

ORIGINAL ARTICLE

Association between copy number variations in parkin (*PRKN*) and schizophrenia and autism spectrum disorder: A case-control study

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Abstract

Aim: The present study aimed to examine the association between copy number variations (CNVs) in parkin (*PRKN*) and schizophrenia (SCZ) and autism spectrum disorder (ASD) in a large case-control sample.

Method: Array comparative genomic hybridization was performed on 3111 cases with SCZ, 1236 cases with ASD, and 2713 controls. We systematically prioritized likely pathogenic CNVs (LP-CNVs) in *PRKN* and examined their association with SCZ and ASD.

Results: In total, 3014 SCZ cases (96.9%), 1205 ASD cases (97.5%), and 2671 controls (98.5%) passed quality control. We found that monoallelic carriers of LP-CNVs in *PRKN* were common (70/6890, 1.02%) and were not at higher risk of SCZ ($p=0.29$) or ASD ($p=0.72$). We observed that the distribution pattern of LP-CNVs in the Japanese population was consistent with those in other populations. We also identified a patient diagnosed with SCZ and early-onset Parkinson's disease carrying biallelic pathogenic CNVs in *PRKN*. The absence of Parkinson's symptoms in 10 other monoallelic carriers of the same pathogenic CNV further reflects the lack of effect of monoallelic pathogenic variants in *PRKN* in the absence of a second hit.

Conclusion: The present findings suggest that monoallelic CNVs in *PRKN* do not confer a significant risk for SCZ or ASD. However, further studies to investigate the association between biallelic CNVs in *PRKN* and SCZ and ASD are warranted.

KEYWORDS

autism spectrum disorder, DNA copy number variations, parkin, Parkinson disease 2, schizophrenia

1 | INTRODUCTION

The encoding gene of parkin, *PRKN* (OMIM *602544), formerly known as *PARK2*, was originally discovered in 1998 by Kitada et al.¹ and named after its role in the pathogenesis of autosomal recessive juvenile Parkinson disease-2 (*PARK2*, MIM 600116). Soon thereafter, further works established the genetic variants of *PRKN* as a common cause of early-onset Parkinson's disease (EOPD).^{2,3} Since the original discovery of exonic deletions in *PRKN*, copy number variations (CNVs) have been the main focus of genetic studies in *PRKN* because they likely lead to functional loss of Parkin. As studies have indicated a substantial contribution of CNVs to the etiology of neuropsychiatric disorders (NPDs),⁴⁻⁷ the identification of CNVs in *PRKN* among patients with schizophrenia (SCZ) and autism spectrum disorder (ASD) has brought the possible involvement of *PRKN* in NPDs to light.^{8,9} This is further supported by neurobiological evidence that knockdown of Parkin causes reduced surface levels of AMPA and NMDA receptors and alters glutamatergic synaptic transmission, which is a common feature of synaptopathy in SCZ and ASD.¹⁰⁻¹² Thereafter, several reports of patients with NPDs carrying CNVs in *PRKN* were published,¹³⁻¹⁵ and case-control association studies attempted to clarify the role of heterozygous CNVs in *PRKN* in NPDs, including

attention-deficit/hyperactivity disorder (ADHD)^{16,17} and ASD.^{18,19} While these early approaches implicated a possible association of CNVs in *PRKN* with NPDs, the genotyping methods used in these studies may have missed some of the CNVs, and the results were inconsistent or lacked sufficient power because of their limited sample sizes (ranging from about 340 to 880¹⁶⁻¹⁹). Accompanying the limited sample sizes was the lack of proper filtering. As CNVs are rare events, studies tend to adopt loose filtering to preserve more CNVs, resulting in the inclusion of not only likely pathogenic CNVs (e.g., CNVs at internal exons), but also less likely pathogenic CNVs²⁰ (e.g., intronic CNVs,^{8,16} duplications spanning terminal [first/last] exons) in the same study. These defects in study design may have led to inaccurate interpretations of the role of CNVs in *PRKN* in the pathogenesis of NPDs. Therefore, a case-control association study with an adequate sample size and appropriate prioritization using a sensitive and reliable detection method is needed to clarify the role of CNVs in *PRKN* in NPDs. To address this issue, we performed array comparative genomic hybridization (aCGH) on 4347 NPD cases (3111 SCZ and 1236 ASD) and 2713 healthy controls. We adopted a systematic prioritization for likely pathogenic CNVs (LP-CNVs) (Figure 1) and examined the association between LP-CNVs in *PRKN* and NPDs. Additionally, we analyzed the frequency and distribution of LP-CNVs in *PRKN* in

