation of recurrent pathogenic deletions of exon 10 in the*SPAST* gene. These different mutational mechanisms may be inferred by the presence of different breakpoint sequence features(Kidd et al., 2010).

Both gross deletions and microdeletions are non-randomly distributed in the human genome and are known to be strongly influenced by the local DNA sequence environment(Del Mundo, Zewail-Foote, Kerwin, & Vasquez, 2017; Georgakopoulos-Soares, Morganella, Jain, Hemberg, & Nik-Zainal, 2018). Previous studies

have found that both gross deletions and microdeletions originate through the formation and resolution of aberrant DNA secondary structures, and we now know that the process of secondary structure formation is strongly sequence-mediated(Férec et al., 2006; Kouzine et al., 2017; Krawczak & Cooper, 1991; Wu et al., 2014). Previous studies have found that the breakpoints of deletions often possess a significant number of identical nucleotides, indicating the involvement of direct repeats(Kato et al., 2008), while replication slippage is recognized as a common cause of microdeletions(MacLean, Favaloro, Warne, & Zajac, 2006). A more recent study has revealed that replication-based mechanisms are frequently involved in gross duplications and deletions(Ankala et al., 2012; C. M. Carvalho & J. R. Lupski, 2016; Geng et al., 2021; Marey et al., 2016; Seo et al., 2020). Analyzing 8,399 microdeletions in 940 genes from HGMD, one early study found that 81% of microdeletions (<21 bp) were located in the vicinity of direct, inverted, or mirror repeats(Ball et al., 2005). Another study attempted to relate the occurrence of microdeletions to the presence of non-B DNA structures by employing a set of 17,208 microdeletions (defined as being of length <21 bp), and found that 56% of microdeletions harbored either direct repeats or mirror repeats near the breakpoints(Kamat, Bacolla, Cooper, & Chuzhanova, 2016). An analysis of 11 gross deletions associated with autosomal dominant polycystic kidney disease, early-onset Parkinsonism, Menkes disease, α^{+}thalassemia, adrenoleukodystrophy, and hydrocephalus, respectively, concluded that these large deletions were mediated by negative supercoiling-dependent non-B DNA conformations(Bacolla et al., 2004). Sequence motifs capable of forming non-B DNA structures contribute to the genome-wide instability responsible for both small- and large-scale copy number variants(Brown & Freudenreich, 2021; Guiblet et al., 2021). Arlt et al.(Arlt et al., 2009) reported that replication stress induces genome-wide copy number changes resembling pathogenic deletions and duplications. Most deletion breakpoint junctions were characterized by microhomologies suggesting that the deletion breakpoint junctions were formed by non-homologous end joining (NHEJ) or a replication-coupled process(Seo et al., 2020). Marely et al.(Marey et al., 2016) illustrated the important role of NHEJ in the formation of *DMD* gene deletions.

Different forms of sequence capable of forming non-B DNA structures predispose certain genomic regions to instability causing pathogenic rearrangements (Zhao, Bacolla, Wang, & Vasquez, 2010). The relationship between deletions and non-B DNA structures has been investigated in terms of the molecular properties of the deletion breakpoints (the breakpoints being defined as the junctions between the normal and rearranged DNA sequences)(Bacolla, Wojciechowska, Kosmider, Larson, & Wells, 2006; Damas, Carneiro, Amorim, & Pereira, 2014; Keegan, Wilton, & Fletcher, 2019). Verdin et al. identified various genomic architectural features, including sequence motifs, putative sites of non-B DNA conformations, and repetitive elements in breakpoint regions(Verdin et al., 2013). Recurrent gross chromosomal rearrangements, including large deletions of several hundred kb are mediated by non-allelic homologous recombination NAHR (Demaerel et al., 2019; Dittwald et al., 2013; Harel & Lupski, 2018; Hillmer et al., 2016; Inoue & Lupski, 2002; Liu, Carvalho, Hastings, & Lupski, 2012; P. H. Vogt et al., 2021). More recently, 8,943 non-pathogenic deletion breakpoints from 1,092 healthy humans were analyzed, revealing that NAHR-mediated breakpoints are associated with open chromatin(Abyzov et al., 2015). To our knowledge, however, no study has been performed that systematically explores the range of structural features associated with, and the mechanisms underlying, the full spectrum of human pathogenic gene deletions of different lengths, extending from the smallest of microdeletions to gross deletions. Such a study is needed to determine how microdeletions differ from gross deletions in terms of their underlying generative mechanisms, and whether there is a natural threshold or cut-off between these two entities or if they simply form the discrete ends of a continuum.

Besides a relationship between non-B DNA structure-forming motifs and deletion mutagenesis, several studies show that increasing GC content is associated with elevated rates of mutation and recombination(Kiktev, Sheng, Lobachev, & Petes, 2018; Romiguier, Ranwez, Douzery, & Galtier, 2010). Deletion rates also vary between species in relation to genomic GC content(Hardison et al., 2003; Lindsay, Rahbari, Kaplanis, Keane, & Hurles, 2019). A study of eutherian genomes found that increased GC content was associated with an increase in germline deletion frequency(Hardison et al., 2003). In a similar vein, an analysis of 33 mammalian genomes found that GC-rich sequences were prone to deletion(Romiguier et al., 2010). These discoveries have indicated the importance of GC content in the formation of deletions in several different contexts. However,

all these studies have either been inter-species comparisons or intra-genome comparisons in healthy humans and did not investigate pathogenic deletions. Importantly, to our knowledge, no study has yet investigated the relationship between GC content and deletion length in a disease context. Thus, here we formally investigate the relationship between GC content and pathogenic deletion length.

