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의학박사 학위논문

Lewy body dementia의 유전적 위험인자 발굴

Investigating genetic risk factors for Lewy body dementia

울산대학교 대학원

의학과

조성양

Investigating genetic risk factors for Lewy body dementia

지도교수 정선주

이 논문을 의학박사 학위논문으로 제출함

2022년 2월

울산대학교 대학원

의학과

조성양

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Contents

Abstract

Background and Aims: Lewy Body Dementia (LBD) is an umbrella term to include Parkinson's disease with dementia (PDD) and dementia with Lewy bodies (DLB). Both PDD and DLB share clinical symptoms including dementia and parkinsonism, and they have common neuropathology. The cognitive decline is heterogeneous in PDD and DLB, and genetic risk factors might explain the heterogeneity and pathophysiology of dementia. Although genome-wide association studies revealed genetic variants associated with PD susceptibility, there was a small number of genetic studies dealing with cognitive decline in PDD and DLB. In this study, we investigated the genetic mutations related to the development of dementia in PDD and DLB. Because LBD overlap with Alzheimer's disease (AD) and Parkinson's disease (PD), we used both healthy controls and patients with AD as controls. We used a microarray chip, which was developed based on a customized platform utilizing variants identified in previous genetic studies.

Method: We prospectively enrolled 313 PD patients with dementia, 321 PD patients without dementia, 232 patients with AD, 11 patients with dementia with Lewy bodies, and 635 healthy controls. For the primary analysis, genome-wide association studies were performed using a multiple logistic regression model adjusted for age and sex. In addition, we investigated rare variants and analyzed genotypes associated with the early development of dementia in PD using Cox regression analysis.

Results: *SNCA* single nucleotide polymorphism (SNP) rs11931074 was most significantly associated with PD (odds ratio = 0.66, 95% confidence interval = 0.56 – 0.78, $P = 7.75 \times 10^{-7}$). In an analysis for patients with PD only, *MUL1* SNP rs3738128 (odds ratio = 2.52, 95% confidence interval = 1.68 – 3.79, $P = 8.75 \times 10^{-6}$) was most significantly associated with

PDD. SNPs in *ZHX2* and *ERP29* were also associated with PDD. *ATP7B* SNP rs148399850 was most significantly associated with DLB compared with healthy controls (OR = 18.73, 95% CI = 4.64 – 75.70, $P = 3.92 \times 10^{-5}$), significance of which disappeared after Bonferroni correction. SNPs in *APOE* (rs769449, and rs75627662), *PVRL2* (rs12972156, rs519113, rs3852860, and rs6859), and *TOMM40* (rs59007384, rs405697) were significantly associated with AD. Rare variants in *AK5* and *PIK3CG* were associated with PDD. In Cox regression analysis, *MUL1* SNP rs3738128 was most significantly associated with the development of dementia at young age in PD ($P = 1.63 \times 10^{-9}$).

Conclusion: This microarray genomic study identified new loci of *MUL1* associated with PDD, suggesting an essential role of mitochondrial dysfunction in the development of dementia in patients with PD.

Keywords: genome-wide association study, Parkinson's disease, dementia, Lewy body dementia, dementia with Lewy bodies

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Introduction

Parkinson's disease dementia and dementia with Lewy bodies

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, affecting more than six million people worldwide (1). The diagnosis of PD is based on motor symptoms, including bradykinesia, rigidity, tremor, or gait disturbance (2). However, patients with PD suffer from various non-motor symptoms, such as fatigue, pain, sleep disturbance, dementia, depression, anxiety, and autonomic dysfunction (3). Dementia is one of the disabling non-motor symptoms that substantially impair the quality of life of patients with PD, increasing caregiver burden and economic costs (4). The prevalence of dementia in PD increases with age and disease duration. According to the longitudinal cohort studies, the prevalence of dementia in patients is 17% at 5 years after PD diagnosis, 46% at 10 years after PD diagnosis, and 83% at 20 years from PD diagnosis (Table 1) (5, 6). The risk of developing dementia in PD is 2.5—6 times higher than people without dementia with similar age. The neuropathologic hallmark in PD is Lewy bodies in the substantia nigra, which was first described in 1912 by Frederick Lewy. (7) Cortical Lewy bodies were first reported in association with dementia in 1961, and recent studies showed concomitant tau and amyloid-beta pathology in a subgroup of Parkinson's disease with dementia (PDD). (8)

Table 1. Longitudinal cohort studies (n>100) reporting the prevalence of dementia, or MCI in Parkinson's disease (Adapted from Aarsland et al. Nature reviews Disease premiers, 2021)(9)

Study	Cohort selection	n (at baseline)	Cognitive outcome	Frequency (%)
Sydney Multicenter Study(6)	Research, de novo	136	Dementia	83% at 20 years

Stavanger Study(10)	Prevalence	233	Dementia	27% at baseline and 60% at 12 years (80–90% by age 90)
Norwegian ParkWest(11)	Incidence	178	Dementia	17.4% at 4 years
CamPaIGN(12-14)	Incidence	142	Dementia	17% at 5 years and 46% at 10 years
CARPA(15)	Research, de novo	123	Dementia	17% at 5 years
NYPUM	Incidence	134	Dementia	27.6% at 5 years
Pennsylvania University(16)	Convenience	141	Dementia	0.7% at 1 year, 3.5% at 2 years, 7.5% at 3 years, 12.9% at 4 years and 28% at 6 years
ICICLE-PD(17)	Incidence	212	MCI	20% at baseline, 14% at 1.5 years and16% at 3 years
PPMI(18)	Research, de novo	423	CI (MoCA < 26)	21% at baseline, 61.8% at 1 year, 69.8% at 2 years, 67.3% at 3 years, 69.9% at 4 years and 68.2% at 5 years

MCI= mild cognitive impairment;

Dementia with Lewy bodies (DLB) is characterized by fluctuation in cognition, hallucination, as well as parkinsonism. In 1961, Ozaki et al reported neuropathologic findings of diffuse Lewy bodies

in two patients who presented with progressive dementia, parkinsonism, and neuropsychiatric symptoms. (8) Both PDD and DLB share common clinical symptoms and neuropathologic findings. However, the presence of dementia within the first year of the onset of parkinsonian symptoms was used to discriminate DLB from PDD. The separation of two diseases might be due to the different features and prognosis of the diseases.

Lewy Body Dementia (LBD) is an umbrella term to include both PDD and DLB. Lewy Body Dementia Association Scientific Advisory Council argued to maintain DLB because (1) patients with DLB might have little or no parkinsonism, and (2) there are some clinical and pathologic differences between DLB and PDD.(19)

Heterogeneity of cognitive decline in Parkinson's disease

The progression of motor features can vary significantly between patients with PD. For example, some patients present with resting tremor at the time of diagnosis, while others present with bradykinesia and rigidity without tremor. The progression rate of motor and non-motor symptoms is also heterogeneous (Figure 1). Especially, cognitive decline varies in clinical severity, cognitive domains involved, and the rate of progression.

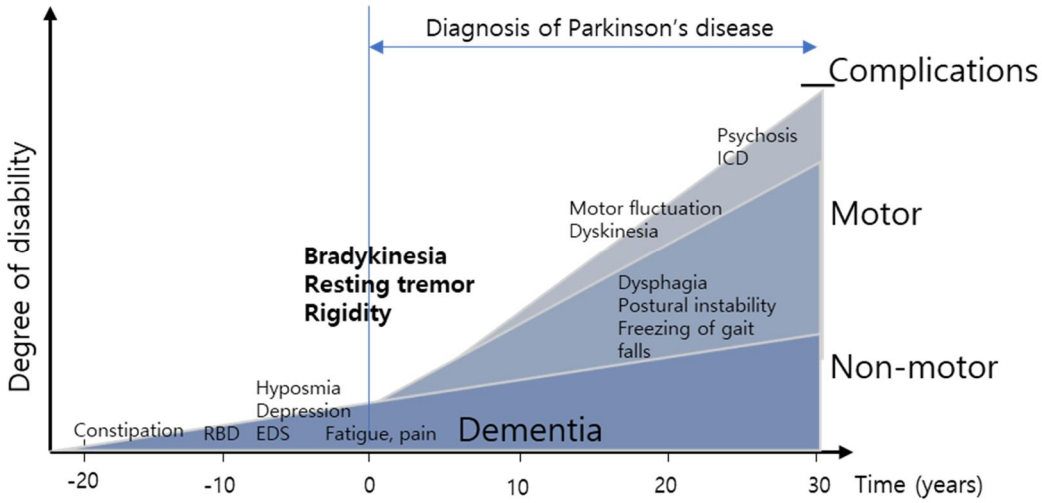


Figure 1. Heterogeneity of progression in Parkinson's disease (Adapted from Kalia et al, Lancet Neurology, 2015)(20)

Therefore, predicting cognitive courses for patients with PD is important. Several demographic, clinical, and genetic risk factors were suggested to affect cognitive decline in PD. Demographic and clinical risk factors include the presence of hallucination, older age, the severity of motor symptoms, presence of speech impairment, older age at PD onset, axial impairment, low level of education, presence of depression, and male sex. (21) The association between the involved cognitive domain and cognitive progression is controversial: CamPaIGN study reported posterior cortical deficit was associated with the development of dementia, (15) while frontal executive dysfunction was associated with the development of dementia in other studies. (22, 23) Modifiable clinical risk factors include heavy alcohol abuse, diabetes mellitus, and hypertension. Increased levels of uric acid, C-reactive protein, high-density lipoprotein cholesterol, and glucose levels were suggested to predict dementia conversion in PD.(24)

Genetic risk factors for Parkinson's disease and Parkinson's disease dementia

Approximately 5% to 10% of the patients with PD have the monogenic disease with Mendelian inheritance. The genetic mutations were found in *SNCA, PARK2, PINK2, DJ-1*, and *VPS35* genes. Another genetic risk factor for PD was the mutations in the *GBA1* gene, the gene responsible for Gaucher's disease. *GBA1* mutation causes a reduction in glucocerebrosidase activity and promotion of alpha-synuclein accumulation leading to the development of PD. However, most of the patients with PD are sporadic, which is affected both by genetic and environmental risk factors.