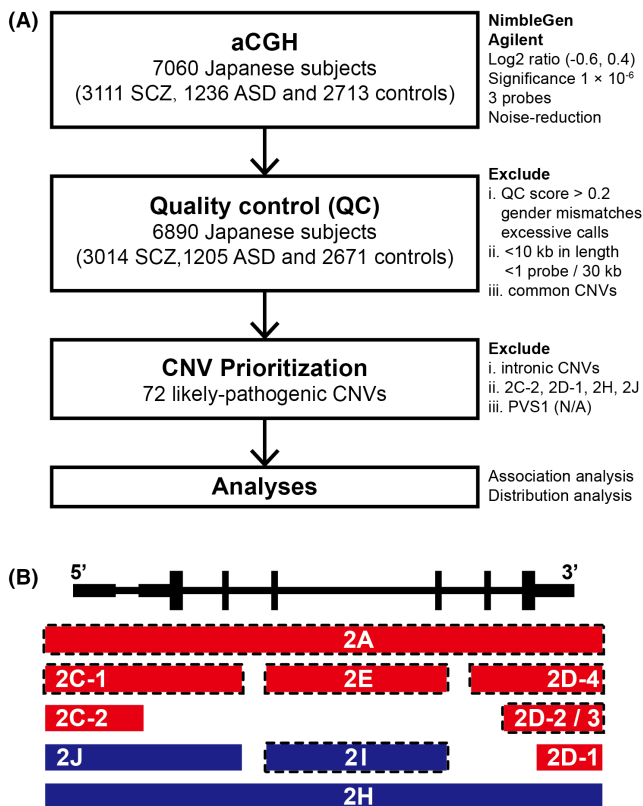


FIGURE 1 Workflow and classifications of CNVs in the present study. (A) Workflow of the present study. (B) Schematic illustration of classifications based on overlapping patterns following the guidelines for the interpretation of CNVs provided by ACMG and ClinGen. Red bars and dark blue bars represent deletions and duplications, respectively. Bars framed by the dotted line represent classifications prioritized as LP-CNVs. 2A: Deletions completely overlapping an established HI gene/genomic region; 2C-1: Deletions partially overlapping with the 5' end (3' end not involved) and the coding sequence is involved; 2C-2: Deletions partially overlapping with the 5' end (3' end not involved) and only the 5' UTR is involved; 2D-1: Deletions partially overlapping with the 3' end (5' end not involved) and only the 3' untranslated region is involved; 2D-2/3: Deletions partially overlapping with the 3' end (5' end not involved) and only the last exon is involved; 2D-4: Deletions partially overlapping with the 3' end (5' end not involved) and includes other exons in addition to the last exon. Nonsense-mediated decay is expected to occur; 2E: Deletions with both breakpoints within the same gene; 2H: Duplications fully containing an HI gene; 2I: Duplications with both breakpoints within the same gene; 2J: Duplications with one breakpoint within the established HI gene. ACMG, American College of Medical Genetics and Genomics; ClinGen, Clinical Genome Resource; HI, haploinsufficient; LP-CNV, likely pathogenic copy number variation.

the Japanese population and compared our findings with previous reports in other populations. We reported a biallelic carrier of pathogenic CNVs in *PRKN* diagnosed with SCZ and EOPD, along with other monoallelic carriers of the same pathogenic CNV to investigate the effect of monoallelic pathogenic variants in *PRKN* in the absence of a second hit.

2 | METHODS

2.1 | Participants

All participants recruited into the present study, including 3111 SCZ cases, 1236 ASD cases, and 2713 healthy controls, were of Japanese ancestry. SCZ and ASD were diagnosed based on the criteria set forth in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. The healthy controls were selected from the general population and confirmed to have no history of mental disorders based on self-reported information and questionnaires. This study was approved by the ethics committee of the Nagoya University and affiliated institutes. Written informed consent was obtained from all participants or accompanying family members before the study began.

2.2 | Array comparative genomic hybridization (aCGH)

Two aCGH platforms were utilized for CNV detection in this study: the NimbleGen 720K Whole-Genome Tiling array (Roche NimbleGen) and the Agilent SurePrint G3 human CGH 400K (Agilent). CNV calls were generated using the Fast Adaptive States Segmentation Technique 2 algorithm implemented in Nexus Copy Number software v9.0 (BioDiscovery). The log₂ ratio thresholds for CNV calls on both platforms were set as follows: (i) 10–500kb: –0.6 (deletion) and 0.4 (duplication); and (ii) >500kb: –0.4 (deletion) and 0.3 (duplication). A significance threshold of 1×10^{-6} was applied to adjust the sensitivity of the segmentation algorithm, and at least three contiguous probes were required for a CNV call in both the NimbleGen and Agilent arrays. To systematically correct for artifacts caused by GC content and fragment length, a noise-reduction algorithm for aCGH data was adopted.²¹ The accuracy of CNVs identified by aCGH was confirmed in our previous study using a quantitative real-time polymerase chain reaction (TaqMan copy number assays) (Applied Biosystems).²² For quality control (QC), a score was calculated for each sample based on the statistical variance of the probe-to-probe log ratios. Lower QC scores indicated higher quality results with less variation, whereas scores >0.2 were excluded from the analysis, as were samples with gender mismatches or excessive autosomal CNV calls. We then filtered out CNV calls that were <10kb in length or had low probe density (<1 probe/30kb). Finally, we removed common CNVs that appeared in $\geq 1\%$ of the total sample).