Various sequence motifs have been reported to be over-represented in the vicinity of microdeletion breakpoints(Ball et al., 2005). For example, purine-pyrimidine sequences and polypurine tracts are significantly enriched in the vicinity of gross gene deletions(Abeysinghe, Chuzhanova, Krawczak, Ball, & Cooper, 2003). Recurrent large deletion of 1.11-Mb in 14q32.2 is catalyzed by large (TGG)n tandem repeats(Béna et al., 2010). One study reporting the sequencing of the breakpoint junctions of 30 rare deletions spanning between 91 bp and 14 kb found that most breakpoints exhibited microhomologies and were associated with specific sequence motifs(Vissers et al., 2009). Currently, we estimate that at least 78 sequence motifs have been found to occur at elevated frequencies in the vicinity of deletion, recombination, or translocation breakpoints(Abeysinghe et al., 2003; Ball et al., 2005; Chuzhanova et al., 2009). Ball et al(Ball et al., 2005). reported 30 motifs, including the heptanucleotide CCCCCTG, DNA polymerase pause sites, and topoisomerase cleavage sites that occurred frequently near deletion breakpoints. Chuzhanova et al(Chuzhanova et al., 2009). Presented DNA sequence motifs are known to be associated with site-specific cleavage/recombination, gene mutations, and various "super-hotspot motifs" that were over-represented in the vicinity of microdeletions. However, to our knowledge, no attempt has as yet been made to analyze a large set of pathogenic deletions, including both microdeletions and gross deletions, in order to systematically explore the relationship between deletion length and occurrence frequency for the different types of sequence motif residing in the vicinity of breakpoints.

Here, we have performed an analysis of pathogenic gene deletions on two originally distinct microdeletion and gross deletion datasets from the Human Gene Mutation Database[43]. Together, these comprise 42,098 breakpoints in a total of 3,685 genes. We used simulated "deletions" matched by length and genomic position as controls. The purpose of this analysis was to assess the combined datasets in terms of the frequencies of six types of non-B DNA-forming repeat, GC content, the frequencies of specific sequence motifs, and microhomologies neighboring the breakpoints. We propose several possible mechanisms for the formation of microdeletions and gross deletions. In addition, we compare generative mechanisms of microdeletions and gross deletions and suggest a new working definition with which to discriminate between microdeletions and gross deletions in terms of their size and underlying mechanisms of formation.

Materials and Methods

Mutation and control datasets

In December 2019, the HGMD(Stenson et al., 2020; Stenson et al., 2014) Professional release 2019.4 [*http://www.hgmd.org*] contained 38,725 microdeletions of [?]20 bp and 3,373 gross (>20 bp) deletions all characterized at base-pair resolution, then constituting about 20% of all sequence-characterized mutations causing human inherited disease. These two deletion datasets were collected from the primary literature in precisely the same way; the 20 bp cut-off employed historically between microdeletions and gross deletions were entirely arbitrary and did not influence collation efficiency in any way. For the purposes of this study, these datasets were merged and together termed the 'HGMD-deletion dataset'. In total, 42,098 deletions were included in the HGMD-deletion dataset. Of these deletions, 40,037 (95.1%) have a length$\leq$106 bp whilst 2,061 (4.9%) deletions have a length between 107 and 28,394,429 bp. Figure S15 displays the log values of deletion numbers (length <107 bp) along deletion lengths. Supplementary Table S6 includes the number of deletions with a specific length.

In order to assess the non-randomness of the HGMD-deletion dataset, we generated 100 simulated breakpoints for each deletion; these were randomly sampled within 3000 bp of the upstream region of each pathogenic deletion breakpoint. This process yielded 4,209,800 random breakpoints for the HGMD-deletion dataset. Then, according to the coordinates of the 100 simulated breakpoints, we generated random deletions that matched each pathogenic deletion in terms of its length. By centering each simulated breakpoint

around a 1-kb bin, we generated a sequence around the breakpoint and included it in the control0 dataset. In total, the control0 dataset includes 4,209,800× 2 breakpoints and 4,209,800 ×2 flanking sequences. By randomly sampling 10 deletions for each pathogenic deletion from control0, we generated the simulated dataset, termed control1 that contained 420,980 deletions. If the simulated sequences contained undefined bases (N), these sequences were excluded from the analysis, and new random breakpoints and flanking sequences were generated by resampling. The coordinates of the simulated sequences were retrieved from a genome sequence file in version hg19 that was downloaded from*https://www.gencodegenes.org/human/*. Supplementary Table S7 shows the coordinates of the control1 dataset.

Searching for non-B DNA-forming repeats in flanking sequences

Non-B DNA-forming repeats within each flanking sequence were obtained from the non-B DB database(Cer et al., 2011; Cer et al., 2013) with custom filters for mirror repeats (Table S1). As shown in Table S1, the mirror repeats were filtered by triplex-motif that is predicted by non-B DB as subset=1. In this study, six types of non-B DNA-forming repeat were considered, specifically direct repeats (DR), inverted repeats (IR), mirror repeats (MR), G-quartets (GQ), short tandem repeats (STR), and Z-DNA (Z)(Ghosh & Bansal, 2003; Kondrashov & Rogozin, 2004; Wells, 2007). More detailed information on each type of non-B DNA-forming repeat is to be found in the Supplementary Material (Table S1). The frequencies of the non-B DNA-forming repeats in the flanking sequences of the pathogenic deletions were compared with the frequencies of these repeats in the simulated data, the control1 dataset. Statistical significance was assessed by means of the Student's t-test, and a Bonferroni correction was applied to allow for multiple testing.

Specific sequence motifs in deletion flanking sequences

From previous publications(Abeysinghe et al., 2003; Ball et al., 2005; Chuzhanova et al., 2009), we collected a total of 78 sequence motifs (Table S4) that have been reported to occur in the vicinity of deletion/rearrangement breakpoints and are thought to play a role in the breakage and rejoining of DNA molecules. Briefly, Abeysinghe *et al* . (2003)(Abeysinghe et al., 2003) listed 36 sequence motifs known to be associated with site-specific recombination, mutation, and DNA cleavage. In their later study, Ball et al. (2005)(Ball et al., 2005) collected an additional 24 sequence motifs thought to be involved in site-specific recombination and putative deletion/insertion hotspots. Finally, Chuzhanova et al. (2009)(Chuzhanova et al., 2009) reported 18 further motifs associated with deletions and recombination. We computed the frequency for each motif in the 1 kb-long sequences flanking the pathogenic deletions from the HGMD-deletion dataset and in the control0 dataset using the R package Biostrings(Gentleman & DebRoy, 2019). We utilized the simulated deletions to determine whether the number of any type of motif in the vicinity of each breakpoint was higher than expected by computing an "experience hit" (eH-value), i.e., the number of times the number of the motifs in the vicinity of the simulated breakpoints of the control dataset was larger than the number of motifs in the vicinity of the pathogenic deletion breakpoints, divided by 100. The relationship between deletion length and motif frequency was then explored by calculating the average motif frequency for each deletion length.