Genome-wide association studies (GWAS) investigate hundreds to million single nucleotide polymorphisms (SNPs) for association with a disease in hundreds of thousands of people. GWAS has been applied to common complex diseases, which are affected by several genetic and environmental factors, in contrast with single-gene disorders.(25) In multifactorial diseases, such as sporadic PD, myocardial infarction, diabetes, and age-related macular degeneration, GWAS enabled identifying genotype-phenotype association. (26, 27). The first GWAS was conducted for aged-related macular

degeneration in 2005. (28) The associations between genetic variants and common diseases led to the identification of disease susceptibility, pathophysiology, and might be applied to personalized medicine (for example, risk prediction and personalized therapy based on genotype).

In PD, GWAS have expanded the scope of genetic knowledge and identified more than 90 genetic loci that are associated with the development of PD (29-35). However, most previous GWAS have focused on the susceptibility of PD, and GWAS that specifically investigated motor or non-motor features, including dementia, of PD has been limited. In a recent GWAS, we reported *RYR2* and other genetic loci were associated with cognitive impairment in PD, but the assessment of cognitive function was only based on the Mini-Mental Status Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) scores (36).

Genetic risk factors for dementia with Lewy bodies

Most recent GWAS reported genomic variants, including *GBA*, *APOE*, *SNCA*, and *CNTN1,* that were associated with DLB (37, 38). Some studies conducted GWAS in patients with LBD, a combination of PDD and DLB. (39, 40) Although PDD and DLB share clinical, neurochemical, and morphological features, there have been debates about the consideration of two extremes on one continuous spectrum of Lewy body disease (41). Interestingly, in a large multinational cohort of patients with PD, PDD, and DLB, parkinsonism, and dementia showed two distinct association profiles, respectively, with the 3' or the 5' of the *SNCA* gene, suggesting PD, PDD, and DLB have distinct genetic etiology. Therefore, further studies of genome-wide investigation are necessary to identify distinct genetic variants associated with the development of PDD, independent of DLB.

Overlap between Lewy body dementia and Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disease featuring gradually progressive cognitive and functional deficits. The neuropathologic hallmarks of AD are amyloid and

tau deposition, which are found in patients with PDD and DLB. Recent studies showed that some of the genetic variants associated with LBD overlap with those associated with AD and PD (39). Indeed, genetic risk scores of AD or PD in patients with LBD show a continuum of the disease. However, little is known about the distinct genetic variants associated with LBD apart from PD or AD, which could contribute to the identification of the specific dementia pathogenesis in PD.

Genome-wide survival studies for Lewy body dementia

Since the first GWAS was conducted in 2005, genetic variants linked to susceptibility for the common disease were identified through two group comparisons. The progression and prognosis for the disease are fundamental to patients, and genetic variants could identify the progression rate. Therefore, recent few studies conducted genome-wide survival studies, which estimate the influence of common and low-frequency genetic variants on time from the onset of PD to progression to the PDD.(42, 43)

In this study, we have used a new customized microarray platform to comprehensively investigate the genetic variants that are associated with LBD. We investigated distinct genetic variants associated with PDD, and DLB, compared to healthy controls and AD. In addition, we investigated the genotype associated with the early development of dementia in PD.

Methods

Study population

We prospectively enrolled patients with PDD, patients with PD without dementia (PD-ND), patients with DLB, patients with AD, and healthy controls at Asan Medical Center, Seoul, South Korea. All participants were ethnic Koreans. The diagnosis of PD was based on the UK Brain bank criteria, which includes bradykinesia, rigidity, resting tremor, and postural instability (2). Exclusion criteria for PD are described in Table 2.

Table 2. Diagnostic criteria for Parkinson's disease (adapted from Hughes et al. JNNP, 1992(2))

Step 1. Diagnosis of Parkinsonian Syndrome
• Bradykinesia
• At least one of the following
o Muscular rigidity
o 4-6 Hz rest tremor
o postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction
Step 2 Exclusion criteria for Parkinson's disease
• history of repeated strokes with stepwise progression of parkinsonian features
• history of repeated head injury
• history of definite encephalitis
• oculogyric crises
• neuroleptic treatment at onset of symptoms
• more than one affected relative
• sustained remission • strictly unilateral features after 3 years

• supranuclear gaze palsy
• cerebellar signs
• early severe autonomic involvement
• early severe dementia with disturbances of memory, language, and praxis
• Babinski sign
• presence of cerebral tumor or communication hydrocephalus on imaging study
• negative response to large doses of levodopa in absence of malabsorption
• MPTP exposure
Step 3 supportive prospective positive criteria for Parkinson's disease
Three or more required for diagnosis of definite Parkinson's disease in combination with step one
• Unilateral onset
• Rest tremor present
• Progressive disorder
• Persistent asymmetry affecting side of onset most
• Excellent response (70-100%) to levodopa • Severe levodopa-induced chorea • Levodopa response for 5 years or more • Clinical course of ten years or more

The diagnosis of DLB was based on the 4th consensus report of the DLB consortium criteria for probable DLB. (44) Probable DLB was diagnosed if 1) two or more core clinical features of DLB are present, or 2) only one core clinical feature is present but with one or more indicative biomarkers. (Table 3)

The diagnosis of AD was based on the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria for probable AD dementia (Table 4). (45)

Table 3. Diagnostic criteria for probable dementia with Lewy bodies (adapted from McKeith et al. Neurology, 2017(44))

Essential for a diagnosis of DLB is dementia, defined as a progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational functions, or with usual daily activities. Prominent or persistent memory impairment may not necessarily occur in the early stages but is usually evident with progression. Deficits on tests of attention, executive function, and visuoperceptual ability may be especially prominent and occur early.
Core clinical features (The first 3 typically occur early and may persist throughout the course.)
Fluctuating cognition with pronounced variations in attention and alertness.
Recurrent visual hallucinations that are typically well formed and detailed.
REM sleep behavior disorder, which may precede cognitive decline.
One or more spontaneous cardinal features of parkinsonism: these are bradykinesia (defined as slowness of movement and decrement in amplitude or speed), rest tremor, or rigidity.
Indicative biomarkers
Reduced dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET.
Abnormal (low uptake) 123iodine-MIBG myocardial scintigraphy. Polysomnographic confirmation of REM sleep without atonia.

Table 4. Diagnostic criteria for probable Alzheimer's disease dementia (adapted from McKhann et al. Neurology, 1984(45))

Meets criteria for dementia and has the following characteristics
A. Insidious onset. Symptoms have a gradual onset over months to years, not sudden over hours or days;
B. Clear-cut history of worsening of cognition by report or observation; and

C. The initial and most prominent cognitive deficits are evident on history and examination in one of the following categories.

a. Amnestic presentation: It is the most common syndromic presentation of AD dementia. The deficits should include impairment in learning and recall of recently learned information. There should also be evidence of cognitive dysfunction in at least one other cognitive domain, as defined earlier in the text.

b. Nonamnestic presentations:

• Language presentation: The most prominent deficits are in word-finding, but deficits in other cognitive domains should be present.

• Visuospatial presentation: The most prominent deficits are in spatial cognition, including object agnosia, impaired face recognition, simultanagnosia, and alexia. Deficits in other cognitive domains should be present.

• Executive dysfunction: The most prominent deficits are impaired reasoning, judgment, and problem solving. Deficits in other cognitive domains should be present.

D. The diagnosis of probable AD dementia should not be applied when there is evidence of (a) substantial concomitant cerebrovascular disease, defined by a history of a stroke temporally related to the onset or worsening of cognitive impairment; or the presence of multiple or extensive infarcts or severe white matter hyperintensity burden; or (b) core features of Dementia with Lewy bodies other than dementia itself; or (c) prominent features of behavioral variant frontotemporal dementia; or (d) prominent features of semantic variant primary progressive aphasia or nonfluent/ agrammatic variant primary progressive aphasia; or (e) evidence for another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition

Healthy controls were recruited from the spouses of the patients, and inclusion criteria were those

who did not have a neurological disease including PD or dementia.

Clinical information

All participants underwent blood sampling for genetic tests, and the clinical information including age, sex, and education years was collected at the time of sampling. Mini-Mental Status Examination (MMSE) was performed for the screening of cognitive function.

Patients with PD visited the outpatient clinic at 3- to 6-month intervals. Patients with PD and/or their caregivers were asked questions about cognitive decline, and daily functioning such as the patient's ability to manage finances, use pieces of equipment, and cope in a social situation. If significant cognitive changes and subsequent impairment on daily life activities are detected, patients underwent MMSE (level I), according to the recommendation from the Movement Disorder Society Task Force (46) (Table 5). Level II assessment was performed when the patients were compatible with PDD at level I to specify the pattern and severity, or when the diagnosis of PDD is uncertain or equivocal at the end of the Level I process (Table 6). We used Seoul Neuropsychological Screening Battery (SNSB) test for level II tests, which includes digit span, verbal fluency test, trail making test, Stroop test, free and cued recall test, Boston naming test, and NPI. (47) The diagnosis of PDD was made by 2 neurologists, based on the clinical diagnostic criteria proposed by the Movement Disorder Society Task Force (46). At 3- to 6- months intervals, conversion to dementia was assessed.

