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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.

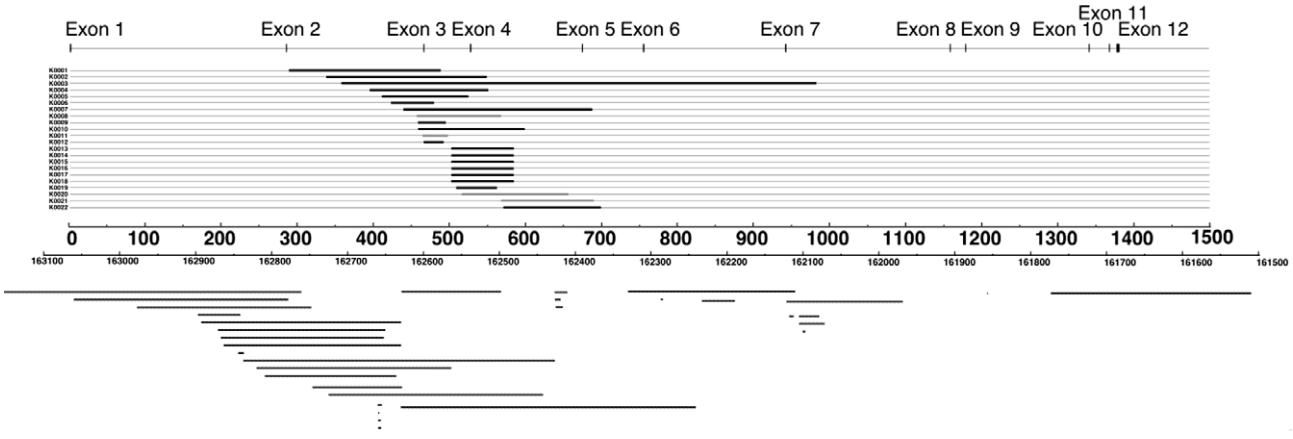

Fig. 1. Deletion mutations and CNVs in the 1.4-Mb parkin gene. The major coordinate (0–1500 kb) starts from the proximal end of BAC clone KB-1F5 (AP001576), which is 1333 bp upstream of the start codon. The minor coordinate (161500–163100 kb) was adapted from the human reference sequence in Build36. 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.