GC content

GC content was calculated for sequences in 1-kb bins centered at the breakpoints of the pathogenic deletions and simulated deletions using custom R codes. GC content was calculated for each deletion and each location from breakpoints, respectively. We explored the relationship between GC content and deletion length by considering average GC content centered around the deletion breakpoint for each deletion length.

Microhomology analysis

To determine the extent to which microhomologies are associated with deletion variants, we used MH-cut(Grajcarek et al., 2019) to search for homologous sequences at the junction sequences of deletion variants, thereby yielding a score with which to evaluate any microhomology present. For each deletion entry, microhomology was tested for both flanking configurations (5' flanking region with 3' variant sequence and 3' flanking region with 5' variant sequence), from which we selected the one with the highest score. The

enrichment of microhomologies in the flanking sequence of deletions was assessed by means of the Chi-square test.

Results

Non-B DNA-forming repeats and deletion breakpoints

A major goal of this work was to ascertain whether gene deletions causing human inherited disease occur disproportionately at sites that are capable of adopting non-B DNA structures, including hairpin and looped-out bases (direct repeats (DR) and short tandem repeats (STR)), cruciform (inverted repeats (IR)), mirror repeats (MR), G4 DNA (G-quartets (GQ)), and left-handed Z-DNA (Z-DNA (Z)). Using criteria defined in previous studies(Cer et al., 2011; Cer et al., 2013) and in Table S1, we searched for uninterrupted versions of each type of repeat within a 1-kb window centered at each deletion breakpoint. We found that most of the identified repeat sequences were less than 50 bp in length (Figure 1). As shown in Figure 1, more IR and STR were found in the deletion flanking sequences than other types of repeats.

We compared the total numbers of repeats within 1-kb bins centered at the breakpoints for the HGMD-deletion data and the simulated deletion dataset. All repeats occurred with a higher frequency in the vicinity of the gross deletions (length >20 bp) than in the control1 dataset (Table 1). However, when we combined the gross deletions and microdeletions, we found that the numbers of repeats in the individual DR, IR, MR, STR, and Z DNA categories around the pathogenic deletion breakpoints were lower than those around the simulated data (Table 1, Figure 2). Table S2 shows the detailed comparison of frequencies of different types of non-B DNA-forming repeats in the vicinity of breakpoints of deletions of different lengths. The frequencies of GQ around the pathogenic deletion breakpoints were higher than around the simulated data when the GQ was about 150 bp away from the deletion breakpoints (Figure 2D). However, when the GQ was close to the deletion breakpoints, the frequency of this repeat around the pathogenic deletion breakpoints was lower than around the simulated data (Figure 2D). We also partitioned the GQs around the breakpoints of deletions into G-rich GQs (15,931/32,067, 49.68%) and C-rich GQs (16,136/32,067, 50.32%), and compared their frequencies around pathogenic deletion breakpoints with the simulated data, control1. We found that the frequencies of C- and G-rich GQs around breakpoints of pathogenic deletions were rather similar and generally higher, than around the simulated deletion breakpoints of control1 (Figure S1A and B).

To ascertain whether we could identify a cut-off that would help to functionally distinguish gross deletions from microdeletions based on the occurrence of non-B DNA-forming motifs, we determined the average frequency of all types of non-B DNA-forming repeat in the 1-kb bins centered at the deletion breakpoints. As shown in Figure 3A, as the length of the pathogenic deletions increased, so too did the average frequency of non-B DNA-forming repeats around the deletion breakpoints. When the deletion length was ≤8 bp, the frequency of occurrence of non-B DNA-forming repeats in the vicinity of deletion breakpoints was lower than random expectation. Here, only 40,037 deletions shorter than 106 bp in length were analyzed because beyond this length the number of deletions of each length is less than 4 and the number of deletions is only 4.9% of the total. When we used a 10 bp sliding window to separate the deletions into bins and computed the average frequency of non-B DNA-forming repeats around the deletion breakpoints for the deletions in each bin, we found that deletion length was positively correlated with the frequency of non-B DNA-forming repeats but was not significant (Pearson Correlation Coefficient (PCC)=0.33, p=0.32) (Figure 3B).

We then tested the correlation between deletion length and the frequency of non-B DNA-forming repeats. When the deletion length was [?]9 bp, the PCC of deletion length and average non-B DNA-forming repeat frequency was 0.79 (P-value = 1.10E-2). When the deletion length was less than [?]27 bp, the PCC attained its maximal value, 0.91 (P-value = 3.39E-11), whereas when the deletion length was less than [?]30 bp, the PCC was 0.80 (P-value = 9.06E-8) (Figure 3C). These findings indicate that the non-B DNA-forming repeat frequency in the vicinity of the breakpoints of deletions [?] 27 bp in length was significantly and positively correlated with deletion length. When the deletion length was >30 bp, no significant correlation was observed between deletion length and the average non-B DNA-forming repeat frequency. Thus, we speculate that 30 bp could represent a natural cut-off that serves to separate the pathogenic deletions into

two relatively distinct (albeit overlapping) groups, with the larger deletions (with length >30 bp) having more complicated mechanisms of formation than the shorter deletions.