Table 5. Algorithm for diagnosing Parkinson's disease dementia at Level 1 (adapted from Dubois B. et al. Movement disorders, 2007)

1. A diagnosis of Parkinson's disease based on the Queen's Square Brain Bank criteria for PD
2. PD developed prior to the onset of dementia
3. MMSE below 26
4. Cognitive deficits severe enough to impact daily living (Caregiver interview or Pill

Questionnaire)
5. Impairment in at least two of the following tests: Months reversed or Seven backward Lexical fluency or Clock drawing MMSE Pentagons Word recall

Table 6. Summary of Tests at Level II testing for Parkinson's disease dementia (adapted from Dubois B. et al. Movement disorders, 2007)

Global efficiency	Mattis DRS
Executive functions	
Working memory	Digit span Spatial span (CANTAB) Digit ordering test
Conceptualization	Similarities (WAIS-III) Wisconsin CST
Set activation	Verbal fluency (C, F, L)
Set shifting	TMT
Set maintenance	Stroop test Odd man out test
Behavioral control	Prehension behavior
Memory	RAVLT Free and cued recall test
Instrumental functions	
Language	Boston naming test

Visuo-constructive	Copy of the clock
Visuo-spatial	Benton line orientation test
	Cube analysis (VOSP)
Visuo-perceptive	Benton face recognition test
	Fragmented letters (VOSP)
Neuropsychiatric functions	
Apathy	Apathy scale
Depression	MADRS
	Hamilton
	Beck depression inventory
	GDS-15
Visual hallucination	PPQ6
	Psychosis
	NPI

For patients with PD and those with DLB, age at disease onset, age at latest follow-up, Hoehn and Yahr (H&Y) stage at the time of study enrollment were assessed using medical chart review. Age at disease onset was defined as the age at the first detection of motor symptom for patients with PD, and as the age at the first detection of motor symptom or cognitive decline, whichever comes first for patients with DLB. We investigated the time at the diagnosis of dementia in patients with PD, and the latest MMSE scores through medical chart review.

Development of microarray genotyping platform

We designed a microarray genotyping platform that contained genetic variants with biological plausibility for PD, suggested by previous our GWAS or other previous genetic studies: 1) Genetic

variants that showed the high level of association (P value < 10^{-4}) with PD in our previous GWAS using ethnicity-specific Korean Chip (K-CHIP). K-CHIP was designed by the Center for Genome Science, Korea National Institute of Health (4845-301, 3000-3031) (www.cdc.go.kr) (36, 48). K-CHIP consists of an imputation GWAS grid [505,000 Asian-based grid with minor allele frequency (MAF) > 5% in Asians]; exome contents [84,000 Korean-based grid with MAF > 5%, in Koreans; 149,000 coding single-nucleotide polymorphisms (cSNPs); and insertions and deletions on the basis of data from 2000 whole-exome sequences and 400 whole-genome sequences with MAF > 0.1%]; new exome/loss of function contents (44,000 variants); expression quantitative trait loci (17,000 variants); absorption, distribution, metabolism, and excretion genes; and other miscellaneous variants. 2) Genetic variants that showed significant association with PD in previous GWAS (29-35). 3) Genetic mutations that were reported to be a cause of monogenic familial PD with Mendelian inheritance (https://www.omim.org/). 4) Genetic variants that showed significant association with DLB in previous GWAS (37, 38, 49). 5) Genetic variants that showed significant association with Alzheimer's disease (AD) in previous GWAS (50-53). 6) Genetic variants associated with neuroinflammation in previous GWAS (33, 54, 55). The characteristics of the markers used in the microarray are described in Table 7.

Table 7. Characteristics of the markers used in the microarray.

Source	Not Design	Design	Submit	Design Rate
AD (PMID:30820047)	689	244	933	26.2%
AD_IGAP	9754	6474	16,228	39.9%
ADPD cRE	5412	533	5945	9.0%
DLB (PMID:31065058)	2	1	3	33.3%
ENDOSOME	451	714	1165	61.3%
GWAS category AD	2827	2319	5146	45.1%
GWAS category DM	484	332	816	40.7%
GWAS category PD	1356	1183	2539	46.6%
GWAS category_T2DM	6248	5261	11,509	45.7%
KEGG_LYSOSOME	946	1289	2235	57.7%
KEGG TOLL LIKE RECEPTOR SIGNALING PATHWAY	817	1166	1983	58.8%

MITOCHONDRION	2389	3458	5847	59.1%
NEURON_DEVELOPMENT	423	715	1138	62.8%
PD (Nall_2019_Biorxiv)	663	615	1278	48.1%
PD (PMID:28892059)	6	6	12	50.0%
PD (Foo)	3681	3604	7285	49.5%
PD (Nall)	13158	10,132	23,290	43.5%
REACTOME ACTIVATION OF_NF_KAPPAB_IN_B_CELLS	378	545	923	59.0%
SLEEP	142	282	424	66.5%
SLEEP (PMID:18820697)	3	6	9	66.7%
SLEEP (PMID:19412176)	5	5	10	50.0%
SLEEP (PMID:21170044)	4	3	7	42.9%
SLEEP (PMID:22257907)	7	4	11	36.4%
SLEEP (PMID:26507264)	34	50	84	59.5%
SLEEP (PMID:29535854)	139	107	246	43.5%
T2D (PMID:30718926)	9	2	11	18.2%
T2D_Xue	9223	8059	17,282	46.6%
UBIQUITIN_CYCLE	262	426	688	61.9%
Other GWAS category	50,292	61,726	112,018	55.1%
Total	109,804	109,261	219,065	49.9%

Annotation of variants was performed using nspEff tool to confirm the distribution of gene effect (56). In a total of 219,065 variants, we excluded 109,804 'novel-not recommended and neutral' markers in the score data, because the performance or efficacy of genotyping might be low (Table 7). The final selection was performed by excluding duplicate markers, markers not included in the 1000 genome project phase 3 data, markers with minor allele frequency of zero in East Asian GWAS data, and proxy SNPs (tagging r^2>0.8) (Table 8 and Table 9). The final candidate markers consisted of 74,224 markers.

Table 8. Staged verification of the markers.

Step-by-step Marker Check for Tagging			
Steps	Description	Number of Markers	Number of QC Marker
Step0	Raw	219,065	-
Step1	Remove duple Marker	179,344	39,721
Step2	1000genome	165,822	13,522

Step3	MAF = 0	151,641	14,181
Step4	Tagging r^2 0.8	59,920	91,721
	Tagging SNP Coverage		
Step4	Tagging r2 0.8	59,920	14,811
Step5-1	independent Marker	45,109	-
Step6-1	Design Possible	32,379	12,730
Step5-2	Non Independent Group	14,807	-
Step6-2	Design Possible Group	13,880	927
Step7	Final Tagging SNP	46,259	-

Table 9. Additional selection according to selection priority.

	Description	Number of Markers	Cumulative Numbers	Priority
Step0	Tagging SNP	46,259	46,259	Top
Step1	Priority1 Uniq Marker	12,088	58,347	Middle
Step2	Priority2 Uniq Marker	15,210	73,557	Bottom
Step3	Includes markers of interest and multi-allelic markers	667	74,224	Top

Sample quality control

First, samples with a low call rate (< 0.95%) were excluded due to the possibility of low DNA quality or experimental error. Also, high heterozygosity was excluded due to low DNA quality or possible contamination of samples. We checked the entire sample distribution, and low-quality samples were excluded if they have deviated significantly from the whole sample distribution. SNP pruning was also performed. Only representative SNP information based on linkage disequilibrium was selected from the data. Considering the population stratification, samples that deviated from the whole sample were excluded from the analysis by assessing the multidimensional scaling.

Secondary sample quality control consisted of genotype calling, excluding samples with low quality based on the primary sample quality control criteria and sex-inconsistent samples. We excluded samples that did not satisfy the quality control criteria after a repeat sample quality control. We excluded SNP data from the cryptic first-degree relative analysis.

SNP quality control

We performed a SNPolisher analysis to exclude low-quality SNPs. SNPs with low call rates (call rate was < 95%) were excluded because errors might be due to probe design and clustering analysis problems. SNPs with Hardy–Weinberg equilibrium (HWE) test *P*-value > 10^{-4} were excluded, because it indicates a probable error in the genotype clustering process. We excluded SNPs with minor allele frequency < 1% in both cases and controls. We performed cluster quality control, to include SNPs with $P < 0.001$.

Genome-wide association studies

The association between genetic variants and PD or PDD was analyzed using a multiple logistic regression model after adjusting for age, sex, and education years. For each genetic variant, we calculated the odds ratios (OR), 95% confidence interval (CI), and two-tailed P-value. Bonferroni correction was applied to adjust for multiple comparisons. Manhattan plots and quantile-quantile plots (Q-Q plots) were constructed for the P-values for all genotyped variants that passed quality controls.

Genetic variants associated with the onset of dementia

Log-rank test and Cox proportional hazard analysis were used to estimate the genotype associated with the onset of dementia after the onset of motor symptoms or age at dementia after birth. Gender and education years were included as a covariate in the Cox proportional hazard analysis. To calibrate the test statistics, we used a saddlepoint approximation using SPACox (version 0.1.2).

Gene-based rare variant association analysis

Sequence kernel association-optimized (SKAT-O) analysis (57) was conducted to determine the

difference in the aggregate burden of rare variants between PD, PDD, AD, compared with healthy controls. For this analysis, we used a minor allele frequency threshold of ≤1% and a minor allele count ≥ 3 as filters. This analysis was performed using R SKAT (version 2.0.1).

Statistical analysis

We compared demographics and clinical characteristics between PDD, PD-NC, DLB, AD, and healthy controls using Kruskal-Wallis tests for continuous variables which did not meet the assumption of homogeneity of variance, and chi-square for categorical variables. Post-hoc analysis was performed using Dunnett's posthoc tests and Bonferroni's correction.

