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Replication-based rearrangements are a major mechanism for SNCA duplication in Parkinson disease

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Background

Approximately 10% of Parkinson's disease (PD) cases are linked to genetic causes. SNCA gene multiplication is a genomic cause of familial PD, showing dosage-dependent toxicity and increased mRNA expression. Until now, non-allelic homologous recombination (NAHR) was suggested as the mechanism of SNCA duplication based on various repetitive elements in the 4q22 region. However, only one case had breakpoint analysis at the base-pair level, showing homology between repetitive elements. This study aimed to elucidate the exact mechanism of SNCA duplication.

Methods

A total of 408 PD patients were screened for SNCA multiplication at Seoul National University Hospital. Six samples with confirmed SNCA duplication underwent whole genome sequencing (WGS) with mean depth coverage of 35.4X. Duplicated regions were defined with nucleotide-resolution breakpoints, confirmed by junction PCR and Sanger sequencing. Repetitive elements were searched using RepeatMasker, stem-loop structure predictions were conducted with Mfold, and haplotype analysis was performed using 12 SNPs encompassing the SNCA gene.

Results

Detection and Characterization

- SNCA duplication frequency: 0.30% (6 out of 2,031 patients)
- Duplication sizes ranged from 718.3 kb to 4,162 kb
- All duplications were in tandem and direct orientation

Key Junction Sequence Findings

Repetitive elements:
Eight of 12 breakpoint sequences mapped within repetitive elements (LINEs, SINE, LTR), but none of the pairs shared overt homologies between proximal and distal breakpoints - inconsistent with NAHR mechanism

Microhomology:
Five of six junctions had microhomologies at the breakpoint, ranging from 2-4 bp

Sequence insertions:
Three junctions had short stretch insertions (1-23 bp). Cases 1 and 4 showed homology to adjacent sequences; Case 5 contained repeated TGs

Stem-loop structures:
All junctions except one were located within or next to stem-loop structures - suggesting FoSTeS/MMBIR mechanism

Haplotype Analysis

Cases 3 and 6 shared the exact same breakpoint sequence but differed by two SNPs in haplotype analysis, suggesting unlikely common founder effect

Clinical Features

- Cognitive impairment in all cases except Case 2
- Visual hallucination in 4 patients (Cases 1, 4, 5, 6)
- Correlation between number of risk alleles and onset age
- No correlation between duplication size and disease severity

Conclusion

This study demonstrated that the major mechanism of SNCA duplication is FoSTeS/MMBIR rather than NAHR. Although repetitive elements were present, no homology was found between proximal and distal breakpoints; instead, microhomology and stem-loop structures were consistently observed.

This indicates that SNCA duplication occurs through a replication-based rearrangement mechanism involving template switching with minimal base pairing when replication forks stall. Stem-loop structures may induce DNA damage by preventing strand reannealing during replication, or serve as sites for restarting stalled replication forks.

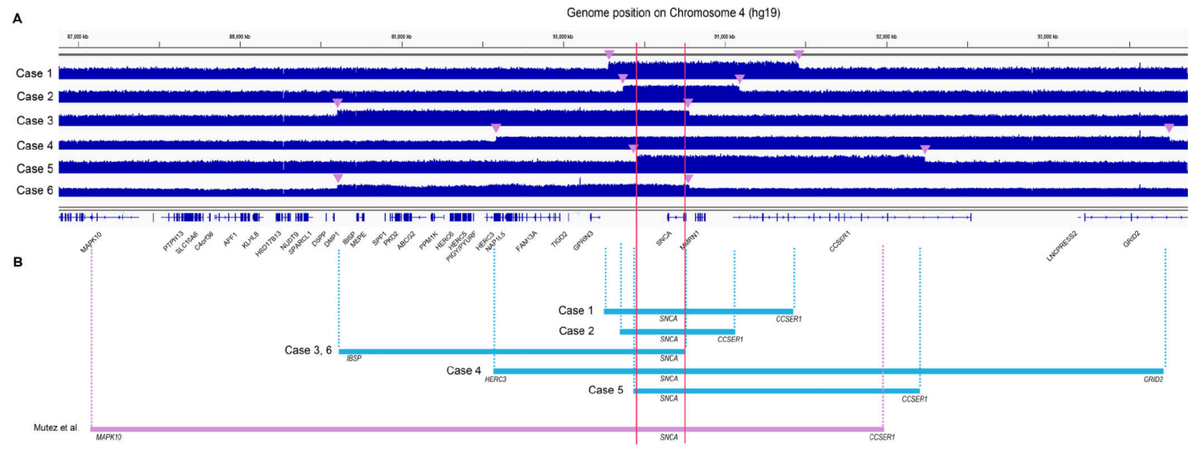

Figure 1.