The relationship between the frequencies of the different types of non-B DNA-forming repeats and the deletion length is shown in Figure S2. For G-quadruplex-forming (GQ) sequences, a strong correlation (PCC=0.87, p=3.48E-10) was observed between deletion length and repeat frequency when the deletion length was ≤30 bp. For IR, DR, and STR, strong correlations (PCC=0.72 and p=1.3E-2, PCC=0.76 and p=5E-6, and PCC=0.73 and p=1.57E-5, respectively) were observed when the deletion length was≤11 bp, ≤27 bp, and ≤27 bp, respectively. However, no strong correlation was observed between deletion length and the average frequencies of MR and Z-DNA-forming repeats. Taken together, for DR, GQ, and STR the frequencies of these repeats were significantly correlated with deletion length when the deletions were ≤30 bp; for IR, the repeat frequencies were significantly correlated with deletion length when the deletions were ≤10 bp. These results suggest that a more precise cut-off to separate deletions mechanistically into microdeletions and gross deletions might lie between 10 bp and 30 bp.

To further investigate the non-B DNA-forming repeat frequency and distribution in the vicinity of breakpoints of deletions of different lengths, we used 30 bp as a cut-off to divide the pathogenic deletions in the HGMD-deletion dataset into gross deletions and microdeletions and analyzed the frequency of DR, GQ, and STR repeats in the vicinity of the breakpoints. We observed two frequency peaks of DR and STR repeats for deletions >30 bp and two frequency valleys for deletions [?]30 bp (Figure 4 A and C). However, no obvious frequency peak or valley was observed for GQ repeats flanking deletions >30 bp whereas a valley was found around the breakpoint location of deletions [?]30 bp (Figure 4B). When we divided the GQ repeats into G-rich and C-rich, we found that the frequencies of G-rich GQ repeats and C-rich GQ repeats around breakpoints of short and long pathogenic deletions are close, and show valleys around the breakpoints of deletions with length [?]30 bp (Figure S1C). The underlying reason for the absence of any obvious frequency peak of GQ repeats for deletions with length >30 bp appears to be due to the fact that G4 structures arising from GQ repeats may cause DNA polymerase pausing when associated with certain short motifs, which in turn promotes short deletions. Indeed, when we analyzed the probability of co-occurrence of GQ around deletions with short motifs found at DNA polymerase pause sites (Supplementary Table S3), 91.1% of the GQs co-occurred with such short motifs.

We also used 10 bp as a cut-off to divide the deletions into microdeletions and gross deletions and to analyze the frequency of IR in the vicinity of breakpoints. The frequencies of IR repeats showed a peak around the breakpoint of deletions with length >10 bp, and a valley at the breakpoint of deletions with length [?]10 bp (Figure 4D). These results suggest that the deletions separated by a cut-off into two groups had different properties in terms of the frequencies of non-B DNA-forming repeats in the vicinity of breakpoints. The patterns observed for the frequencies of non-B DNA-forming repeats in the vicinity of deletion breakpoints contrasted with the flat lines seen in controls (Figure 4), supporting the conclusion that a 30 or 10 bp cut-off can functionally distinguish microdeletions from gross deletions.

In summary, the frequency and distribution of non-B DNA forming repeats in the vicinity of pathogenic deletion breakpoints were clearly different when comparing deletions [?]30 and >30 bp (Figure 4). These differences may represent heterogeneity in the underlying causative mechanisms responsible for both groups of deletion. For the breakpoints of deletions [?]30 bp, the number of non-B DNA-forming repeats increased in the breakpoint flanking regions in a "mirror image" fashion, suggesting that these breakpoints are either rarely located within non-B DNA forming sequences or that limited resection occurs before repair. Nevertheless, the increase in the frequency of these repeats at breakpoint flanking regions supports the view that non-B DNA structures induced nearby DNA breakage or polymerase stalling. Indeed, a comparable pattern of non-B DNA-forming sequences were not observed in the control dataset or in pathogenic deletions >30 bp. Rather, the most striking difference between the [?]30 bp and >30 bp deletions was observed from the distribution of direct repeats, which exhibited the highest frequency directly at breakpoints, suggesting replication slippage as the initiating event for the genetic alteration.

Non-B DNA-forming repeat motifs associated with deletions

We wished to ascertain whether the short deletions and long deletions were associated with different types of repeat motifs. Six types of non-B DNA-forming repeat, DR, GQ, IR, MR, Z-DNA, and STR, were investigated in this study. For each type of repeat, we obtained the top 10 most frequent sequences occurring in the vicinity of breakpoints of deletions with length >30 bp or ≤30 bp (Figure S3). Interestingly, most repeat motifs occurring in the vicinity of short deletions were different from the repeat motifs occurring in the vicinity of the long deletions (Figure S3). For DR (Figure S3A and B), all of the top 10 repeat motifs in deletions >30 bp were single nucleotide repeats whereas in deletions ≤30 bp, only one of the top 10 repeats in DR was a single base repeat. Meanwhile, for MR, we observed six single nucleotide repeat motifs (all motifs were nucleotide poly-A repeats) among the deletions >30 bp whereas only three single nucleotide repeats were found in the deletions≤30 bp (Figure S3 E and F). Thus, there may be a preference for single nucleotide repeats [poly A, poly T, poly C, or poly G] around deletion breakpoints [?]30 bp. From Figure S3I and J, we can see that seven of the top 10 repeat motifs occurring in STR are shared between the long deletions and the short deletions. We also noted that the sequence preference of Z-DNA repeats in long deletions is similar to the sequence preference associated with short deletions (Figure S3K and L). The underlying reason may be that for the STR and Z-DNA repeats, the cut-off in terms of partitioning the deletions into short and long groups does not lie around 30 bp (Figure S2). Frequencies of Z-DNA repeats were not found to correlate with the deletion length. When Z-DNA was divided into two groups according to deletion length, a frequency peak was observed at the breakpoints (Figure S4F) of long deletions (length >20 bp) but not at the breakpoints of short deletions ([?]20 bp). Thus, if we use the frequency of Z-DNA to define the gross deletions, 20 bp may be the appropriate cut-off.