The statistical analysis was performed using R (version 3.1.2, Free Software Foundation, Inc., Boston, MA, USA), the PLINK program (version 1.90, NIH-NIDDK Laboratory of Biological Modeling, Bethesda, MD, USA), Haploview (version 4.2, Daly Lab at the Broad Institute, Cambridge, MA, USA), and LocusZoom (version 1.4, University of Michigan, Department of Biostatistics, Center for Statistical Genetics, Ann Arbor, MI, USA).

Results

Subject characteristics

We enrolled 318 patients with PDD, 326 patients with PD-ND, 12 patients with DLB, 248 patients with AD, and 648 healthy controls. In the process of quality control, 5 patients with PDD, 5 patients with PD-NC, 1 patient with DLB, 16 patients with AD, and 13 healthy controls were excluded. The final study population included 313 patients with PDD, 321 patients with PD-NC, 11 patients with DLB, 232 patients with AD, and 635 healthy controls. Age at the sample was significantly different between the five groups ($P < 0.001$) (Table 10). In posthoc analysis, age at the sample was significantly lower in controls compared with all other groups. Female was significantly lower in patients with DLB than in other groups ($P < 0.001$). Education years were significantly lower in patients with PDD and patients with AD, compared with controls (median 6.0 years, 9.0 years, 12.0 years, $P < 0.001$). Age at disease onset, and disease duration, were not significantly different between PDD and PD-ND. The median disease duration was 12.0 years for both PDD and PD-ND groups.

Genome-wide association study for Lewy body dementia

1) Parkinson's disease vs. healthy controls

The 41,534 genetic variants that passed quality controls were genotyped and analyzed. Multiple logistic regression with additive coding schemes were performed.

First, we compared patients with PD (combined PDD and PD-ND) and controls. Manhattan plot is described in Figure 2. Among top 10 genetic variants associated with PD, five SNPs were in the loci of *SNCA* (rs11931074, rs12642514, rs35691, rs80184884, and rs75876872) (Table 11), and two *SNCA* SNPs (rs11931074 and rs12642514) showed statistical significance after Bonferroni correction. *SNCA* SNP rs11931074 was the most significantly associated with PD (OR = 0.66, 95% CI = 0.56 – 0.78, $P = 7.75 \times 10^{-7}$). *SPHK1* SNP rs2247856 (OR = 0.65, 95% CI = 0.53 – 0.80, $P = 4.35 \times 10^{-5}$), and *FYN* SNP rs7772036 (OR = 0.72, 95% CI = 0.61–0.85, $P = 9.74 \times 10^{-5}$) were also associated with PD.

Table 10. Baseline clinical characteristics of study subjects

	PD with dementia (n=313)	PD without dementia (n=321)	DLB (n=11)	Controls (n=648)	AD (n=232)	P value
Age at sample, years	70.0 (65.0−74.0)	69.0 (64.5−73.0)	72.5 (68.0−77.5)	64.0 (62.0−67.0)[a]	70.0 (64.0−73.0)	<0.001
Female, n (%)	179 (57.2 %)	166 (51.7 %)	3 (27.3 %)[b]	289 (44.6 %)	94 (40.5 %)	<0.001
Education, years	6.0 (2.0−12.0)[c]	12.0 (6.0−16.0)	12.0 (9.0−14.0)	12.0 (9.0−16.0)	9.0 (6.0−12.0)[c]	<0.001
Latest MMSE	17.0(13.0−20.0)[c]	27.0 (26.0−29.0)	21.5 (20.5−23.5)	28.0 (26.0−29.0	18.0(14.0−22.0)[c]	<0.001
Hoehn and Yahr stage	3.0 (2.0-3.0)[d]	2.0 (2.0-3.0)	2.5 (0.0-3.5)	-	-	<0.001
Age at onset, years	63.5 (57.0−69.0)	63.0 (57.0−68.0)	69.5 (64.0−76.0)[d]	-	-	0.002
Age at latest follow-up, years	76.0 (72.0-81.0)	75.0 (72.0−80.0)	-	-	-	0.119
Disease duration, years	12.0 (9.0−17.0)	12.0 (9.0−16.0)	-	-	-	0.896
Age at dementia, years	73.0 (69.0−78.0)	-	-	-	-	-

[a]Significant difference compared with all other groups, using Dunn's posthoc test
[b]Significant difference compared with all other groups, using Dunn's posthoc test
[c]Significant difference compared with PD without dementia, DLB, and controls, using Dunn's posthoc test
[d]Significant difference compared with PD without dementia, using Dunn's posthoc test

AD, Alzheimer′s disease; DLB, dementia with Lewy bodies; PD, Parkinson's disease; MMSE, Mini-Mental Status Examination

Figure 2. Genetic variants associated with Parkinson's disease compared with healthy controls

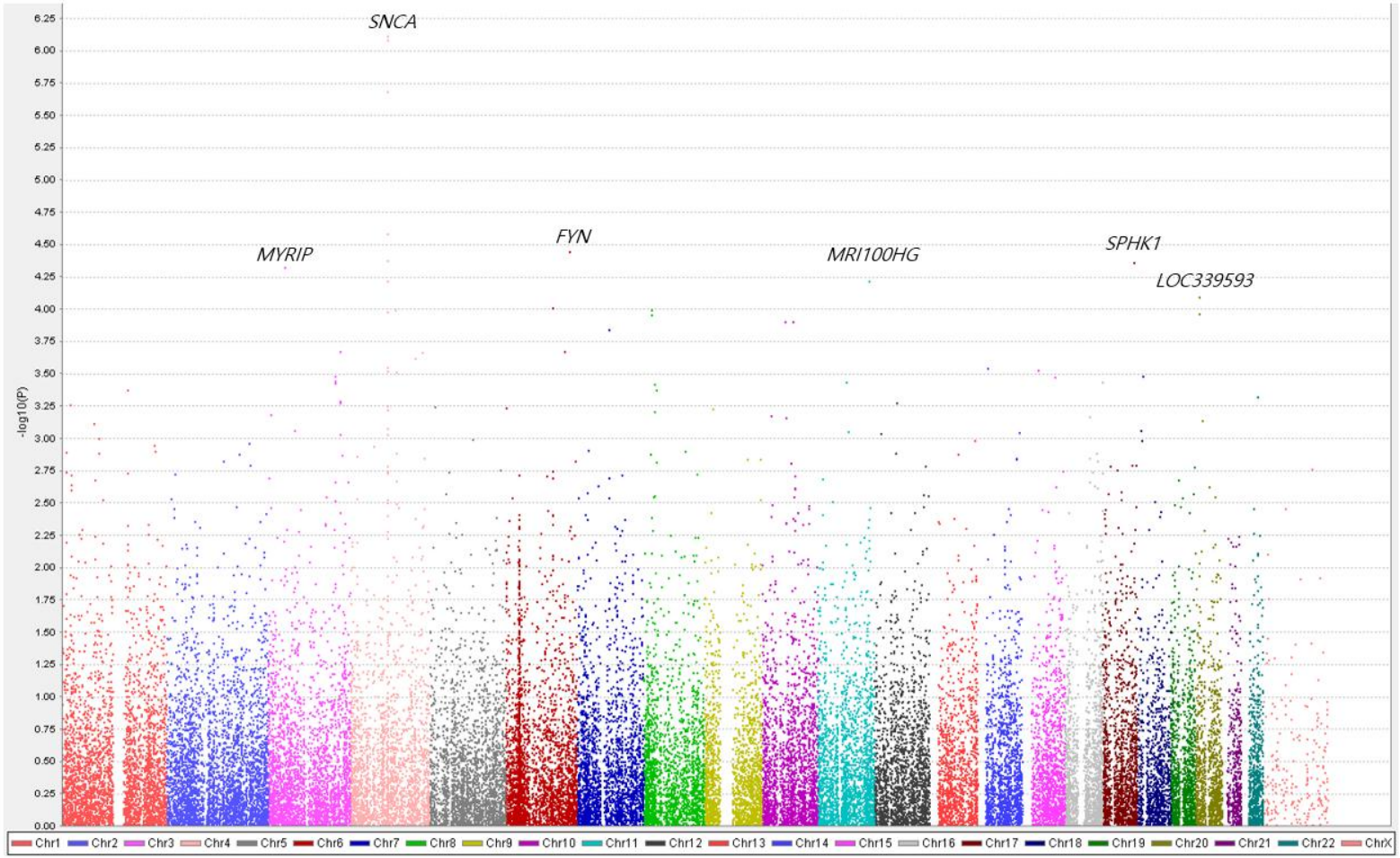


Table 11. Top 10 genetic variants that were associated with Parkinson's disease compared with healthy controls in the order of statistical significance

Gene	SNP	Chr	Position	Allele (minor/major)	Minor allele frequency (case/control)	OR (95% CI)	*P* value
SNCA,GPRIN3	rs11931074	4	89718364	G/C	0.37/0.46	0.66 (0.56, 0.78)	7.75×10^{-7}
SNCA,GPRIN3	rs12642514	4	89708246	A/C	0.36/0.46	0.69 (0.58, 0.79)	2.08×10^{-6}
SNCA	rs356191	4	89766969	A/G	0.06/0.10	0.52 (0.38, 0.70)	2.64×10^{-5}
SNCA,GPRIN3	rs80184884	4	89705068	G/A	0.06/0.10	0.52 (0.38, 0.71)	4.24×10^{-5}
SPHK1	rs2247856	17	76385474	A/G	0.16/0.22	0.65 (0.53, 0.80)	4.35×10^{-5}
MYRIP	rs6599077	3	40055127	A/G	0.43/0.35	1.42 (1.20, 1.68)	4.81×10^{-5}
MRI100HG	rs577924	11	122264399	C/T	0.43/0.35	1.41 (1.19, 1.67)	6.05×10^{-5}
SNCA,GPRIN3	rs75876872	4	89705795	G/A	0.04/0.08	0.49 (0.35, 0.69)	6.07×10^{-5}
LOC339593	rs1473702	20	11253884	C/T	0.51/0.44	1.38 (1.18, 1.62)	8.05×10^{-5}
FYN	rs7772036	6	111739596	G/A	0.32/0.39	0.72 (0.61, 0.85)	9.74×10^{-5}

Chr, chromosome; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism;

2) Parkinson's disease dementia vs. healthy controls

Second, we compared genetic variants between PDD and controls, and between PDD and PD-ND using multiple logistic regression with additive coding schemes after adjusting for age, sex, and education years. The Manhattan plot is described in Figure 3. Among the top 10 SNPs associated with PDD compared with controls, two SNPs were in the loci of *SNCA* (rs11931074 and rs12642514) (Figure 3A and Table 12). *SATB2* SNP rs1456522 (OR = 2.05, 95% CI = 1.49 – 2.83, $P = 8.75 \times 10^{-6}$) was the most significantly associated with PDD compared with healthy controls.