2.3 | Prioritization of likely pathogenic CNVs in *PRKN*

We started by considering all CNVs in the *PRKN* locus (chr6: 161691121-163068690, NCBI36) as potential candidates. To identify CNVs that were likely pathogenic, we performed a systematic

prioritization process (Figure 1A). First, we excluded all intronic CNVs because they were deemed less likely to be pathogenic. Next, to prioritize the pathogenic CNVs that cause functional loss of one copy of *PRKN*, we followed the guidelines for interpreting CNVs provided by the American College of Medical Genetics and Genomics and the Clinical Genome Resource (Figure 1B),²³ under the assumption of *PRKN* being a haploinsufficient gene, although this is still under debate. We excluded CNVs falling in categories 2C-2, 2D-1, 2H, and 2J, which have insufficient evidence to support their pathogenicity, and retained CNVs falling in categories 2A, 2C-1, 2D-2, 2D-3, 2D-4, 2E, and 2I. We further evaluated the CNVs in categories 2E and 2I based on PVS1 specifications.²⁴ 2E and 2I CNVs disrupting reading frames and predicted to undergo nonsense-mediated decay (NMD) were classified as PVS1. 2E CNVs preserving reading frames were classified as PVS1_Strong (if removing >10% of protein) or PVS1_Moderate (if removing <10% of protein). 2I CNVs preserving reading frames were classified as N/A. CNVs classified as PVS1, PVS1_Strong, or PVS1_Moderate were retained, whereas those classified as N/A were excluded. After this prioritization process, we retained the CNVs considered to have moderate to very strong evidence supporting their pathogenicity and labeled these as likely pathogenic CNVs (LP-CNVs; Table S1).

2.4 | Association analysis

We conducted association analysis on carriers of monoallelic (heterozygous) and biallelic (homozygous or compound heterozygous) CNVs separately (Figure 1A). To investigate the associations between LP-CNVs in *PRKN* and SCZ or ASD, we used Fisher's exact test (two-tailed) to calculate odds ratios (ORs) and *P*-values for the contingency table. In a secondary analysis, we investigated the association between LP-CNVs that spanned specific exon(s) and SCZ or ASD using Fisher's exact test (two-tailed).

3 | RESULTS

3.1 | Identification of *PRKN* CNVs by aCGH

Of the 7060 individuals (3111 SCZ cases, 1236 ASD cases, and 2713 controls) analyzed with aCGH, 3014 SCZ cases (96.9%), 1205 ASD cases (97.5%), and 2671 controls (98.5%) passed our QC. CNV analysis of 6890 subjects detected 176 CNVs in the *PRKN* region. After prioritization, intronic CNVs ($n=84$) and unlikely pathogenic exonic CNVs ($n=20$, Table S1) were excluded from further analyses. As a result, 71 participants were identified as carriers of LP-CNV in *PRKN* (36 SCZ cases, 12 ASD cases, and 23 controls), including one biallelic carrier among the SCZ cases (Figure 2A). Monoallelic carriers of LP-CNVs in *PRKN* were common in our sample set (70/6890, 1.02%). The frequency was slightly higher in SCZ (35/3014, 1.16%) and ASD (12/1205, 1.00%) than in controls (23/2671, 0.86%). Meanwhile, biallelic carriers of LP-CNVs in *PRKN* were rare (1/6890). The only

biallelic carrier was identified with two LP-CNVs in *PRKN* (CNV23 and CNV56, Table S1, Figure 2A) and diagnosed with SCZ and EOPD. CNV23 is a duplication in exon 2, while CNV56 is a deletion in exon 6 of *PRKN*, both resulting in a premature stop codon and potential degradation of transcripts by NMD (Figure 2C). Ten other individuals were found to carry similar duplications in exon 2 with uniform length as CNV23 (CNV18–20, CNV24–25, and CNV65–68), but none exhibited Parkinson's symptoms.

3.2 | Association analysis

With adequate identification of CNVs and a systematic prioritization, we revisited the question regarding whether CNVs in *PRKN* increase the risk of developing NPD, specifically SCZ or ASD. Our initial association analysis conducted on 6889 participants with zero or one LP-CNV revealed that monoallelic carriers of LP-CNVs in *PRKN* were not at a higher risk of developing SCZ (OR=1.35, 95% confidence interval [CI]=0.80–2.30, $p=0.29$) or ASD (OR=1.16, 95% CI=0.57–2.34, $p=0.72$) (Table 1). We also conducted a secondary analysis to examine whether monoallelic CNVs spanning specific exon(s) (e.g., exon 2, exons 5–12) of *PRKN* confer an increased risk for NPDs, but no statistically significant results ($p < 0.05$) were found (Table 1). Furthermore, we conducted an association analysis on biallelic carriers and non-carriers, but due to the limited discovery of only one biallelic carrier (a SCZ case) among all participants, no statistically significant result was found (Table 1).