Relationship between GC content and deletion length

We next determined the GC content within the 1-kb bins centered at the breakpoints in the HGMD-deletion dataset and the control1 dataset. As shown in Figure 5A, the GC content was at its maximum at precisely 1 bp from the breakpoint. Further, the GC content was invariably higher for pathogenic deletions than for the control1 dataset (Student's t-test p< 2.2E-16). In addition, the GC content distribution for control1 was remarkably constant irrespective of the breakpoint location and did not show a peak or valley at the breakpoint. The average GC content was then determined for deletions of different lengths. The results are shown in Figure 5B. When the deletion length was ≤29 bp, the correlation between deletion length and GC content reached the highest value, with PCC=0.87 (p=6.0E-10). The GC content was found to correlate significantly (PCC=0.71 and p=7.3E-7) with deletion length when the deletion length was [?]38 bp but not when it was >38bp. These results suggest that, in relation to GC content, 29-38 bp represents a potential cut-off that can serve to divide pathogenic deletions into gross deletions and microdeletions. When we used either 29 bp or 38 bp as a cut-off to partition the deletions into two groups, the GC content of the short deletions was higher than that of the longer deletions at the breakpoint (Figure S5). Thus, the short and long deletions partitioned by the cut-off exhibit differences in GC content at the breakpoints.

Motif frequency and deletion length

The motif analysis was performed to determine the frequencies of a series of specific DNA sequence motifs around the breakpoints of the pathogenic deletions. In total, 78 motifs (Table S4) were surveyed from previous publications(Abeysinghe et al., 2003; Ball et al., 2005; Chuzhanova et al., 2009). For each deletion from the HGMD dataset, we calculated the motif frequency at each location in 1-kb bins centered at the breakpoints. Each deletion in the HGMD dataset had 100 simulated deletions in the control0 dataset, for which we also calculated the frequency of motifs. Considering all motifs together, we compared the motif frequencies in the vicinity of the breakpoints of the pathogenic deletions (HGMD-deletion dataset) to the motif frequencies in the vicinity of breakpoints in deletions from the control0 dataset. We found that the motif frequencies flanking the pathogenic breakpoints decreased gradually with distance from 150 bp to the breakpoint, and then attained their highest values precisely one base from the breakpoint itself (Figure S6), reflecting the likely contributions of these motifs to the formation of the deletions. By contrast, the motif frequencies in the vicinity of the deletion breakpoints from the control0 dataset were remarkably similar irrespective of their distances from the breakpoints.

When we considered the frequencies of individual motifs in the vicinity of breakpoints, the distributions could be classified into four subtypes (Table S5), "Valleys", "Peaks", "M shapes" and "Others" (Figure S7-S12). In total, 22 motifs were grouped as "Valleys" (Figure S7 and S8); their frequencies decreased with decreasing distance to the breakpoints and reached their lowest values at the breakpoints themselves; 28 motifs were grouped as "Peaks" (Figure S9, Figure S10); their frequencies increased with decreasing distance to the breakpoints and reached their highest values precisely at the breakpoints; 14 motifs were grouped in an "M shape" (Figure S11) being characterized by frequencies that were distributed as an "M" shaped curve; finally, 11 motifs were grouped as "Others" (Figure S12) and were characterized by frequencies that were unrelated to distance from the breakpoints. Many of these "patterns" are exclusive to the pathogenic deletion dataset and hence may indicate specific sequence differences between both datasets that are functionally relevant and predispose these regions to instability.

We counted the frequency of each motif in 10-bp bins centered at each breakpoint of the HGMD-deletion dataset and the 100 simulated breakpoints. Then, we calculated the "experience hit" eH-values to assess the significance of each motif in the vicinity of the control breakpoints and the average eH-value of this motif over all the deletion breakpoints in the HGMD-deletion dataset. The eH-value indicates the number of times the number of the motifs in the vicinity of the simulated breakpoints of the control dataset was larger than the number of motifs in the vicinity of the pathogenic deletion breakpoints, divided by 100. We found that 23 motifs occurred more frequently (eH-value < 0.05) in 10 bp bins centered at the breakpoints of the pathogenic deletion dataset than at the breakpoints from the simulated dataset (Figure 6A). These motifs were "CTY", "RNYNNCNNGYNGKTNYNY", "GCCCWSSW", "GCTGGTGG", "GCWGGWGG", "GGAGGTGGGCAGGARG", "AGAGGTGGGCAGGTGG", "GAAAATGAAGCTATTTACCCAGGA", "TGRRKM", "CAGR", "GCS", "WGGAG", "CTGGCG", "RGAC", "RAG", "ACYYMK", "CCG", "GTAAGT", "CGGCGG", "TTCTTC", "CCACCA", "GCCCCG", "GGAGAA" (Table 2), which included four motifs identified by Ball et al.(Ball et al., 2005). The one-sided Fisher's exact test was used to examine if the motifs identified by Ball et al. overrepresented as motifs occurred more frequently in 10 bp bins centered at the breakpoints of the pathogenic deletion dataset than at the breakpoints from the simulated dataset. No significant result was identified with OR = 0.35 and P-value = 0.055. We calculated the average frequencies of all 78 motifs in 1-kb bins centered at the deletion breakpoints to explore the relationship between motif frequency and deletion length (Figure 6B) and identified six motifs for which the frequencies significantly correlated with deletion length (PCC>0.7 and p< 1E-6) (Figure S13).