3) Parkinson's disease dementia vs. Parkinson's disease without dementia

Third, when we compared PDD and PD-ND, we found distinct genetic variants as shown in Figure 3B. Two SNPs were in the loci of *MUL1* (rs3738128 and rs12566937) (Figure 3B and Table 13). *MUL1* SNP rs3738128 (OR = 2.68, 95% CI = 1.78 – 4.01, $P = 2.01 \times 10^{-6}$) was the most significantly associated with PDD. In linkage analysis, *MUL1* SNP (rs12566937) showed moderate linkage disequilibrium with the *MUL1* SNP rs3738128, which was the SNP with the lowest *P*-value (Figure 4). SNPs in *ZHX2* (OR = 0.56, 95% CI = 0.43 – 0.74, $P = 3.65 \times 10^{-5}$) and *ERP29* (OR = 3.05, 95% CI = 1.77 – 5.27, $P = 6.41 \times 10^{-5}$) were also associated with PDD. However, none of the SNPs remained significant after Bonferroni correction.

4) Dementia with Lewy bodies vs. healthy controls

Fourth, we compared genetic variants associated with DLB compared with healthy controls. *ATP7B* SNP rs148399850 (OR = 18.73, 95% CI = 4.64 – 75.70, $P = 3.92 \times 10^{-5}$) was the most significantly associated with DLB compared with healthy controls. SNPs in *HIF1AN* (OR = 6.97, 95% CI = 2.65 – 18.39, $P = 8.62 \times 10^{-5}$) and *TRAK1* (OR = 15.17, 95% CI = 1.77 – 5.27, $P = 6.41 \times 10^{-5}$) were also associated with DLB (Figure 5 and Table 14). However, none of the SNPs remained significant after Bonferroni correction.

Figure 3. Genetic variants associated with Parkinson's disease (PD) dementia (PDD) compared with healthy controls and PD without dementia

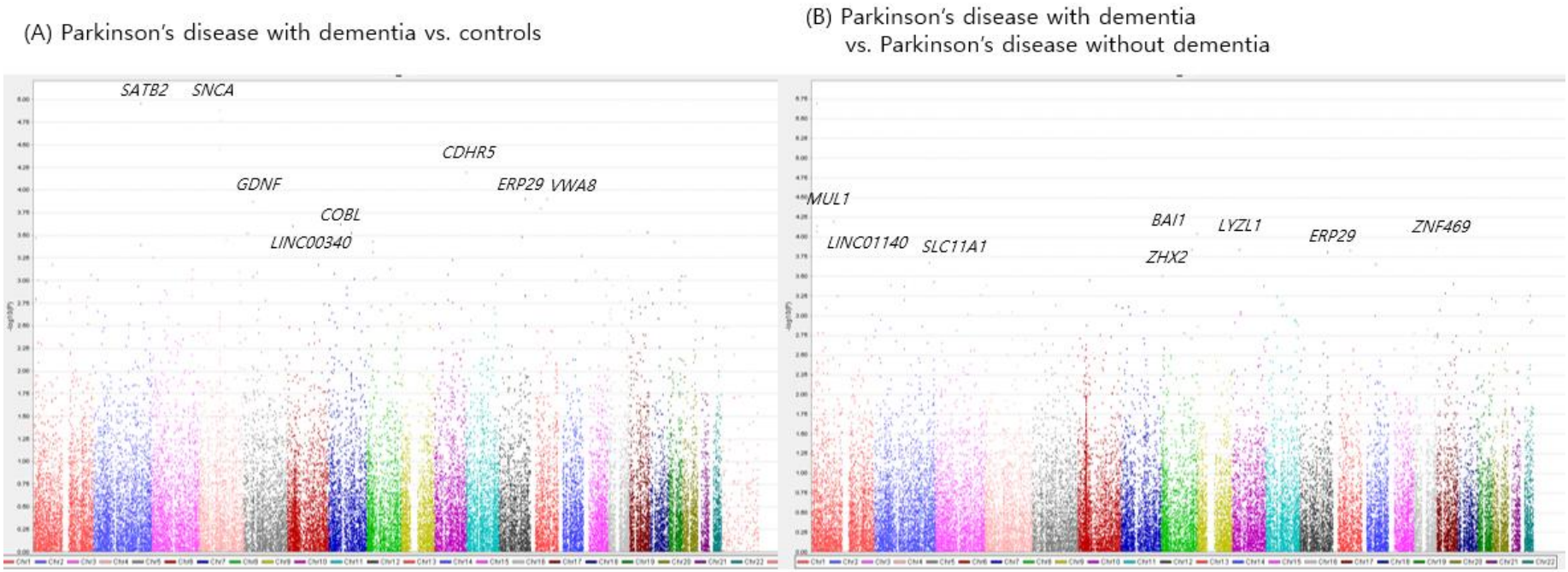


Table 12. Top 10 genetic variants that were associated with Parkinson's disease dementia compared with healthy controls in the order of statistical significance

Gene	SNP	Chr	Position	Allele (minor/major)	Minor allele frequency (case/control)	OR (95% CI)	P value
SATB2,LOC101927619	rs1456522	2	198904459	A/G	0.15/0.10	2.05 (1.49, 2.83)	1.10×10^{-5}
SNCA,GPRIN3	rs11931074	4	89718364	G/C	0.36/0.46	0.62 (0.50, 0.77)	1.31×10^{-5}
SNCA,GPRIN3	rs11931074	4	89718364	G/T	0.37/0.47	0.63 (0.51, 0.77)	1.71×10^{-5}
SNCA,GPRIN3	rs12642514	4	89708246	A/C	0.36/0.40	0.64 (0.52, 0.79)	3.56×10^{-5}
CDHR5	rs3758650	11	616865	A/G	0.12/0.08	2.01 (1.43, 2.84)	6.31×10^{-5}
ERP29,NAA25	rs4767293	12	112025492	A/G	0.08/0.05	2.27 (1.49, 3.44)	1.26×10^{-4}
GDNF	rs76568852	5	37837968	A/G	0.10/0.06	2.2 (1.47, 3.30)	1.34×10^{-4}
VWA8	rs9566819	13	41678098	T/C	0.09/0.05	2.16 (1.45, 3.21)	1.59×10^{-4}
COBL,POM121L12	rs1949829	7	51470190	T/C	0.20/0.15	1.68 (1.27, 2.21)	2.39×10^{-4}
LINC00340	-	6	22004680	T/C	0.27/0.20	1.58 (1.24, 2.02)	2.49×10^{-4}

Chr, chromosome; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism;

Table 13. Top 10 genetic variants that were associated with Parkinson's disease dementia compared with Parkinson's disease without dementia in the order of statistical significance

Gene	SNP	Chr	Position	Allele (minor/major)	Minor allele frequency (case/control)	OR (95% CI)	*P* value
MUL1	rs3738128	1	20499992	G/C	0.16/0.07	2.68 (1.78, 4.01)	2.01×10^{-6}
LINC01140	rs7553864	1	87147675	T/C	0.23/0.14	1.92 (1.39, 2.64)	6.34×10^{-5}
MUL1	rs12566937	1	20506181	G/T	0.21/0.13	1.95 (1.40, 2.71)	7.28×10^{-5}
MUL1	rs12566937	1	20506181	G/T	0.21/0.13	1.93 (1.39, 2.68)	8.55×10^{-5}
BAI1	-	8	143611669	T/C	0.26/0.17	1.77 (1.33, 2.35)	9.15×10^{-5}
ZNF469	rs883284	16	88502831	A/G	0.16/0.10	2.05 (1.42, 2.96)	1.37×10^{-4}
LYZL1,C10orf126	rs1889714	10	29099710	A/G	0.06/0.13	0.43 (0.28, 0.67)	1.46×10^{-4}
ZHX2	rs11779459	8	122968311	T/C	0.24/0.33	0.60 (0.46, 0.78)	1.46×10^{-4}
ERP29,NAA25	rs4767293	12	112025492	A/G	0.08/0.04	2.84 (1.65, 4.87)	1.56×10^{-4}
SLC11A1	rs1059823	2	218395121	G/A	0.38/0.28	1.62 (1.25, 2.08)	2.12×10^{-4}

Chr, chromosome; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism;