3.3 | Distribution pattern of CNVs in *PRKN*

Among the LP-CNVs identified in 6890 Japanese individuals, we observed a clustering toward exons 1–4 encoding Ubl and RINGO domains of Parkin (Figure 2B). Overall, no obvious difference in the distribution of CNVs in *PRKN* was found between NPD cases and controls. CNVs involving exon 2, 3, or 4 accounted for the majority 62/72 (86%) of all LP-CNVs. Exon-wise, exon 2 was observed with most of the LP-CNVs, including 33 CNVs spanning exon 2 and 26 single-exon CNVs. Notably, 11 of the 13 single-exon duplications in exon 2 (CNVs 18–20, 23–26, and 65–68) were uniform in width (~300kb), accounting for 85% of the duplications in exon 2 of *PRKN*. This finding was consistent with a recent large-scale study in the European population showing that CNVs in exons 2–4 of *PRKN* are common and that duplications found in exon 2 were uniform in size.²⁵ Intriguingly, the duplications identified in our sample set of the Japanese population were larger (~300kb) than those identified in that study (~200kb). Given the high-resolution of CNV detection methods in both our study (NimbleGen or Agilent) and the previous study (NeuroX²⁶ or NeuroChip²⁷), the 100-kb difference in length suggested that the two duplications were likely distinct variants. However, whether the 300-kb duplication discovered in our study is population-specific requires further investigation.