Microhomology analysis for deletions and control1 data

To ascertain microhomologies, we used MHcut, which searches for homologous sequences within the flanking sequences of deletion variants. Of the 15,453 deletions with a minimum size of 3 bp, 40% (6,195) were flanked by microhomologies of at least 3 bp, which is significantly higher than the corresponding probability (7.3%±0.2%) from control1 (t-test P-value <2.2E-6). For the remaining deletions, 59.4% of 1 bp deletions were found with at least 1 bp flanking microhomologies (control1 28.2%± 0.2%), and 71.3% of 2 bp deletions were detected with at least 2 bp flanking microhomologies (control1 8.7%±0.1%), implicating microhomologies as a common enriched characteristic feature of pathogenic deletion breakpoints. When we divided the pathogenic deletions in the HGMD dataset into two groups by using 30 bp as a cutoff, we found that the sequence flanking of 42% deletions with deletions of length <30 bp have microhomologies while 29% sequence flanking of longer deletions have microhomologies. The Chi-square test indicated that the short deletions (length <30 bp) enriched (P-value < 2.2E-16) with microhomologies comparing to the longer deletions. However, there was no significant correlation between the frequency of microhomologies and deletion length.

Gross deletions and microdeletions are naturally partitioned

Our analysis indicates that the frequencies of non-B DNA-forming repeat, GC content, and specific sequence motifs all correlated with the length of the deletions when deletion length was shorter than a given threshold. The PCC values against deletion lengths are shown in Figure 7A. Here, PCC represents the extent of the correlation between deletion length and the frequencies of non-B DNA-forming repeats, GC content, and the frequencies of the sequence motifs being explored. As indicated in Figure 7A, when the deletion length

was <25 bp, the PCC values pertaining to motif frequency and deletion length were negatively correlated. The PCC of the correlation between non-B DNA-forming repeat frequencies and deletion length attained its maximum value when the deletion length was 25 bp. The highest PCC value for the correlation between the deletion length and GC content was observed when the deletion length was 29 bp. Thus, we conclude that 25-30 bp may be a natural threshold to functionally distinguish gross deletions from microdeletions in terms of the underlying generative mechanisms.

Can we score the deletions so as to separate the gross deletions and microdeletions naturally?

For each deletion, we calculated the non-B DNA-forming repeat frequency, GC content, and motif frequency in the region around it. Subsequently, we obtained the percentile ranking of the deletions in the HGMD-repeat database according to the cumulative non-B DNA-forming repeat frequency, GC content, and motif frequency. Then, each deletion was scored by summing the percentile ranking of the deletion in terms of the frequency of non-B DNA-forming repeats, GC content, and motif frequencies in the HGMD-deletion database. This score was termed the percentile ranking (PR) score. We then investigated the correlation between the PR scores of deletions and the deletion lengths. As shown in Figure 7B, when the deletion length was less than 46 bp, the average PR score for deletions of each length was significantly (PCC = 0.71 and P-value = 4.1E-8) correlated with deletion length. When the deletion length was >46 bp, no significant correlation was observed between the average PR score for deletions of each length and the deletion length. When we investigated the relationship between PR scores and deletion length with respect to repeat frequencies, GC content, and motif frequencies, respectively, we found that the deletion length (<31 bp) was significantly (P-value = 8.8E-9) correlated with the PR scores of non-B DNA-forming repeat frequency, and the deletion length (<47 bp) was significantly (P-value = 5.0E-8) correlated with the PR scores of GC content (Figure S14). These findings suggest that the deletion length around 30-47 bp could serve as a possible natural cutoff to partition microdeletions and gross deletions on the basis of their PR scores calculated from the non-B DNA-forming repeat frequency, GC content, and motif frequency.

Discussion

Irrespective of whether we consider microdeletions or gross deletions, the mechanisms underlying pathogenic deletions appear to be strongly influenced by the local DNA sequence environment(Kondrashov & Rogozin, 2004; Krawczak & Cooper, 1991). The role of non-B DNA structures in the formation of cancer-associated deletions as well as deletions in the germline and in mitochondrial sequences has been appreciated for some time(Bacolla, Tainer, Vasquez, & Cooper, 2016; Bacolla, Ye, Ahmed, & Tainer, 2019; Damas et al., 2014; Dong et al., 2014; Fontana & Gahlon, 2020; Pabis, 2021; Svetec Miklenic & Svetec, 2021; Zhao et al., 2010). Such non-B DNA structures often have key regulatory functions in DNA replication and transcription but may also cause genomic instability (Lemmens, van Schendel, & Tijsterman, 2015; Zhao et al., 2010). Furthermore, many deletions in the human genome are mediated by retrotransposon repeat-dependent mechanisms(Fujimoto et al., 2021; Mendez-Dorantes, Tsai, Jahanshir, Lopezcolorado, & Stark, 2020; Morales et al., 2021; Vocke et al., 2021). Similarly, many studies have indicated a role for GC content and DNA motif sequences in the formation of microdeletions and gross deletions(Cooper, Ball, & Mort, 2010; Visser, Shimokawa, Harada, Niikawa, & Matsumoto, 2005). However, the role of these sequence features in the formation of deletions of different lengths has not yet been methodically examined by robust statistical analyses. Meanwhile, the somewhat arbitrary definitions traditionally employed to distinguish between microdeletions and gross deletions have become blurred. We, therefore, collected 42,098 pathogenic deletions that display a length continuum stretching from 1 to 28,394,429 bp, from which we used 40,037 deletions with length <107 bp to perform a comprehensive analysis of the relationship between deletion length and non-B DNA-forming sequences, GC content, specific sequence motifs, and microhomologies.

To our knowledge, this is the first study to demonstrate that very short deletions (≤8 bp) have a low probability of co-occurrence with non-B DNA-forming repeats. However, when the deletion length is >8 bp but ≤30 bp, the non-B DNA-forming repeat frequency neighboring deletion breakpoints is significantly and positively correlated with deletion length (Figure 3). By contrast, no significant correlation was observed between deletion length and repeat frequencies for deletions >30 bp, a finding that distinguishes the complexity

of the mechanisms of formation associated with long deletions versus short deletions.