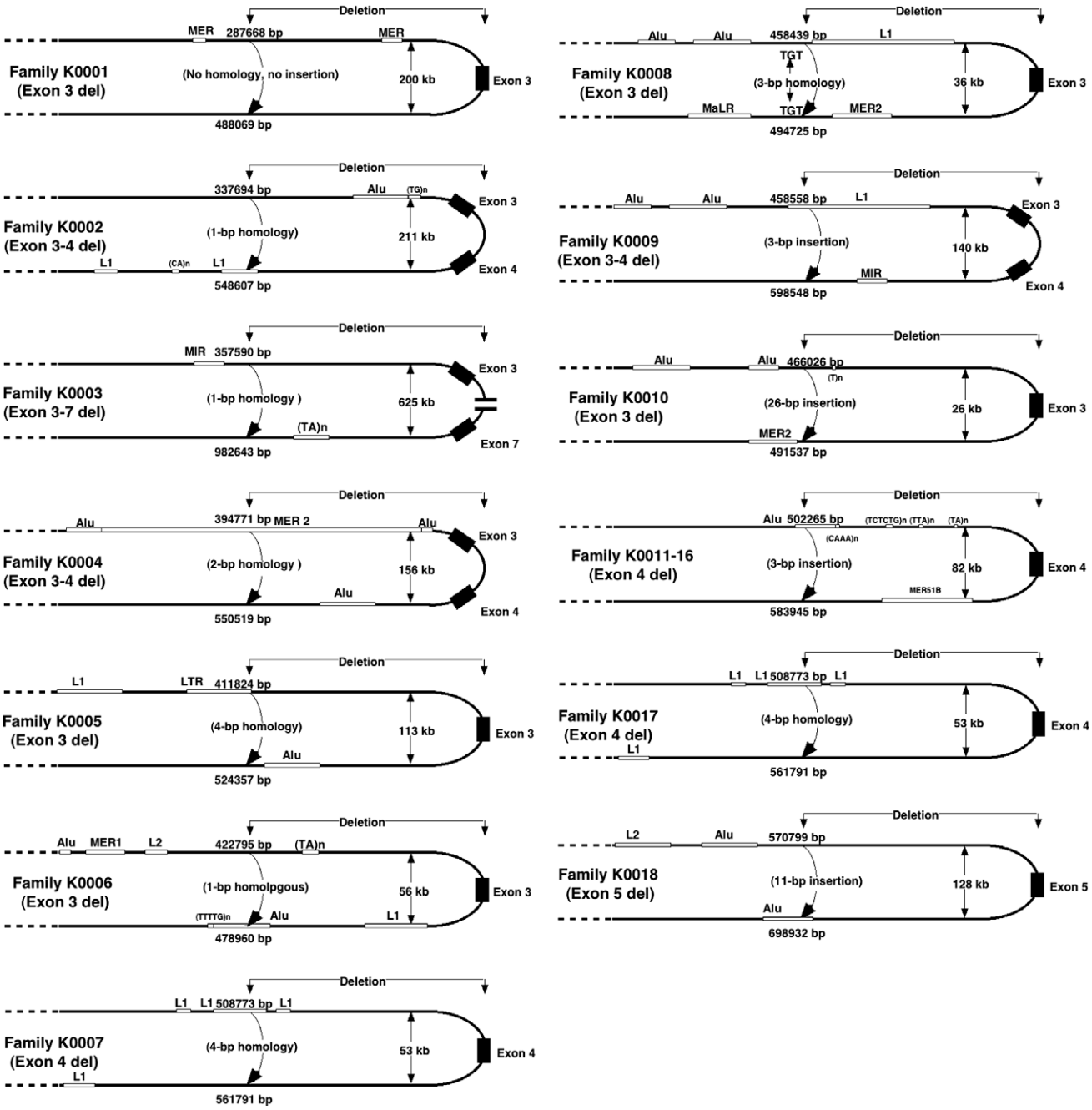

Fig. 2. Structures of 13 deletion breakpoints in the parkin gene. Position of the breakpoints (the starting point is the same as in Fig. 1 legend), size of the deletions, short homologous sequences or insertions at the breakpoints, and flanking repetitive elements are shown.

Possible association between deletion hotspot and meiotic recombinations

The very short homologous sequences (1–4 bp) at the joining points of the deletions implied that these junctions were generated by nonhomologous end joining (NHEJ) [14]. NHEJ is one of the pathways for repairing double-strand breaks (DSBs) of chromosomal DNA. As DSBs occur systematically during meiotic recombination, we examined the possible association between the frequency of the deletion breakpoints and that of the DSBs in meiosis (Fig. 3). The frequency of the meiotic DSBs should be strongly associated with the crossover frequency, which can be estimated in terms of the linkage distance; therefore, we examined the linkage maps for the parkin gene. We found a rather high recombination rate within the deletion hot spot regions (Fig. 3A, D, G, H). A region between D6S1599 and D6S980 just corresponded to the deletion hot spot. The physical distance between D6S1599 and D6S980 was 183 kb, whereas the linkage distance between these two markers was 2.17 cM on the Marshfield map [15], showing a very high recombination rate, 11.9 cM/Mb, between the two markers (Fig. 3G).

Because the Marshfield map was generated from the data on only 188 meioses, we further investigated the recombination rate within the parkin gene by using another linkage map, deCODE [16], with higher resolution. As it was generated from the data of 1257 meioses, the map should have been more suitable for this type of high-resolution analysis. In fact, on using the deCODE map, the recombination rate of the deletion hot spot (D6S1599–

