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Analysis of eighteen deletion breakpoints in the parkin gene

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ABSTRACT

Parkin mutations are responsible for the pathogenesis of autosomal-recessive juvenile parkinsonism (AR-JP). On initial screening of Japanese patients with AR-JP, we had found that approximately half of the parkin mutations are deletions occurring between exons 2 and 5, forming a deletion hot spot. In this study, we investigated the deletion breakpoints of the parkin mutations in 22 families with AR-JP and examined the possible association between these deletion events and meiotic recombinations. We identified 18 deletion breakpoints at the DNA nucleotide sequence level. Almost all these deletions were different, indicating that the deletion hot spot was generated by recurrent but independent events. We found no association between the deletions and specific DNA elements. Recent copy number variation (CNV) data from various ethnic groups showed that the deletion hot spot is overlapped by a highly polymorphic CNV region, indicating that the recurrent deletion mutation or CNV is observable worldwide. By comparing Marshfield and deCODE linkage maps, we found that the parkin deletion hot spot may be associated with a meiotic recombination hot spot, although such association was not found on comparison with recent high-resolution genetic maps generated from the International HapMap project. Here, we discuss the possible mechanisms for deletion hot spot formation and its effects on human genomes.

Introduction

Parkin has been identified as the causative gene of autosomal-recessive juvenile parkinsonism (AR-JP) [1]. It is a gigantic gene occupying a 1.4-Mb genomic DNA sequence and consists of 12 exons with a 1.4-kb coding sequence [2]. The gene encodes a type of ubiquitin ligase (E3), which is associated with ubiquitin-conjugating enzymes (E2s) UbcH7 or UbcH8 [3]. Some proteins such as the *O*-glycosylated form of α-synuclein [4] and the Pael receptor [5] serve as substrates for parkin and accumulate in parkin-deficient patients.

Parkin mutations frequently cause early onset Parkinson's disease, especially in the case of a family history and an autosomal-recessive mode of transmission. The frequency of such mutations has been estimated as 40–50% in familial cases and 10–20% in idiopathic cases of early onset Parkinson's disease [6]. In our initial screening of the parkin mutations in Japanese patients with AR-JP, we found frequent occurrences of exon 3, exon 4, or both exon 3 and exon 4 deletions [7]. To date, 95 different mutations have been reported, and the exonic deletions have been commonly observed worldwide [8]. To precisely analyze the deleted regions, we had previously determined the 1.4-Mb genomic DNA sequence of the parkin gene in collaboration with the Sanger Centre [2]. Thus far, the DNA sequences of deletion breakpoints have been reported for only two cases of exon 4 deletions [9]. In this study, we investigated the deletion breakpoints of parkin mutations in 22 families with AR-JP and examined the possible association between these deletion events and meiotic recombinations.

Materials and methods

AR-JP families. This study included 16 Japanese families, one Korean family, one Taiwanese family, one Israeli family, and three Turkish families, totally comprising 27 individuals with AR-JP. Molecular analysis was performed for the 22 unrelated families, and all the patients showed some exonic deletions of the parkin gene. The findings on five of these families have been reported previously [7]. DNA samples were obtained from the patients following informed consent, and the study was approved by the ethical committee of the Juntendo University School of Medicine, Japan.

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Analysis of the parkin genomic sequence. The accession numbers of the sequence data used in this study are AP001576, AP000888, AP000887, AL008631, AP001578, AP001577, AP002091, AP000886, AP003699, AL078583, AL035697, AL138716, AL445215, and AL132982, directed from the 5′-end to the 3′-end of the parkin gene. Because we had employed the sequence data of BAC clones determined by us (AP00XXXX), some clones were different from those incorporated into the reference sequence (Build36). Therefore, the distances from the start codon of the parkin gene to each exon were slightly different between our data and the reference sequence; however, these differences ranged from −121 bp to 77 bp, which is negligible when compared with the gene size (1.4 Mb). Therefore, we considered that they had no effect on the conclusions of our study.

Detections of tandem repeats and inverted repeats were performed using equicktandem (http://bioweb.pasteur.fr/seqanal/interfaces/equicktandem.html) and palindrome (http://bioweb.pasteur.fr/seqanal/interfaces/palindrome.html), respectively. Matrix attachment regions were predicted using MAR-wiz (http://www.futuresoft.org/MAR-Wiz/). We also used DNASIS (Hitachi Software, Japan) to search for specific DNA elements.

Identification of the deletion breakpoints. The primer pairs to examine each exon of the parkin gene were designed as described previously [1], and we designed new PCR primer pairs for some intronic sites of the gene to examine whether the sites are deleted. In total, 191 sites were examined for the deletion mapping. After narrowing down the deletion breakpoints to a few kilobases, we attempted to amplify the DNA fragment covering the junctions of the breakpoints by a long PCR and used the PCR products as templates for direct sequencing.