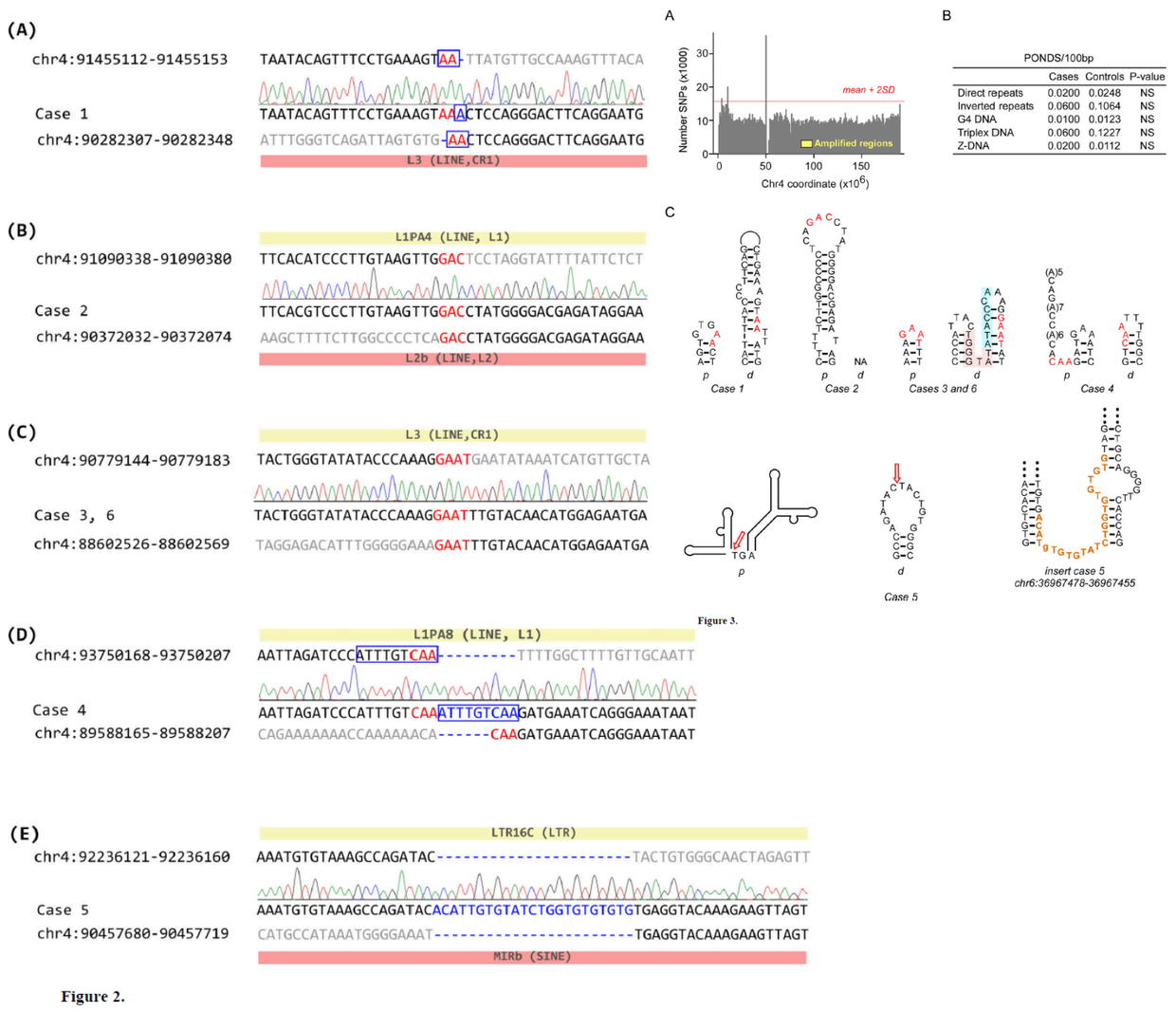

Figure 2.

Figure 3.

Table 1.