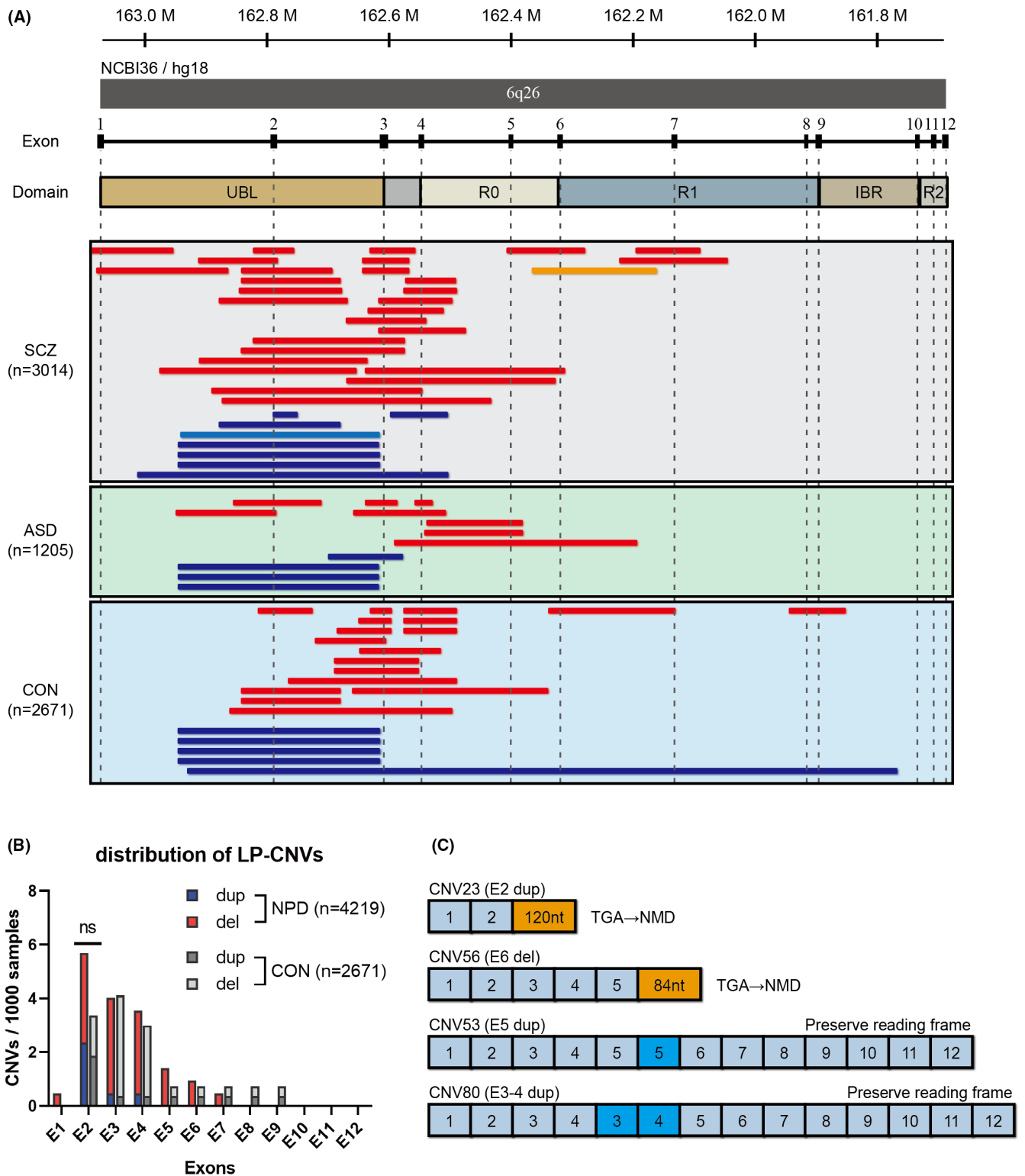


FIGURE 2 Genomic locations, distribution, and consequences of LP-CNVs in *PRKN*. (A) Genomic locations of LP-CNVs in *PRKN*. Red bars and dark blue bars represent deletions and duplications identified in the monoallelic carriers, respectively. The yellow bar and light blue bar represent the deletion (CNV56) and the duplication (CNV23) identified in the biallelic carrier. The genomic coordinates correspond to the NCBI36/hg18 build of the human genome assembly. (B) Distribution of LP-CNVs in the 12 exons of *PRKN* in NPD cases ($n=4219$) and controls ($n=2671$). (C) Predicted consequences of the mRNA structure due to CNVs in *PRKN*. Duplication or deletion disrupting reading frames and resulting in a premature stop codon that leads to NMD (e.g., CNV23 and CNV56). Duplication spanning one or more exons preserving reading frames and leading to mRNA with exon repetition (e.g., CNV53, and CNV80). ASD, autism spectrum disorder; CON, healthy controls; LP-CNV, likely pathogenic copy number variation; del, deletion; dup, duplication; NMD, nonsense-mediated mRNA decay; NPD, neuropsychiatric disorder; nt, nucleotide; SCZ, schizophrenia.



TABLE 1 Association analysis of LP-CNVs in PRKN with risk of SCZ or ASD.

LP-CNV type	Genotype counts (allelic/non)			ORs (95% CI)			p-value		
	SCZ	ASD	CON	SCZ	ASD	NPD	SCZ	ASD	NPD
Monoallelic									
DEL	28/2985	8/1197	18/2653	1.38 (0.76-2.51)	0.99 (0.43-2.27)	1.27 (0.72-2.24)	0.30	1.00	0.48
DUP	7/3006	4/1201	5/2666	1.24 (0.39-3.92)	1.78 (0.48-6.63)	1.39 (0.48-4.02)	0.78	0.47	0.62
DEL or DUP	35/2978	12/1193	23/2648	1.35 (0.80-2.30)	1.16 (0.57-2.34)	1.30 (0.79-2.14)	0.29	0.72	0.33
E2 DEL ¹⁶	12/3001	2/1203	4/2667	2.67 (0.86-8.28)	1.11 (0.20-6.06)	2.22 (0.73-6.75)	0.09	1.00	0.23
E2 DUP ¹⁶	6/3007	3/1202	5/2666	1.06 (0.32-3.49)	1.33 (0.32-5.58)	1.14 (0.38-3.41)	1.00	0.71	1.00
E2 DEL or DUP ¹⁶	18/2995	5/1200	9/2662	1.78 (0.80-3.96)	1.23 (0.41-3.69)	1.62 (0.75-3.51)	0.18	0.77	0.28
E5-12 DEL ¹⁹	6/3007	3/1202	3/2668	1.77 (0.44-7.10)	2.22 (0.45-11.01)	1.90 (0.51-7.03)	0.52	0.38	0.39
Region A (E3-4) ¹⁸	17/2996	5/1200	14/2657	1.08 (0.53-2.19)	0.79 (0.28-2.20)	1.00 (0.51-1.95)	0.86	0.81	1.00
Region B (E5) ¹⁸	3/3010	3/1202	2/2669	1.33 (0.22-7.97)	3.33 (0.56-19.96)	1.90 (0.38-9.43)	1.00	0.18	0.50
Region C (E6-7) ¹⁸	4/3009	1/1204	2/2669	1.77 (0.32-9.69)	1.11 (0.10-12.24)	1.58 (0.31-8.17)	0.69	1.00	0.71
Biallelic									
DEL or DUP	1/3012	0/1205	0/2671	∞	NaN	∞	1.00	1.00	1.00

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; CON, healthy controls; DEL, deletion; DUP, duplication; E(n): exon (n); LP-CNVs, likely pathogenic copy number variations; NPD, neuropsychiatric disorder; ORs, odds ratios; SCZ, schizophrenia.