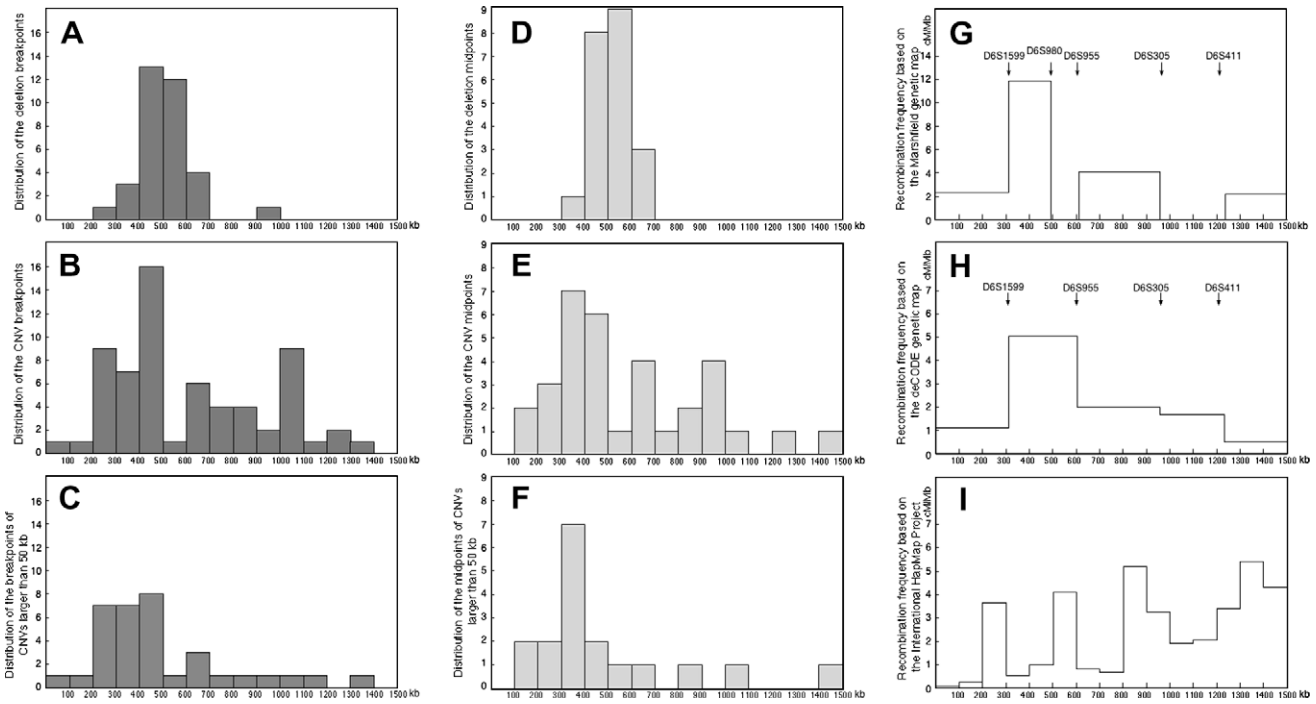

Fig. 3. Comparison of the deletion mutations, CNVs, and recombination frequency in the parkin gene. We divided the gene into fourteen 100-kb segments and counted the number of breakpoints for both the proximal and distal ends, and the midpoints of the deletions and CNVs. (A) Distribution of the deletion breakpoints. (B) Distribution of the CNV breakpoints. (C) Distribution of the breakpoints of the CNVs larger than 50 kb. (D) Distribution of the deletion midpoints. (E) Distribution of the CNV midpoints. (F) Distribution of the midpoints of CNVs larger than 50 kb. (G) Recombination frequency based on the Marshfield genetic map; the STS markers used are also shown. (H) Recombination frequency based on the deCODE genetic map; the STS markers used are also shown. (I) Recombination frequency based on the International HapMap Project. Because the HapMap linkage data were presented with very high-resolution, we divided the parkin gene and calculated the recombination frequency along every 100 kb.

D6S955, 296-kb length) was high (5.0 cM/Mb; Fig. 3H). In contrast, the average recombination rate per megabase along chromosome 6 is 0.99 cM/Mb in the deCODE map. We also examined the recombination rate of the parkin gene generated by statistical analysis with the International HapMap Project (http://www.hapmap.org/index.html.en) [13,17] (Fig. 3I); however, the recombination rate of the region covering the deletion hot spot showed no specific values when compared with other regions of the gene. The Marshfield and deCODE maps were generated by direct observations of genetic recombination, whereas the HapMap linkage map was based mainly on indirect statistical analysis, possibly causing the discrepancy between the linkage maps. Indeed, such discrepancy has been reported for MHC and other loci [18].