This study confirmed and extended previous observations that deletions of all sizes tend to be concentrated in GC-rich regions of the genome. Indeed, high GC content has been associated with a high level of mutation in general, not just deletions(Abeysinghe et al., 2003; Albano et al., 2010; Kiktev et al., 2018; Zheng et al., 2013). Furthermore, we found that when deletion length was less than 38 bp, the deletion length and GC content were positively correlated; the correlation attained its highest value (PCC=0.87, p=6.0E-10) when the deletion length was≤29 bp. A previous study found that increased GC content contributes to the stabilization of non-B DNA structures, thereby enhancing the propensity of deletions to occur(Tanay & Siggia, 2008). This may partially explain our findings that deletion length was positively correlated with both non-B DNA-forming motifs and GC content. A recent study discovered that GC content is associated with both increased and decreased mutation rates depending upon the nucleotide motif(Carlson et al., 2018). Our previous analysis showed that the free energy ([?]G) of fold-back structures increases with increasing GC content, and so does the number of SNPs(Abeysinghe et al., 2003; Cooper et al., 2011). The underlying reason may be that the triple bonds of G:C pairs may lead to more stable hairpins, although since GC-rich sequences are also more flexible than AT-rich ones, this may also contribute to relative stability(Abeysinghe et al., 2003; Cooper et al., 2011).

Previous studies have reported the involvement of a number of different sequence motifs in the DNA breakage events leading to microdeletions and microinsertions(Ball et al., 2005). Several studies have been performed pertaining to sequence motifs in the vicinity of large genomic rearrangement breakpoints including also large deletions(Abeysinghe et al., 2003; Dittwald et al., 2013; Ferec et al., 2006; Hillmer et al., 2017; Jahic et al., 2017; Visser, Shimokawa, Harada, Kinoshita, et al., 2005; J. Vogt et al., 2014). Here we collected a large number of inherited pathogenic deletions, representing a continuum of lengths from 1 bp to 28,394,429bp, and determined the frequency of occurrence of 78 sequence motifs known to be over- or under-represented in the vicinity of breakpoints or sites of gene conversion in the human genome(Abeysinghe et al., 2003; Ball et al., 2005; Chuzhanova et al., 2009). We found that the sequence motif frequency was significantly and negatively (PCC=-0.62, p=3.2E-2) correlated with deletion length when deletions were ≤12 bp. However, the relationship between motif frequency and deletion length may well be dependent upon the type of motif in question. As shown in Figures S7-S12, the motif frequencies are distributed quite differently in the vicinity of the deletion breakpoints; thus, further studies are required to identify the underlying reasons responsible for the relationship between deletions and the frequencies of specific motifs.

Here we observed that non-B DNA-forming sequences such as DR, IR, and STR were less abundant at the breakpoints and in breakpoint flanking regions of deletions [?]30 bp than of deletions >30 bp (Figure 4). These repeats may form non-B DNA structures that cause replication stalling followed by replication fork repriming downstream, thereby leading to the deletions, a mechanism described as Fork Stalling and Template Switching (FoSTeS)(Lee et al., 2007). Replication errors mediated by these repeats may more frequently cause deletions >30 bp than deletions [?]30 bp in length. In particular, direct repeats were overrepresented immediately at the breakpoints of deletions >30 bp (Figure 4A), indicative of a specific role for these repeats in deletion formation. Direct repeats may form slipped structures if they are base-paired with the complementary strand in a misaligned fashion, causing hairpins or looped-out bases which may cause replication slippage(Zhao et al., 2010). By contrast, G- quadruplex (GQ) -forming repeats were not overrepresented at the breakpoints of deletions >30 bp (Figure 4B). However, the frequency of GQ-forming repeats was increased in regions flanking the breakpoints of deletions [?]30 bp. The highest frequency of these repeats was observed in regions ˜150 bp flanking the breakpoints on both sides (Figure 4B), suggesting the involvement of stem-loop formations and microhomology-mediated break-induced replication (MMBIR) in the deletion process.

In addition to MMBIR, microhomology-mediated end joining (MMEJ) plays an important role in double-strand repair and causes pathogenic deletion and translocation variants in the human genome(McVey & Lee, 2008; Verdin et al., 2013). MMEJ repairs DNA breaks via the use of substantial microhomology and creates precise deletions without insertions or other mutations at the breakpoint. We identified microhomologies

within the breakpoint flanking regions of 60% of the HGMD deletions indicating that MMEJ is an important mechanism underlying pathogenic deletions in humans. This is in accord with the findings of Grajcarek et al.(Grajcarek et al., 2019) who identified microhomologies at the breakpoints of 57% of the deletions included in ClinVar. Additionally, we found that more than 42% of the breakpoints flanking regions of short deletions (< 30bp) have microhomologies, somewhat higher than for those (29%) within long deletions. This is the first investigation in comparing the occurrence of microhomologies in short and long deletions.

It is well known that replication-based mechanisms are often involved in the formation of deletions and duplications of various sizes(Ankala et al., 2012; Geng et al., 2021; Seo et al., 2020; Vissers et al., 2009; Zhao et al., 2010). Our findings suggest that these mechanisms also contribute to the formation of pathogenic microdeletions <30 bp and gross deletions [?]30 bp. However, the different frequencies and distribution profiles of non-B DNA-forming sequence motifs at the breakpoints and within breakpoint-flanking regions of both groups of deletions suggest that the replication errors underlying the deletions are induced by different types of non-B DNA structure.

Overall, this study suggests 25-30 bp as a potential threshold that can be used to distinguish gross deletions and microdeletions in terms of their likely underlying mechanisms of mutagenesis. This notional threshold is based on the observation of the correlations between deletion length, non-B DNA-forming repeats frequencies, GC content, and sequence motif frequencies (Figure 7A). For deletion lengths greater than 30 bp, correlations start to weaken, and they tend to disappear at lengths greater than 50 bp. Although establishing a threshold to distinguish gross deletions from microdeletions is to some extent dependent on the intended research purpose, there is value in being able to draw distinctions based upon objective analyses. The approach and results reported here provide a path that should allow us to move away from arbitrary dividing lines and arrive at information-based knowledge concerning the rather different generative mechanisms underlying microdeletions and gross deletions.

Availability of data and materials

A pipeline is available at https://github.com/Qimengling/deletion_score_pipe for anyone having novel deletions. This pipeline enables users to calculate the frequency of non-B-forming DNA repeats, GC content, and specific motif frequency, and to obtain a deletion score according to the percentile ranking in the HGMD-deletion database.