4 | DISCUSSION

In the present study, we identified a greater number of LP-CNVs in *PRKN* in NPD cases compared with previous studies.^{16,18,19} However, after systematic prioritization and examination, we found that monoallelic carriers of LP-CNVs in *PRKN* were not at a higher risk of developing SCZ or ASD (Table 1), which contradicts previous findings that monoallelic CNVs may confer an increased risk of developing NPDs.^{16–19} Yin et al.¹⁸ reported a higher frequency of *PRKN* CNVs in ASD cases compared with controls (0.94% vs. 0.14%, respectively; $p=0.014$) by screening 1428 participants (335 ASD cases and 1093 controls) for CNVs in *PRKN* and genotyping on designated regions for replication in 301 ASD cases and 301 controls. Jarick et al.¹⁶ reported a similar result in ADHD cases (1.04% vs. 0.13%, respectively; $p=0.043$) in the replication samples of 386 ADHD cases and 781 controls. However, the frequency of CNVs in *PRKN* in their control samples was much lower than that in our study (0.86%) and other large-scale studies (0.55–0.95%^{25,28,29}). This omission of CNVs may have been caused by limitations in the detection methods employed, and may have influenced their results. Moreover, we were unable to replicate the excess of CNVs among NPD cases in the risk regions suggested by their studies (i.e., exon 2, exons 3–4, exon 5, and exons 6–7) (Table 1). Instead, our observations were more consistent with the results of Conceição et al.,¹⁹ who reported that CNVs in exons 1–4 of *PRKN*, which encode the Ubl and RING0 domains of Parkin, were frequently observed in both controls and NPD cases. We found that LP-CNVs in exons 2–4 were the most common, whereas LP-CNVs in exons 5–12 were infrequent. Notably, exons 5–12 of *PRKN* encode the RBR domain, which is the main functional domain of Parkin, and an excess of CNVs in this region has been reported in NPD cases.¹⁹ However, our association analysis did not yield any statistically significant evidence to suggest that monoallelic LP-CNVs in exons 5–12 of *PRKN* are associated with an increased risk of NPDs (Table 1). Overall, while the possibility of monoallelic CNVs in *PRKN* conferring a higher risk of SCZ or ASD remains, as low-penetrance from monoallelic CNVs in *PRKN* for Parkinson's disease (PD) has been suggested,³⁰ our data showed that any potential risk should be limited.

Only one carrier of biallelic pathogenic CNVs in *PRKN* was identified among the NPD cases in our study (none in the control group); however, due to limited number of cases, statistical significance was not achieved. In fact, the low prevalence of biallelic CNVs in *PRKN* (1/6890) makes achieving statistical significance challenging. To achieve a statistical power of 0.8, a minimum of 200000 participants is estimated to be required, even when combining other pathogenic variants such as disruptive single-nucleotide variations (SNVs). Future studies will require more complex study designs to investigate the role of biallelic pathogenic variants in *PRKN* in NPDs, rather than relying solely on large sample sizes in a one-stage genetic association study.

Nevertheless, the discovery of this biallelic carrier is valuable, as this carrier was also diagnosed with EOPD, a recessive form of PD commonly caused by pathogenic variants in *PRKN*.³¹ This diagnosis

supports the pathogenicity of the two CNVs (CNV23 and CNV56) carried by the individual, suggesting that they may lead to the functional loss of both copies of *PRKN* in a compound heterozygous manner. This finding is significant because 10 other monoallelic carriers of the same pathogenic CNVs in exon 2 did not exhibit PD symptoms, further highlighting the lack of effect of monoallelic pathogenic CNVs in *PRKN* in the absence of a second hit.

Here, we reported the frequency and distribution of LP-CNVs in *PRKN* in a Japanese population of 6890 participants by systematically screening for CNVs using high-resolution arrays. We found that monoallelic carriers of LP-CNVs in *PRKN* were relatively common (1.02%), whereas biallelic carriers were rare (1/6890). The distribution of CNVs in NPD cases and controls showed no obvious differences, and the clustering toward exons 2–4 was similar to that observed in other populations.²⁵ Notably, we identified 300-kb duplications in exon 2, which were larger than those previously reported in the European population,²⁵ but were also uniform in length and likely had identical consequences, as they both involved only exon 2. Whether this 300-kb duplication is population-specific requires further investigation.