CNVs in the parkin gene

We examined recent copy number variation (CNV) data (http://projects.tcag.ca/variation/) [19–26] of the parkin genomic sequence (Fig. 1) and counted the ends of each reported CNV segment. The sizes of the reported CNV segments ranged from 1 to 410 kb and were highly heterogeneous, as seen in the case of the parkin deletion mutations. We wondered whether the smaller CNV segments are likely to be inconsistent between array platforms and actual experiments; therefore, we used the data of CNV segments larger than 50 kb. Surprisingly, the distribution of the CNV breakpoints coincided with the deletion breakpoints of the parkin gene, although their midpoints were slightly off (Fig. 3A–F). This close association indicated that the parkin deletion mutations are rare polymorphisms (up to nearly 1% in frequency) and that CNV segments are commonly seen among various ethnic groups.

Possible hypothesis explaining the formation of deletion hot spot

In general, DSBs are processed by several mechanisms such as double-stranded break repair (DSBR), synthesis-dependent strand annealing (SDSA), and NHEJ [14]. A high recombination rate between two loci indicates frequent occurrences of DSBs during meiosis. Meiotic DSBs are essentially processed by DSBR or SDSA, but some irregular events may occur and their frequency may correlate to the frequency of DSBs in meiosis. Such an irregular meiotic recombination process would be forced by NHEJ. This hypothesis is supported by our observations that the deletion hot spot in the parkin gene was associated with a high recombination rate and that the junctions of the deletion breakpoints showed the typical feature of NHEJ. This is also supported by an observation in the dystrophin gene that a region in intron 47 (i.e., a major deletion hot spot in the *DMD* gene) generates DSBs during meiosis in yeast and harbors a cluster of previously sequenced deletion breaks [27].

Here, we postulate a molecular mechanism to account for the formation of the deletion hot spot. In meiotic recombination, a pair of homologous chromosomes line up parallel to each other, following which DSBR or SDSA occurs. Both DSBR and SDSA require the existence of a pair of homologous chromosomes. We imagined how the recombination events would occur if one of the homologous chromosomes had a large deletion, as seen in the parkin gene. When a DSB occurs in a normal chromosome, the double strand is sucked, and the resulting single-stranded tail searches for a homologous sequence of the other homologous chromosome to invade the duplex and form heteroduplex DNA during DSBR or SDSA. However, if the homologous sequence is deleted in the counterpart, the tail cannot meet it, and so the chromosome with the double-strand break is forced to repair by NHEJ (Fig. 4A). An interesting result of such deletion has been shown in the mating type switching in yeast, which is a good model to study DSBR