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Conflict of Interest

No conflict of interests.

HGVS Nomenclature Compliance

no variants reported.

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Tables

Table 1. The density of non-B DNA-forming motifs in 1-kb sequences centered at breakpoints as presented by average numbers of repeats per kb.

Repeat type	Deletion (n/kb)	Microdeletion (n/kb)	Gross deletion (Deletions >20 bp) (n/kb)	Contr
ALL	83.22(0˜1095)	81.871(0˜1095)	98.713(0˜735)	89.176(
DR	12.441(0˜881.5)	12.086(0˜881.5)	16.518(0˜621)	13.797(
IR	43.32(0˜394.5)	43.045(0˜394.5)	46.48(0˜313.5)	45.137(
MR	2.352(0˜219)	2.252(0˜219)	3.504(0˜83)	2.935(0
GQ	11.118(0˜511)	11.075(0˜511)	11.618(0˜328)	9.355(0
STR	11.992(0˜257)	11.46(0˜257)	18.108(0˜238)	15.477(
Z	1.996(0˜206)	1.954(0˜206)	2.485(0˜92.5)	2.476(0

Table 2. Sequence motifs present more frequently (eH-value <0.05) in 10 bp bins centered at the breakpoints of the pathogenic deletion dataset (HGMD-deletion) than in the breakpoints from the simulated dataset.

Motif sequence	Motif description	Average eH-valu
GCCCWSSW	Translin target sites	0
GCTGGTGG	χ element	0
GGAGGTGGGCAGGARG	Human hypervariable minisatellite core sequence	0
AGAGGTGGGCAGGTGG	Human hypervariable minisatellite recombination sequence	0
GAAAATGAAGCTATTTACCCAGGA	Mariner transposon-like element (30end)	0
GCS	DNA polymerase α pause site core sequence	0
WGGAG	DNA polymerase arrest site	0
CTGGCG	DNA polymerase α frameshift hotspots	0
RGAC	Murine MHC deletion hotspot	0
RAG	Vertebrate/plant topoisomerase I consensus cleavage site	0
CCG	Fragile X breakpoint cluster repeat	0
GTAAGT	Indel hotspot	0
CGGCGG	Human Fra(X) breakpoint cluster	0
TTCTTC	Hamster and human APRT deletion hotspot	0
GCCCCG	“Super-hotspot” motifs	0
GGAGAA	“Super-hotspot” motifs	0
RNYNNCNNGYNGKTNYNY	Vertebrate topoisomerase II consensus cleavage site	5.00E-04
GCWGGWGG	Human minisatellite conserved sequence/χ-like element	5.00E-04
CTY	Vertebrate/plant topoisomerase I consensus cleavage sites	0.001
CCACCA	“Super-hotspot” motifs	0.001
CAGR	Murine MHC deletion hotspot	0.0015
TGRRKM	Deletion hotspot consensus sequence	0.0035
ACYYMK	Deletion hotspot consensus sequence	0.0035

Figures

Figure 1 . Repeat length distribution in all 1-kb bins centered at the breakpoints of the HGMD-deletion data. "DR", "GQ", "IR", "MR", "STR", and "Z" denote direct repeats, G-quadruplex-forming, inverted repeats, mirror repeats, short tandem repeats, and Z-DNA, respectively.

Figure 2. Frequency of non-B DNA forming repeats occurring near the breakpoints of the HGMD-deletion dataset. X-axis represents the position relative to the breakpoint and Y axis is the repeat frequency. A-F is the frequency for direct repeats (DR), inverted repeats (IR), mirror repeats (MR), G-quadruplex-forming (GQ), short tandem repeats (STR), and Z DNA sequence, respectively. This frequency refers to the proportion of sequences with repeats at each location.

Figure 3. Relationship between deletion length and average non-B DNA-forming repeat frequency. A. The relationship between deletion length and average repeat frequency within a 1-kb bin of breakpoints. B. Correlation were observed between deletion length and the average repeat frequency for each 10-bp bins of deletion lengths. C. Significant correlations were observed between deletion length and repeat frequency in 1-kb sequence centered at breakpoints by different cut-offs for deletions with length [?]9 bp, [?]27 bp, and [?]30 bp, respectively.

Figure 4. Repeats frequency occurring near the breakpoints of deletions of different length. A-D are the average frequencies of direct repeats (DR), G-quadruplex-forming (QG), short tandem repeats (STR), and inverted repeats (IR), respectively.

Figure 5. GC content in the vicinity of breakpoints of deletions and the relationship between deletion length and GC content. A. GC content in the vicinity of all the pathogenic deletion breakpoints and the simulated data. B. Relationship between deletion length and GC content. When deletion length was less than 38 bp, it was significantly correlated with GC content (PCC = 0.71 and P-value = 7.3E-7).

Figure 6 . Sequence motifs around the breakpoints of deletions. A. eH-values for the difference between frequencies of motif occurrence in 10-bp bins centered at breakpoints of the deletion data and the simulated data; we found that 16 motifs occurred more frequently (eH-value < 0.01) in 10 bp bins centered at the breakpoints of the pathogenic deletion breakpoints than in 10 bp bins centred at the breakpoints of the control dataset including simulated breakpoints. B. Relationship between deletion length and average motif frequency; Each point represents the average motif frequency occurring in the vicinity of deletions with a certain length.

Figure 7. The Pearson Correlation Coefficient (PCC) and PR scores for motif frequency, GC content, or repeat frequency against deletion length. A. Distribution of PCC against deletion length. The PCC values represent the correlations between deletion length and motif frequency, GC content, or repeat frequency. B. Relationship between deletion length and PR score.

Appendices

Supplementary file 1. Supplementary Figures. Figure S1-S15 and Table S4.

Supplementary file 2. Supplementary Tables. Table S1-S3 and Table S5-S6.

Supplementary file 3. Supplementary Table. Table S7.