To our knowledge, our case-control study is the first to systematically examine the association of CNVs in *PRKN* with SCZ since the original discovery,⁹ and the largest to examine the association of CNVs in *PRKN* with NPD.^{16,18,19} Therefore, we have provided a better perspective for interpreting CNVs in *PRKN* in patients with NPDs by highlighting the importance of reassessing results from previous genetic studies and reporting negative results, which was also suggested by recent large-scale studies on *PRKN*.^{25,28,32}

This study does have a few limitations worth mentioning. First, while aCGH is a sensitive and reliable method for detecting CNVs, it cannot identify SNVs. Therefore, it is possible that there may be biallelic carriers with another pathogenic SNV that were detected. However, because pathogenic SNVs in *PRKN* are rare,²⁵ this limitation is unlikely to affect the main conclusion of the present study. Second, CNV detection in this study was performed using two platforms, NimbleGen and Agilent. While some may question the validity of this approach, our previous research has demonstrated that the CNV detection resolution of these two platforms is comparable under the settings used in this study.³³

In conclusion, the findings of the present study indicate that monoallelic CNVs in *PRKN* do not confer a significant risk for SCZ or ASD. However, further studies are warranted to investigate the association between biallelic CNVs in *PRKN* and NPDs. We also found that CNVs in *PRKN* were relatively common among the Japanese population and that their distribution was no different from that of other populations.

AUTHOR CONTRIBUTIONS

T.L. and I.K. designed the study. I.K., B.A., and T.L. performed the aCGH and/or validation experiments. T.L. and I.K. analyzed the data. I.K., T.O., Hiroki K., Hidekazu K., T.I., Y.N., Y.T., M.Y., R.K., Y.F., Hirota K., S.N., K.K., Tsukasa S., S.Y., T.M., N.I., Masashi I., R.H., Masanari I., M.A., K.O., Toshiyuki S., Y.W., J.E., T.T., M.S., H.Y., and

N.O. recruited the participants and/or collected DNA samples or phenotype data. T.L. wrote the first draft of the manuscript, and the other authors commented on and refined the subsequent versions. All authors carefully read the manuscript and approved the final version for submission.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supporting information of this article. Due to privacy and ethical considerations, the raw CNV data containing personal information cannot be publicly shared. However, to promote transparency and facilitate reproducibility, a de-identified and aggregated table of identified exonic CNVs in *PRKN*, without any personally identifiable information, is included as a supplemental file.

ETHICS STATEMENT

This study was approved by the ethics committee of Nagoya University Graduate School of Medicine; Genomic study for brain and mental disorders and effectiveness and side effect of pharmacological treatment (2010–1033). This study complied with all the provisions of the Declaration of Helsinki.

Informed consent: Written informed consent was obtained from all the participants.

Registry and the registration No. of the study: N/A.

Animal studies: N/A.

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REFERENCES

- Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*. 1998;392:605–8.
- Puschmann A. Monogenic Parkinson's disease and parkinsonism: clinical phenotypes and frequencies of known mutations. *Parkinsonism Relat Disord*. 2013;19:407–15.
- Lücking CB, Dürr A, Bonifati V, Vaughan J, de Michele G, Gasser T, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. *N Engl J Med*. 2000;342:1560–7.
- Onitsuka T, Hirano Y, Nemoto K, Hashimoto N, Kushima I, Koshiyama D, et al. Trends in big data analyses by multicenter collaborative translational research in psychiatry. *Psychiatry Clin Neurosci*. 2022;76:1–14.
- Kushima I, Nakatochi M, Aleksic B, Okada T, Kimura H, Kato H, et al. Cross-disorder analysis of genic and regulatory copy number variations in bipolar disorder, schizophrenia, and autism Spectrum disorder. *Biol Psychiatry*. 2022;92:362–74.
- Kushima I, Aleksic B, Nakatochi M, Shimamura T, Okada T, Uno Y, et al. Comparative analyses of copy-number variation in autism Spectrum disorder and schizophrenia reveal etiological overlap and biological insights. *Cell Rep*. 2018;24:2838–56.
- Kato H, Kimura H, Kushima I, Takahashi N, Aleksic B, Ozaki N. The genetic architecture of schizophrenia: review of large-scale genetic studies. *J Hum Genet*. 2023;68:175–82.
- Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, et al. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. *Nature*. 2009;459:569–73.
- Xu B, Roos JL, Levy S, van Rensburg EJ, Gogos JA, Karayiorgou M. Strong association of de novo copy number mutations with sporadic schizophrenia. *Nat Genet*. 2008;40:880–5.
- Cortese GP, Zhu M, Williams D, Heath S, Waites CL. Parkin deficiency reduces hippocampal glutamatergic neurotransmission by impairing AMPA receptor endocytosis. *J Neurosci*. 2016;36:12243–58.
- Zhu M, Cortese GP, Waites CL. Parkinson's disease-linked parkin mutations impair glutamatergic signaling in hippocampal neurons. *BMC Biol*. 2018;16:100.
- Onitsuka T, Hirano Y, Nakazawa T, Ichihashi K, Miura K, Inada K, et al. Toward recovery in schizophrenia: current concepts, findings, and future research directions. *Psychiatry Clin Neurosci*. 2022;76:282–91.
- Scheuerle A, Wilson K. PARK2 copy number aberrations in two children presenting with autism spectrum disorder: further support of an association and possible evidence for a new microdeletion/