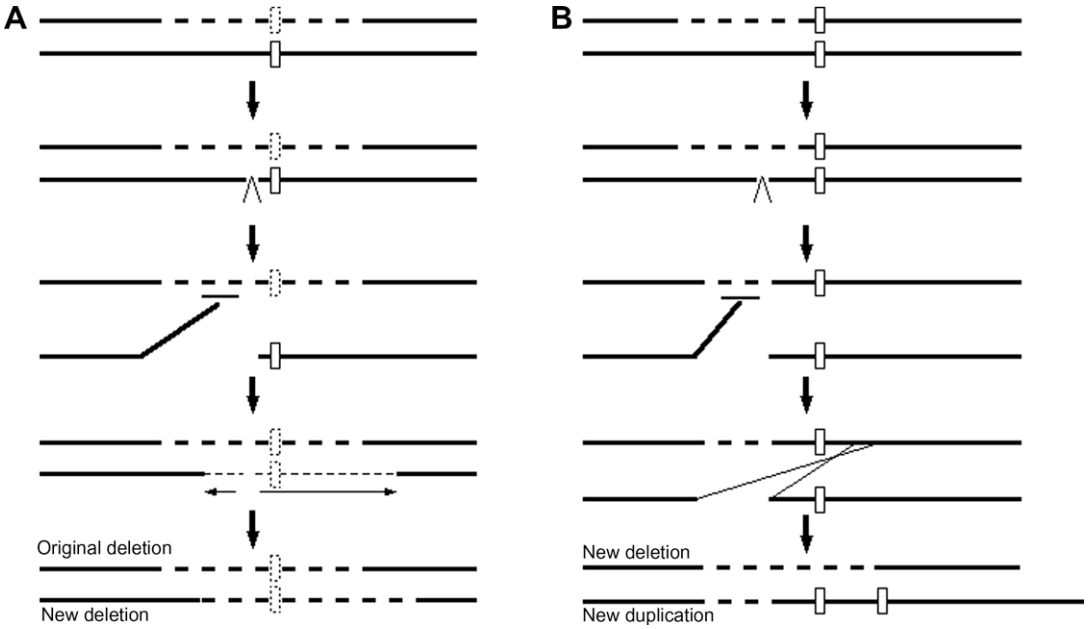

Fig. 4. Hypothetical mechanisms by which a new deletion or duplication is generated by pairing with a deleted homologous chromosome during meiosis. The horizontal bold lines indicate a pair of homologous chromosomes, boxes indicate exons, and dashed lines indicate deletions. (A) Meiotic pairing between a normal chromosome and its counterpart with large deletion (top row). The dashed box indicates a deleted exon. A DSB occurs in the normal chromosome at a position where the corresponding region of the counterpart is deleted (second row). Sucked single-strand tails of the double-strand broken chromosome cannot meet the homologous counterpart because of the deletion (third row). The sucked single-strand tails are forced to delete (thin arrows; fourth row) and ligate each other by NHEJ. Consequently, a new deletion is generated (bottom row). (B) The same process as in (A) except that the deleted region does not contain an exon (top, second, and third rows). The sucked single-strand tails invade into a nonhomologous region and generate crossover illegitimately (fourth row). Consequently, a chromosome containing a new deletion and another chromosome containing duplicated exons are generated (bottom row).

and NHEJ. During mating type switching in yeast, lack of a homologous sequence in the recombination donor causes deletions of up to several kilobases and NHEJ [28] occurs. Interestingly, by FISH and haplotype analysis, we previously found heterogeneous deletions in a consanguineous patient. We believe that this case would validate our hypothesis; however, we could not examine the progenitors of this patient. Occasionally, DSBR may also repair a DSB lacking a homologous donor sequence in its counterpart. In such a case, the sucked tail would invade the duplex illegitimately and act as a primer (Fig. 4B). This process can generate exonic duplication, which has indeed been observed in patients with AR-JP [8,21]. Such genomic alterations are essentially caused by nonpairing of homologous sequences; therefore, large segmentally polymorphic regions are potential sites for such genomic alterations. In fact, many of the low copy repeat regions on 22q11.2 and 8p23 have commonly shown extensive polymorphism with various sizes of subrepeated units [29,30], indicating that once a segmental deletion is generated, the locus tends to accumulate additional mutations and consequently becomes more polymorphic by the mechanisms we have proposed. Indeed, the segmental duplication polymorphisms flanking the deletions in Williams–Beuren syndrome are considered to be a susceptibility factor of *de novo* deletion [31]. The frequency of each CNV is different among ethnic groups; therefore, the frequency of generation of new segmental mutations may differ among them. Recently, enrichment of the CNVs of the parkin gene has been observed in autism spectrum disorders [32]. The differential distribution of CNVs among ethnic groups may be associated with the differential frequency of autism spectrum disorders and Parkinson's disease.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.08.115.

References

[1] T. Kitada, S. Asakawa, N. Hattori, H. Matsumine, Y. Yamamura, S. Minoshima, M. Yokochi, Y. Mizuno, N. Shimizu, Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism, Nature 392 (1998) 605–608.
[2] S. Asakawa, K. Tsunematsu, A. Takayanagi, T. Sasaki, A. Shimizu, A. Shintani, K. Kawasaki, A.J. Mungall, S. Beck, S. Minoshima, N. Shimizu, The genomic structure and promoter region of the human parkin gene, Biochemical and Biophysical Research Communications 286 (2001) 863–868.
[3] H. Shimura, N. Hattori, S. Kubo, Y. Mizuno, S. Asakawa, S. Minoshima, N. Shimizu, K. Iwai, T. Chiba, K. Tanaka, T. Suzuki, Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase, Nature Genetics 25 (2000) 302–305.
[4] H. Shimura, M.C. Schlossmacher, N. Hattori, M.P. Frosch, A. Trockenbacher, R. Schneider, Y. Mizuno, K.S. Kosik, D.J. Selkoe, Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease, Science 293 (2001) 263–269.
[5] Y. Imai, M. Soda, H. Inoue, N. Hattori, Y. Mizuno, R. Takahashi, An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of parkin, Cell 105 (2001) 891–902.
[6] S. Kubo, N. Hattori, Y. Mizuno, Recessive Parkinson's disease, Movement Disorders 21 (2006) 885–893.
[7] N. Hattori, T. Kitada, H. Matsumine, S. Asakawa, Y. Yamamura, H. Yoshino, T. Kobayashi, M. Yokochi, M. Wang, A. Yoritaka, T. Kondo, S. Kuzuhara, S. Nakamura, N. Shimizu, Y. Mizuno, Molecular genetic analysis of a novel parkin gene in Japanese families with autosomal recessive juvenile parkinsonism: evidence for variable homozygous deletions in the parkin gene in affected individuals, Annals of Neurology 44 (1998) 935–941.
[8] K. Hedrich, C. Eskelson, B. Wilmot, K. Marder, J. Harris, J. Garrels, H. Meija-Santana, P. Vieregge, H. Jacobs, S.B. Bressman, A.E. Lang, M. Kann, G.