Genomic characteristics of the duplicated regions of chromosome 4

Case	Coordinates of duplicated region	Genes included in the duplicated region	Size (kb)
#1	chr4:90282327–91455133	*SNCA, MMRN1, CCSER1**	1,172.8
#2	chr4:90372052–91090360	*SNCA, MMRN1, CCSER1**	718.3
#3	chr4:88602546–90779167	*IBSP, MEPE, SPP1, PKD2, ABCG2, PPM1K, HERC6, HERC5, PIGY, PYURF, HERC3, NAP1L5, FAM13A, TIGD2, GPRIN3, SNCA*	2,176.6
#4	chr4:89588185–93750187	*HERC3**, *NAP1L5, FAM13A, TIGD2, GPRIN3, SNCA, MMRN1, CCSER1, LNCPRESS2, GRID2**	4,162.0
#5	chr4:90457700–92236140	*SNCA, MMRN1, CCSER1**	1,778.4
#6	chr4:88602546–90779167	*IBSP, MEPE, SPP1, PKD2, ABCG2, PPM1K, HERC6, HERC5, PIGY, PYURF, HERC3, NAP1L5, FAM13A, TIGD2, GPRIN3, SNCA*	2,176.6
Mutez, et al. (2011)	chr4:87172377–91943911	*MAPK10**, *PTPN13, SLC10A6, C4orf36, AFF1, KLHL8, HSD17B13, NUDT9, SPARCL1, DSPP, DMP1, IBSP, MEPE, SPP1, PKD2, ABCG2, PPM1K, HERC6, HERC5, PIGY, PYURF, HERC3, NAP1L5, FAM13A, TIGD2, GPRIN3, SNCA, MMRN1, CCSER1**	4,771.5

*Genes localized in the breakpoints

Table 2.

Clinical features of Korean patients with *SNCA* duplication

Case		#1	#2	#3	#4	#5	#6
Sex		M	M	F	F	F	M
Age of onset		40	51	66	55	49	51
Family history		Y	N#	N#	N	N	N
Initial symptoms	Bradykinesia	Y	Y	Y	NA	Y	Y
	Rigidity	Y	Y	Y	Y	NA	NA
	Tremor	Y	Y	Y	Y	Y	Y
	Postural instability	Y	Y	NA	NA	NA	NA
Disease Severity	Disease duration (yr) at the time of evaluation	1	2	2	5	4	4
	UPDRS motor	32	31	32	36	NA	NA
	H&Y scale	3	3	3	3	4	2
Nonmotor symptoms	RBD	NA	NA	NA	Y	Y	Y
	Cognition	Y†	N (MMSE 26 at age 65)	Y †	Y (MMSE 18 at age 60)	Y (MMSE 8 at age 56)	Y (MMSE 11 at age 56)
	Urinary.Symptoms	Y	NA	NA	NA	NA	Y
	Orthostatic hypotension	Y	Y	Y	Y	Y	N
	Depression	NA	NA	Y	Y	NA	Y
	Constipation	NA	Y	Y	Y	NA	Y
	Other NMS	NA	anxiety	insomnia	NA	NA	NA
Clinical features in disease course	Levodopa response	Y	Y	Y	NA	Y	NA
	Wearing-off	Y	Y	NA	NA	Y	Y
	Dyskinesia	Y	Y	NA	NA	Y	NA
	Hallucination	Y	NA	NA	Y	Y	Y
	Freezing of Gait	NA	Y	NA	NA	Y	NA
Pyramidal symptoms		Knee Jerk 3+/3+, Babinski sign (+/+)	NA	NA	NA	NA	NA
Case		#1	#2	#3	#4	#5	#6
Axial symptoms		Dysphonia	NA	NA	NA	NA	NA
EOM		cogwheel pursuit	DBN (Vib, HS)	NA	Hypometric saccade	NA	ocular flutter, hypometric saccade
Brain Imaging	Brain MR	Y(+, cortical atrophy in P-O)	NA	Y(−)	Y(−)	NA	Y(+−, mild cerebellar atrophy)
	CIT.PET	NA	Y(+)	NA	NA	NA	NA
	FDG.PET	NA	NA	NA	NA	Y(+,bilateral F-T-P decreased)	NA

#Asymptomatic carrier among family members (#2: Brother, #3: Daughter)

†MMSE scores were not available. A description of mild cognitive decline was found on the medical record.

Abbreviation: Y, symptom present; N, symptom not present; NA, not available; UPDRS, the Unified Parkinson's Disease Rating Scale; H&Y, Hoehn and Yahr scale; RBD, Rapid eye movment sleep behavior disorder; MMSE, Mini-Mental State Examination; NMS, nonmotor symptoms; DBN (HS, Vib); downbeat nystagmus by head shaking or vibration stimuli; P-O, parietal, occipital lobes; F-T-O, frontal, temporal, occipital lobes