- microduplication syndrome. *Am J Med Genet B Neuropsychiatr Genet.* 2011;156B:413–20.
14. Mariani M, Crosti F, Redaelli S, Fossati C, Piras R, Biondi A, et al. Partial duplication of the PARK2 gene in a child with developmental delay and her normal mother: a second report. *Am J Med Genet B Neuropsychiatr Genet.* 2013;162B:485–6.
 15. Barone R, Cirnigliaro L, Saccuzzo L, Valdese S, Pettinato F, Prato A, et al. PARK2 microdeletion in a multiplex family with autism spectrum disorder. *Int J Dev Neurosci.* 2022;83:121–31.
 16. Jarick I, Volckmar AL, Pütter C, Pechlivanis S, Nguyen TT, Dauvermann MR, et al. Genome-wide analysis of rare copy number variations reveals PARK2 as a candidate gene for attention-deficit/hyperactivity disorder. *Mol Psychiatry.* 2014;19:115–21.
 17. Bradley WE, Raelson JV, Dubois DY, et al. Hotspots of large rare deletions in the human genome. *PLoS One.* 2010;5:e9401.
 18. Yin CL, Chen HI, Li LH, Chien YL, Liao HM, Chou MC, et al. Genome-wide analysis of copy number variations identifies PARK2 as a candidate gene for autism spectrum disorder. *Mol Autism.* 2016;7:23.
 19. Conceição IC, Rama MM, Oliveira B, Café C, Almeida J, Mouga S, et al. Definition of a putative pathological region in PARK2 associated with autism spectrum disorder through in silico analysis of its functional structure. *Psychiatr Genet.* 2017;27:54–61.
 20. Truty R, Paul J, Kennemer M, Lincoln SE, Olivares E, Nussbaum RL, et al. Prevalence and properties of intragenic copy-number variation in mendelian disease genes. *Genet Med.* 2019;21:114–23.
 21. Leprêtre F, Villenet C, Quief S, Nibourel O, Jacquemin C, Troussard X, et al. Waved aCGH: to smooth or not to smooth. *Nucleic Acids Res.* 2010;38:e94.
 22. Kushima I, Aleksic B, Nakatochi M, Shimamura T, Shiino T, Yoshimi A, et al. High-resolution copy number variation analysis of schizophrenia in Japan. *Mol Psychiatry.* 2017;22:430–40.
 23. Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the clinical genome resource (ClinGen). *Genet Med.* 2020;22:245–57.
 24. Abou Tayoun AN, Pesaran T, DiStefano MT, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Hum Mutat.* 2018;39:1517–24.
 25. Zhu W, Huang X, Yoon E, Bandres-Ciga S, Blauwendraat C, Billingsley KJ, et al. Heterozygous PRKN mutations are common but do not increase the risk of Parkinson's disease. *Brain.* 2022;145:2077–91.
 26. Nalls MA, Bras J, Hernandez DG, Keller MF, Majounie E, Renton AE, et al. NeuroX, a fast and efficient genotyping platform for investigation of neurodegenerative diseases. *Neurobiol Aging.* 2015;36(1605):e7–e12.
 27. Blauwendraat C, Faghri F, Pihlstrom L, Geiger JT, Elbag A, Lesage S, et al. NeuroChip, an updated version of the NeuroX genotyping platform to rapidly screen for variants associated with neurological diseases. *Neurobiol Aging.* 2017;57:247.e9–247.e13.
 28. Yu E, Rudakou U, Krohn L, Mufti K, Ruskey JA, Asayesh F, et al. Analysis of heterozygous PRKN variants and copy-number variations in Parkinson's disease. *Mov Disord.* 2021;36:178–87.
 29. Kay DM, Stevens CF, Hamza TH, Montimurro JS, Zabetian CP, Factor SA, et al. A comprehensive analysis of deletions, duplications, and copy number variations in PARK2. *Neurology.* 2010;75:1189–94.
 30. Huttenlocher J, Stefansson H, Steinberg S, Helgadóttir HT, Sveinbjörnsdóttir S, Riess O, et al. Heterozygote carriers for CNVs in PARK2 are at increased risk of Parkinson's disease. *Hum Mol Genet.* 2015;24:5637–43.
 31. Kalia LV, Lang AE. Parkinson's disease. *Lancet.* 2015;386:896–912.
 32. Lubbe SJ, Bustos BI, Hu J, Krainc D, Joseph T, Hehir J, et al. Assessing the relationship between monoallelic PRKN mutations and Parkinson's risk. *Hum Mol Genet.* 2021;30:78–86.
 33. Sekiguchi M, Sobue A, Kushima I, Wang C, Arioka Y, Kato H, et al. ARHGAP10, which encodes rho GTPase-activating protein 10, is a novel gene for schizophrenia risk. *Transl Psychiatry.* 2020;10:247.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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