Figure 4. Regional association plot of the genetic variants of *MUL1*

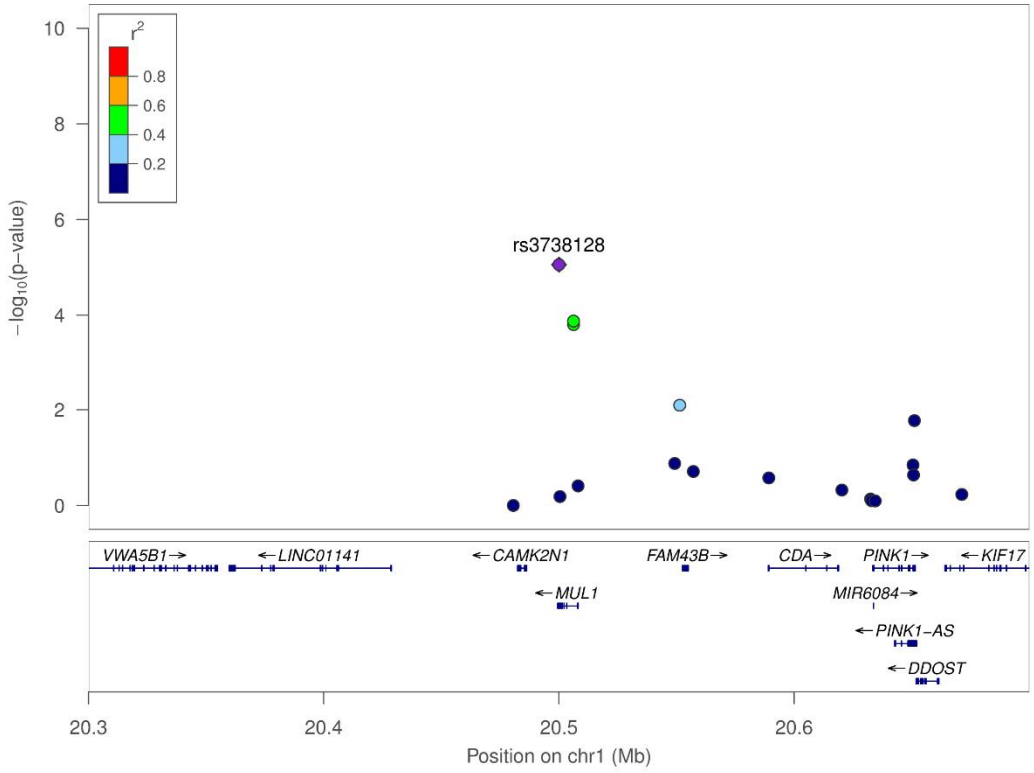


Figure 5 Genetic variants associated with dementia with Lewy bodies compared with healthy controls

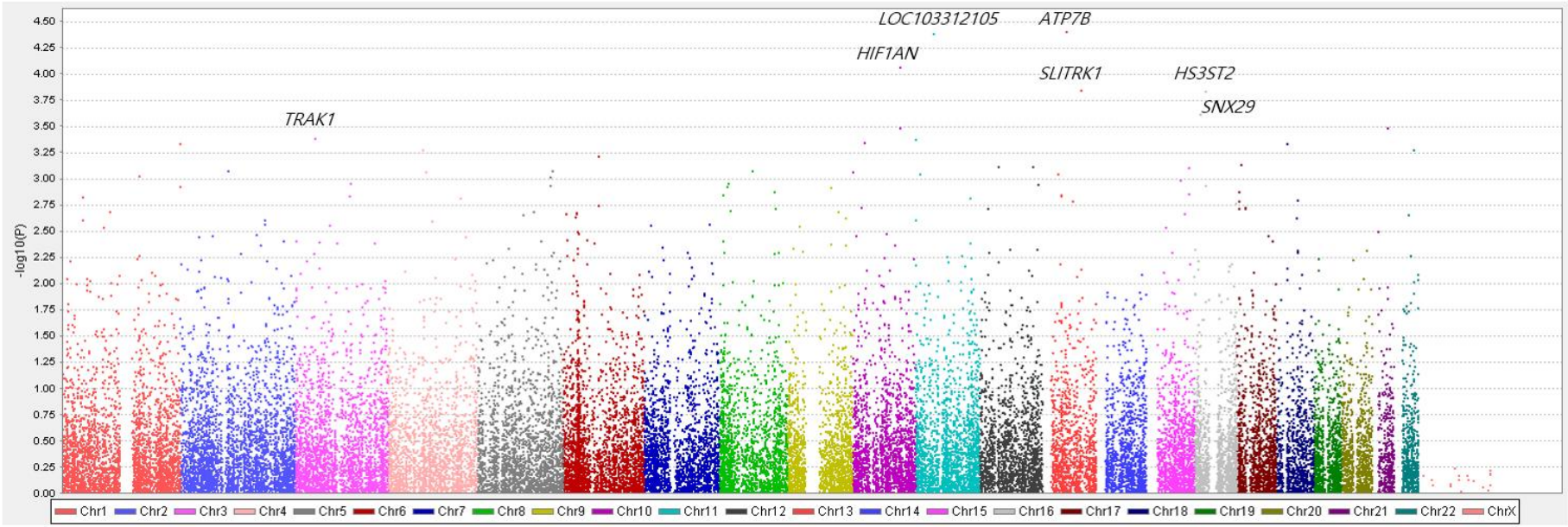


Table 14. Top 10 genetic variants that were associated with dementia with Lewy bodies compared with healthy controls in the order of statistical significance

Gene	SNP	Chr	Position	Region relative to gene	Allele (minor/major)	minor allele frequency (case/control)	OR (95% CI)	*P* value
ATP7B	rs148399850	13	51937490	missense,UTR-3,exon	T/C	0.18/0.015	18.73 (4.64, 75.70)	3.92×10^{-5}
LOC103312105	rs7928395	11	38559490	downstream	T/C	0.36/0.076	8.17 (3.00, 22.30)	4.18×10^{-5}
HIF1AN	rs11190604	10	100542700	intron	G/A	0.41/0.11	6.97 (2.65, 18.39)	8.62×10^{-5}
SLITRK1,RNU6-67P,LINC00564	rs6563353	13	83556097	downstream,upstream	T/C	0.23/0.04	13.71 (3.55, 52.89)	1.44×10^{-4}
HS3ST2,USP31	rs72772480	16	23050620	downstream	A/C	0.14/0.013	20.03 (4.27, 94.09)	1.46×10^{-4}
SNX29	rs3902080	16	12500918	intron	A/C	0.64/0.26	6.36 (2.37, 17.10)	2.42×10^{-4}
HIF1AN	rs10883511	10	100539650	intron	G/A	0.45/0.16	6.47 (2.34, 17.91)	3.26×10^{-4}
	rs11088226	21	32553221	downstream,upstream	G/C	0.77/0.34	7.21 (2.45, 21.19)	3.29×10^{-4}
TRAK1	rs147373791	3	42211402	intron,UTR-3	T/C	0.14/0.01	15.17 (3.35, 68.58)	4.13×10^{-4}
IRF7	rs1061505	11	613297	synon,UTR-3,intron,exon	G/T	0.14/0.02	13.15 (3.14, 55.05)	4.20×10^{-4}

Chr, chromosome; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism;

4) Alzheimer's disease vs. healthy controls

Fourth, we compared genetic variants associated with AD compared with healthy controls, considering the overlapping pathology of AD, PDD, and DLB. SNPs in *APOE* (rs769449, and rs75627662), *PVRL2* (rs12972156, rs519113, rs3852860, and rs6859), and *TOMM40* (rs59007384, rs405697) were significantly associated with AD after Bonferroni correction (Figure 6 and Table 15)

5) Parkinson's disease dementia vs. Alzheimer's disease

When we compared patients with PDD, and AD, the results were similar to the genetic variants associated with AD (Figure 7 and Table 16).

Rare variants associated with Parkinson's disease dementia and Alzheimer's disease

For rare variant analysis, we used a minor allele threshold of ≤ 1% and a minor allele count ≥ 3. Among 57 rare variants in patients with PDD, PD-ND, and healthy controls, we found no significant variants associated with PD (combined PDD and PD-ND) compared with healthy controls (P>0.05, Figure 8A).

Among 140 rare variants in patients with PDD, and PD-ND, we found that variants in *AK5, C2orf80, PIK3CG*, and *ABCA2* genes were associated with PDD compared with PD-ND ($P < 0.05$) (Figure 8B).