Abbruzzese, P. Martinelli, E. Schwinger, L.J. Ozelius, P.P. Pramstaller, C. Klein, P. Kramer, Distribution, type, and origin of parkin mutations: review and case studies, Movement Disorders 19 (2004) 1146–1157.
[9] J. Clarimon, J. Johnson, O. Dogu, W. Horta, N. Khan, A.J. Lees, J. Hardy, A. Singleton, Defining the ends of parkin exon 4 deletions in two different families with Parkinson's disease, American Journal of Medical Genetics Part B-Neuropsychiatric Genetics 133B (2005) 120–123.
[10] J.C. McNaughton, D.J. Cockburn, G. Hughes, W.A. Jones, N.G. Laing, P.N. Ray, P.A. Stockwell, G.B. Petersen, Is gene deletion in eukaryotes sequence-dependent? A study of nine deletion junctions and nineteen other deletion breakpoints in intron 7 of the human dystrophin gene, Gene 222 (1998) 41–51.
[11] C. Nobile, L. Toffolatti, F. Rizzi, B. Simionati, V. Nigro, B. Cardazzo, T. Patarnello, G. Valle, G.A. Danieli, Analysis of 22 deletion breakpoints in dystrophin intron 49, Human Genetics 110 (2002) 418–421.
[12] L. Toffolatti, B. Cardazzo, C. Nobile, G.A. Danieli, F. Gualandi, F. Muntoni, S. Abbs, P. Zanetti, C. Angelini, A. Ferlini, M. Fanin, T. Patarnello, Investigating the mechanism of chromosomal deletion: characterization of 39 deletion breakpoints in introns 47 and 48 of the human dystrophin gene, Genomics 80 (2002) 523–530.
[13] K.A. Frazer, D.G. Ballinger, D.R. Cox, D.A. Hinds, L.L. Stuve, et al., A second generation human haplotype map of over 3.1 million SNPs, Nature 449 (2007) U851–U853.
[14] J.E. Haber, Partners and pathways—repairing a double-strand break, Trends in Genetics 16 (2000) 259–264.
[15] K.W. Broman, J.C. Murray, V.C. Sheffield, R.L. White, J.L. Weber, Comprehensive human genetic maps: individual and sex-specific variation in recombination, American Journal of Human Genetics 63 (1998) 861–869.
[16] A. Kong, D.F. Gudbjartsson, J. Sainz, G.M. Jonsdottir, S.A. Gudjonsson, B. Richardsson, S. Sigurdardottir, J. Barnard, B. Hallbeck, G. Masson, A. Shlien, S.T. Palsson, M.L. Frigge, T.E. Thorgeirsson, J.R. Gulcher, K. Stefansson, A high-resolution recombination map of the human genome, Nature Genetics 31 (2002) 241–247.
[17] S. Myers, L. Bottolo, C. Freeman, G. McVean, P. Donnelly, A fine-scale map of recombination rates and hotspots across the human genome, Science 310 (2005) 321–324.
[18] A.J. Jeffreys, R. Neumann, M. Panayi, S. Myers, P. Donnelly, Human recombination hot spots hidden in regions of strong marker association, Nature Genetics 37 (2005) 601–606.
[19] A.J. Iafrate, L. Feuk, M.N. Rivera, M.L. Listewnik, P.K. Donahoe, Y. Qi, S.W. Scherer, C. Lee, Detection of large-scale variation in the human genome, Nature Genetics 36 (2004) 949–951.
[20] R. Redon, S. Ishikawa, K.R. Fitch, L. Feuk, G.H. Perry, et al., Global variation in copy number in the human genome, Nature 444 (2006) 444–454.