Results and discussions

Features of the deletion breakpoints

We designed primer sets of 191 sequence tagged sites (STSs) along with the 1.4-Mb genomic sequence of the parkin gene and examined for the presence or absence of each STS in the DNA samples from 27 patients with AR-JP. PCR analysis revealed that all these patients had homozygous deletion of at least one exon. Therefore, they represented 44 deletions (88 deletion breakpoints). At least half of these deletion breakpoints (44 deletion breakpoints) were located (Fig. 1). We found six patients (K0013–K0018) of two different ethnic groups (one Korean and five Japanese) with an identical deletion, suggesting that this deletion originated from a common founder. The remaining patients had completely different sequences at the deletion breakpoints, indicating that these mutations are highly heterogeneous even in the same ethnic group (Japanese or Turkish). However, the breakpoints were restricted to the region between exons 2 and 5, forming a deletion hot spot.

We divided the 1.4-Mb gene and its flanking sequence into 14 regions of 100 kb each, counted the common deletion among the six families as one deletion, and then examined the distribution of the breakpoints by chi-square test. The resultant P value was 1.13×10^{-16}, indicating that the distribution of the deletion breakpoint in the 5′-half portion of the parkin gene could not have been generated by chance. Several different mutations such as exonic deletion or duplication, point mutation, and small in/del mutation were found throughout the entire gene, indicating that a mutation in the 3′-half portion of the parkin gene is equally pathogenic to patients with AR-JP. This observation also suggests that the distribution of a large deletion may not be affected by some functional constraint of specific motifs or domains of parkin.

Sequence analysis of the deletion breakpoints

We performed PCR amplifications across the deletion breakpoints and obtained PCR products of proper size for 13 cases of deletions (18 patients). On sequencing the PCR products, we found very short homologous sequences (1–4 bp) in eight cases and small insertions (3–26 bp) in four cases at the breakpoints (Fig. 2, Supplementary Fig. S1). No long homologous sequence was found at the deletion breakpoints, indicating that a homologous recombination mechanism was not involved in the generation of these deletions. This situation is very similar to the deletion breakpoints observed in the dystrophin gene [10–12].

We then investigated possible associations of the breakpoints and their flanking sequences with several known DNA sequences or elements such as interspersed repetitive sequences, palindrome structures, matrix attachment regions, deletion consensus motifs, topoisomerase II recognition sites, purine-rich (PUR) elements, and translation recognition sites, earlier discussed for the dystrophin and other deletion mutations [10–12]. We also examined the distribution of recombination hot spot-associated motifs [13] but found no specific correlations between the breakpoints and these sequences or elements although one breakpoint was located within a tandem repeat sequence.

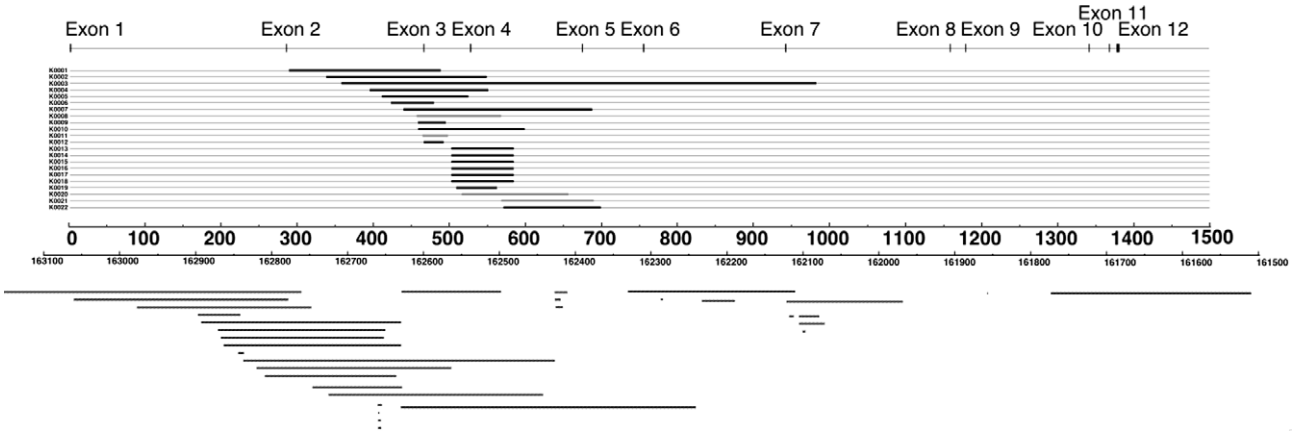

Fig. 1. Deletion mutations and CNVs in the 1.4-Mb parkin gene. The major coordinate (0–1500 kb) starts from the proximal end of BAC clone KB-1F5 (AP001576), which is 1333 bp upstream of the start codon. The minor coordinate (161500–163100 kb) was adapted from the human reference sequence in Build36. The positions of the 12 exons are shown. K0001–K0022 are the AR-JP family identities. The black horizontal bars indicate the deletions for which PCR products were obtained, whereas the gray horizontal bars indicate that no PCR products were amplified. The hatched bars at the bottom of the figure indicate the reported CNV regions.