Among 125 rare variants in patients with AD and healthy controls, rare variants in *EVI5*, and *INPP4B* genes were associated with AD compared with healthy controls (Figure 9)

Figure 6. Genetic variants associated with Alzheimer's disease compared with healthy controls

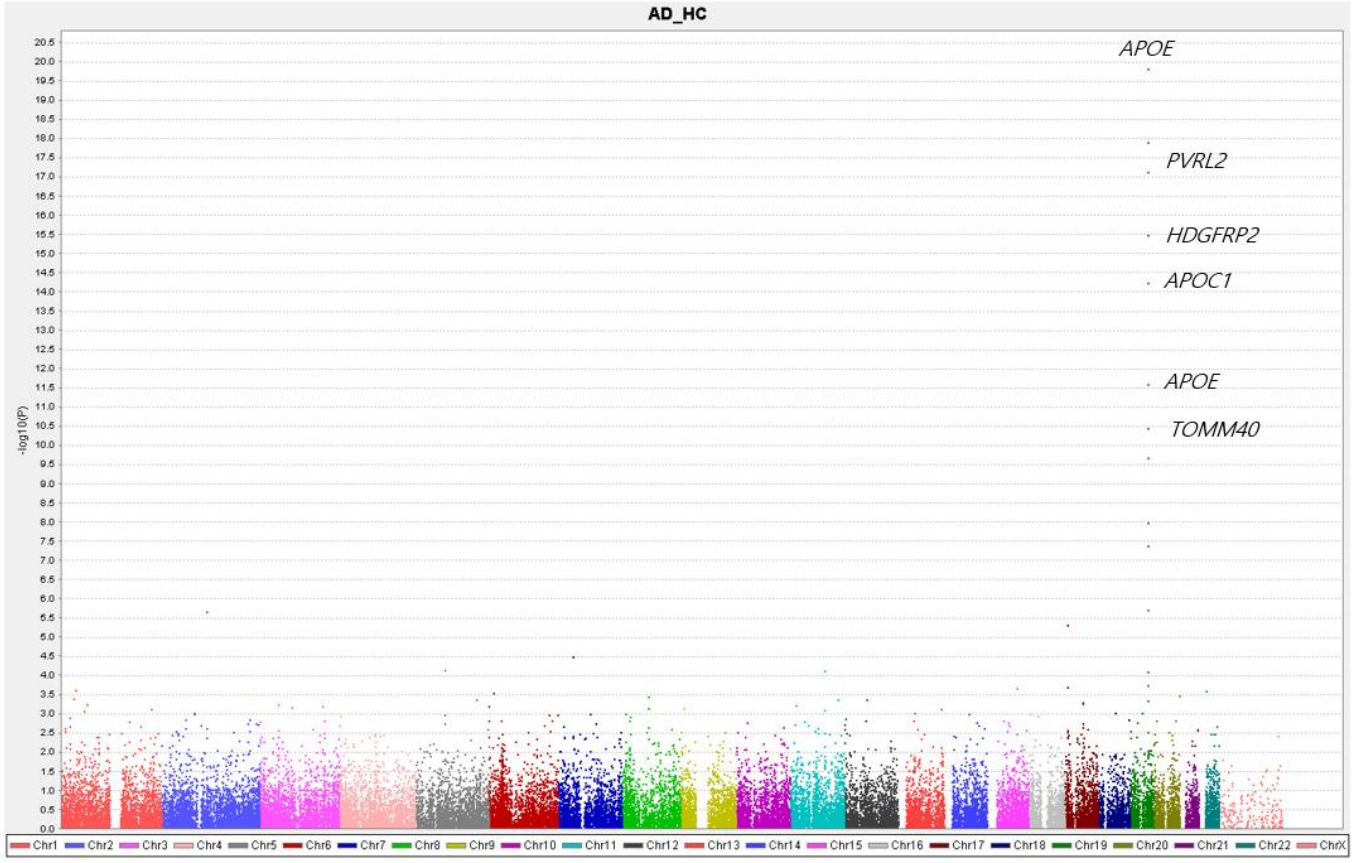


Table 15. Top 10 genetic variants that were associated with Alzheimer's disease compared with healthy controls in the order of statistical significance

Gene	Position	CHR	SNP	Allele (minor/major)	Minor allele frequency (case/control)	OR (95% CI)	*P* value
APOE	rs769449	19	44906745	A/G	0.27/0.08	4.37 (3.20, 5.97)	1.54×10^{-20}
PVRL2	rs12972156	19	44884202	G/C	0.26/0.08	4.11 (3.00, 5.63)	1.31×10^{-18}
PVRL2	rs519113	19	44873027	G/C	0.34/0.13	3.26 (2.49, 4.27)	7.74×10^{-18}
HDGFRP2	rs283815	19	44887076	G/A	0.37/0.17	2.89 (2.24, 3.72)	3.37×10^{-16}
APOC1,APOC4,APOC1P1	rs60049679	19	44926451	C/G	0.36/0.16	2.76 (2.14, 3.56)	5.84×10^{-15}
APOE	rs75627662	19	44910319	T/C	0.31/0.15	2.52 (1.94, 3.26)	2.53×10^{-12}
TOMM40	rs59007384	19	44893408	T/G	0.31/0.16	2.39 (1.85, 3.10)	3.65×10^{-11}
PVRL2	rs3852860	19	44879709	C/T	0.37/0.22	2.19 (1.72, 2.78)	2.17×10^{-10}
PVRL2	rs6859	19	44878777	A/G	0.44/0.30	1.96 (1.56, 2.47)	1.09×10^{-8}
TOMM40	rs405697	19	44901434	G/A	0.53/0.38	1.85 (1.48, 2.30)	4.20×10^{-8}

Chr, chromosome; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism;

Figure 7. Genetic variants associated with Parkinson's disease dementia and Alzheimer's disease

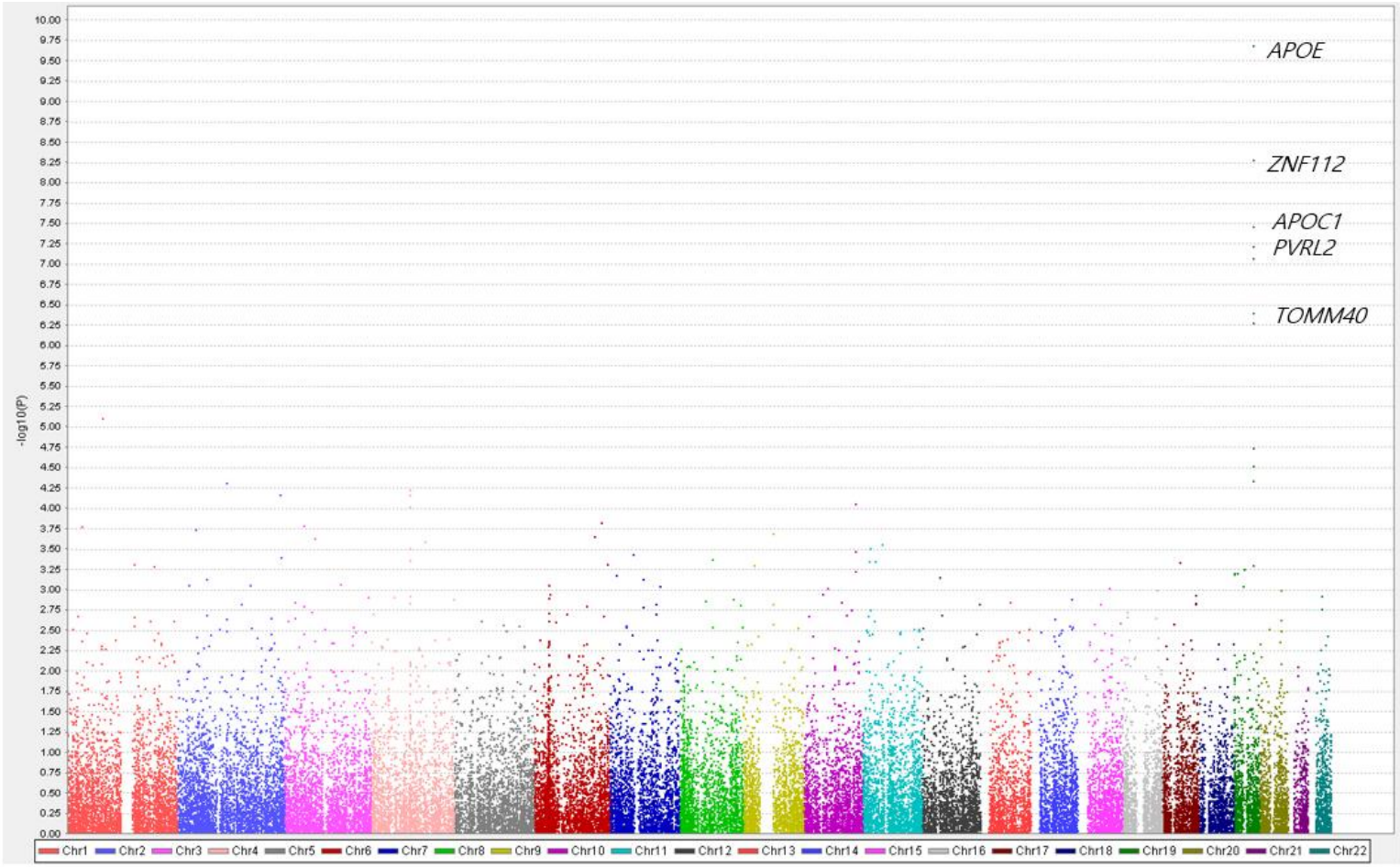


Table 16. Top 10 genetic variants that were associated with Parkinson's disease dementia compared with Alzheimer's disease in the order of statistical significance

Gene	SNP	Chr	Position	Allele (minor/major)	Minor allele frequency (case/control)	OR (95% CI)	*P* value
APOE	rs769449	19	44906745	A/G	0.10/0.27	0.32 (0.23, 0.46)	2.07×10^{-10}
ZNF112	rs283815	19	44887076	G/A	0.20/0.37	0.42 (0.31, 0.56)	5.29×10^{-9}
APOC1,APOC4,APOC1P1	rs60049679	19	44926451	C/G	0.19/0.36	0.44 (0.33, 0.59)	3.42×10^{-8}
PVRL2	rs519113	19	44873027	G/C	0.18/0.34	0.44 (0.33, 0.59)	6.11×10^{-8}
PVRL2	rs12972156	19	44884202	G/C	0.12/0.26	0.40 (0.29, 0.56)	8.57×10^{-8}
APOE	rs75627662	19	44910319	T/C	0.17/0.31	0.45 (0.33, 0.61)	4.04×10^{-7}
TOMM40	rs59007384	19	44893408	T/G	0.17/0.31	0.45 (0.33, 0.62)	5.23×10^{-7}
AK5	rs1166698	1	77926761	A/G	0.50/0.37	1.87 (1.42, 2.46)	7.96×10^{-6}
PVRL2	rs6859	19	44878777	A/G	0.32/0.44	0.56 (0.43, 0.73)	1.84×10^{-5}
TOMM40	rs405697	19	44901434	G/A	0.40/0.53	0.58 (0.45, 0.75)	3.01×10^{-5}

Chr, chromosome; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism;

Figure 8. Rare variants associated with Parkinson's disease (A) rare variants associated with Parkinson's disease compared with healthy controls, (B) rare variants associated with Parkinson's disease dementia compared with PD without dementia. The red line indicated $P=10^{-3}$, and blue line indicates $P=0.05$.