[21] A.J. de Smith, A. Tsalenko, N. Sampas, A. Scheffer, N.A. Yamada, P. Tsang, A. Ben-Dor, Z. Yakhini, R.J. Ellis, L. Bruhn, S. Laderman, P. Froguel, A.I.F. Blakemore, Array CGH analysis of copy number variation identifies 1284 new genes variant in healthy white males: implications for association studies of complex diseases, Human Molecular Genetics 16 (2007) 2783–2794.
[22] J. Simon-Sanchez, S. Scholz, H.C. Fung, M. Matarin, D. Hernandez, J.R. Gibbs, A. Britton, F.W. de Vrieze, E. Peckham, K. Gwinn-Hardy, A. Crawley, J.C. Keen, J. Nash, D. Borgaonkar, J. Hardy, A. Singleton, Genome-wide SNP assay reveals structural genomic variation, extended homozygosity and cell-line induced alterations in normal individuals, Human Molecular Genetics 16 (2007) 1–14.
[23] K. Wang, M.Y. Li, D. Hadley, R. Liu, J. Glessner, S.F.A. Grant, H. Hakonarson, M. Bucan, PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data, Genome Research 17 (2007) 1665–1674.
[24] K.K. Wong, R.J. deLeeuw, N.S. Dosanjh, L.R. Kimm, Z. Cheng, D.E. Horsman, C. MacAulay, R.T. Ng, C.J. Brown, E.E. Eichler, W.L. Lam, A comprehensive analysis of common copy-number variations in the human genome, American Journal of Human Genetics 80 (2007) 91–104.
[25] M. Jakobsson, S.W. Scholz, P. Scheet, J.R. Gibbs, J.M. VanLiere, et al., Genotype, haplotype and copy-number variation in worldwide human populations, Nature 451 (2008) 998–1003.
[26] C. Lee, G.H. Perry, A. Ben-Dor, A. Tsalenko, N. Sampas, L. Rodriguez-Revenga, C.W. Tran, A. Scheffer, I. Steinfeld, P. Tsang, N.A. Yamada, H.S. Park, J.I. Kim, J.S. Seo, Z. Yakhini, S. Laderman, L. Bruhn, The fine-scale and complex architecture of 1086 human Copy Number Variant (CNV) regions as defined by custom high-density oligonucleotide microarrays, Cytogenetic and Genome Research 121 (2008) 34.
[27] M. Sironi, U. Pozzoli, G.P. Comi, S. Riva, A. Bordoni, N. Bresolin, D.K. Nag, A region in the dystrophin gene major hot spot harbors a cluster of deletion breakpoints and generates double-strand breaks in yeast, The FASEB Journal 20 (2006) 1910–1912.
[28] J.K. Moore, J.E. Haber, Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*, Molecular and Cellular Biology 16 (1996) 2164–2173.
[29] I. Dunham, N. Shimizu, B.A. Roe, S. Chissoe, A.R. Hunt, et al., The DNA sequence of human chromosome 22, Nature 402 (1999) 489–495.
[30] C. Nusbaum, T.S. Mikkelsen, M.C. Zody, S. Asakawa, S. Taudien, et al., DNA sequence and analysis of human chromosome 8, Nature 439 (2006) 331–335.
[31] I. Cuscó, R. Corominas, M. Bayés, R. Flores, N. Rivera-Brugués, V. Campuzano, L.A. Pérez-Jurado, Copy number variation at the 7q11.23 segmental duplications is a susceptibility factor for the Williams–Beuren syndrome deletion, Genome Research 18 (2008) B683–B694.
[32] J.T. Glessner, K. Wang, G.Q. Cai, O. Korvatska, C.E. Kim, et al., Autism genome-wide copy number variation reveals ubiquitin and neuronal genes, Nature 459 (2009) 569–573.