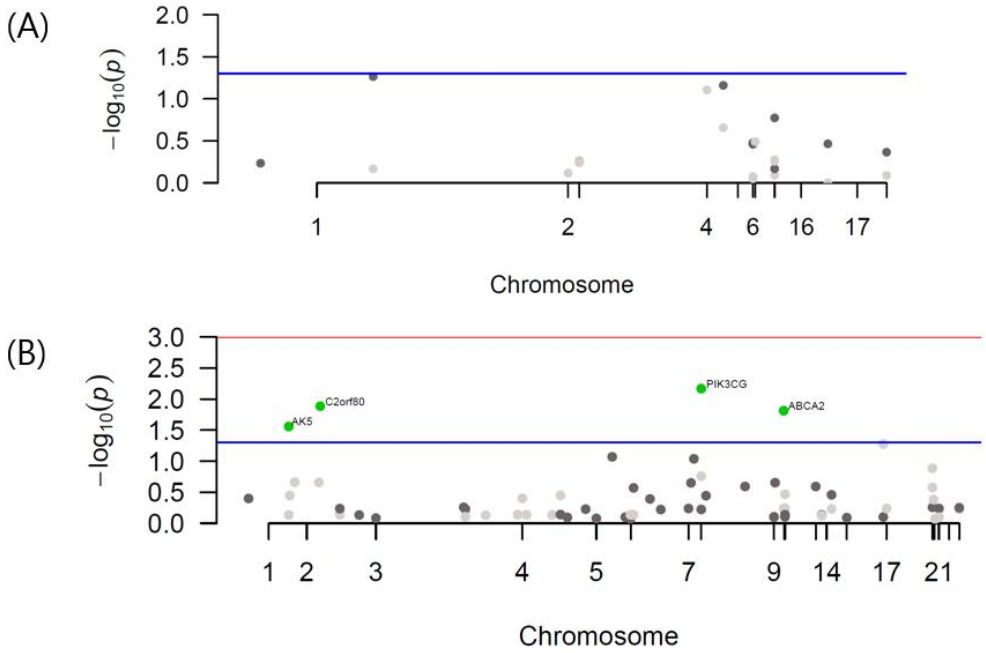
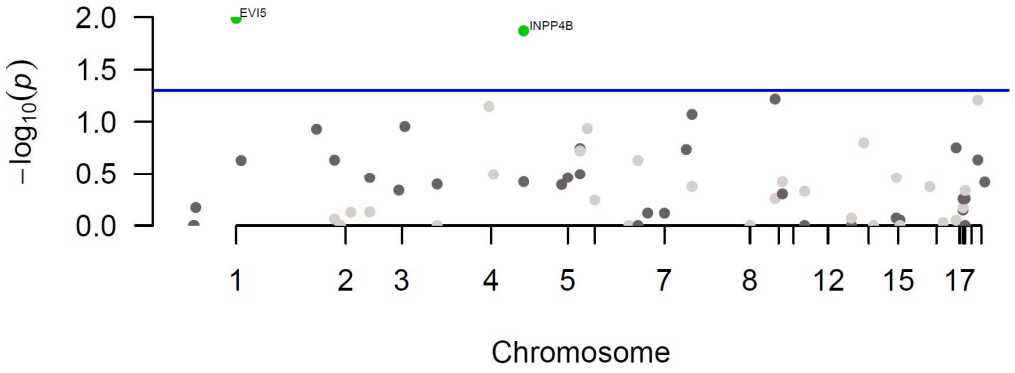


Figure 9. Rare variants associated with Alzheimer's disease compared with healthy controls. The blue line indicates *P*=0.05

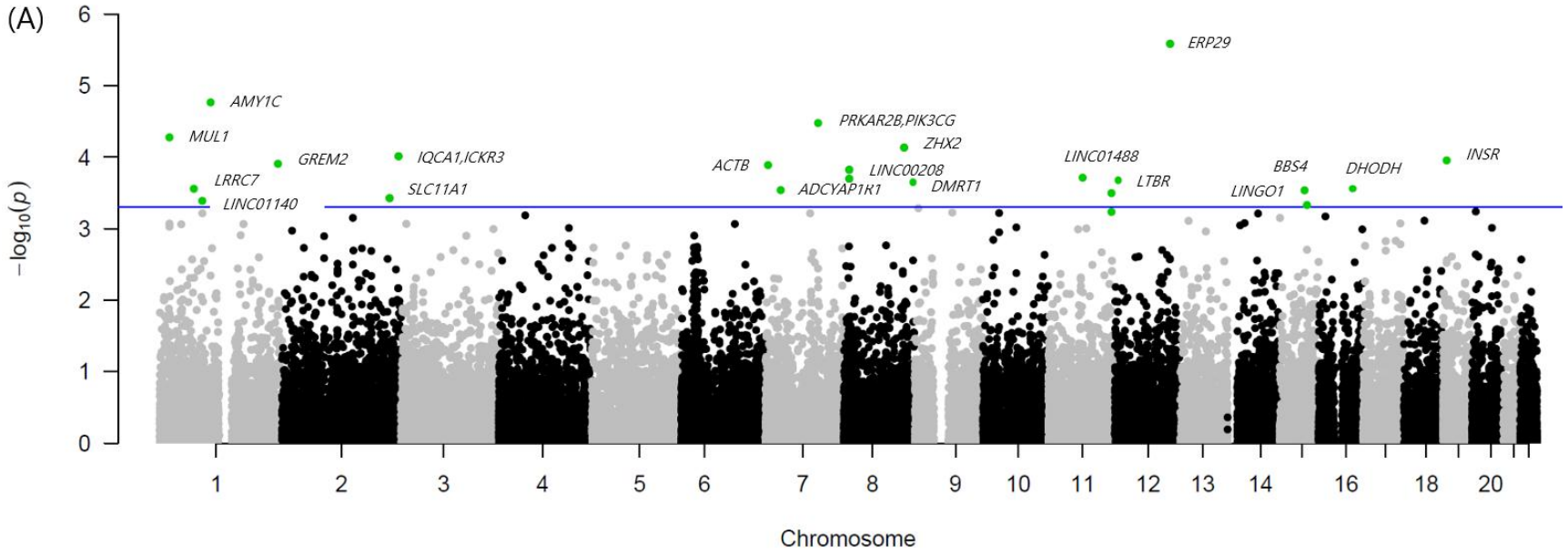


Variants associated with the early development of Parkinson's disease dementia

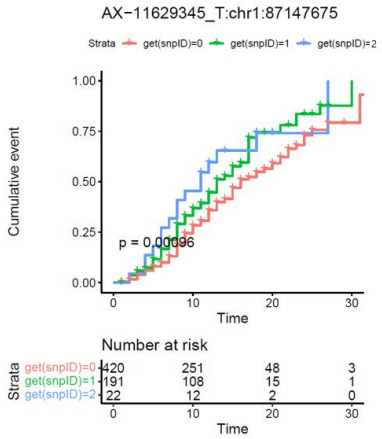
Log-rank test and the Cox proportional hazards statistics were used for dementia onset time analysis after the onset of motor symptom on each genotype detected in 313 patients with PDD and 321 patients with PD-ND (Figure 10). *MUL1* SNP rs3738128 was associated with the early development of PDD ($P = 5.30 \times 10^{-5}$). *LINC01140* SNP rs7553864 was associated with the early development of PDD ($P = 2.83 \times 10^{-6}$). SNPs in *ZHX2, ERP29*, and *SLC11A1* were also associated with the early development of PDD ($P < 0.05$).

We investigated genotype associated with age at dementia after birth in 313 patients with PDD and 321 patients with PD-ND (Figure 11). *MUL1* SNP rs3738128 was most significantly associated with young age at dementia in PD ($P = 1.63 \times 10^{-9}$). *PINK1* SNP rs8064 was associated with young age at dementia in PD ($P = 3.98 \times 10^{-4}$). SNPs in *TLR4,* and *ERP29* were also associated with early development of dementia in PD ($P < 0.05$).

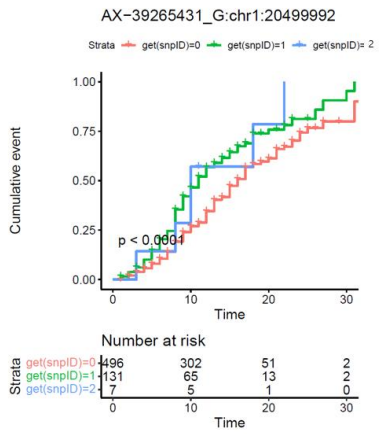
Figure 10. SNPs associated with development of dementia after the diagnosis of Parkinson's disease using Cox regression analysis. Blue line indicates *P*=0.05



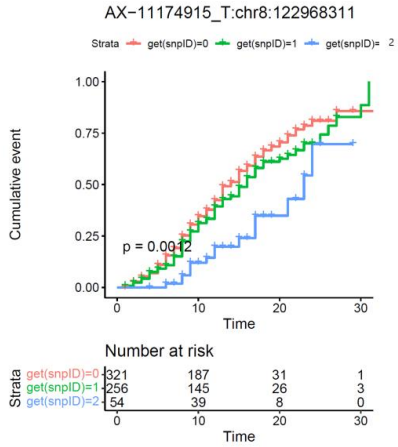
(B) *LINC01140*



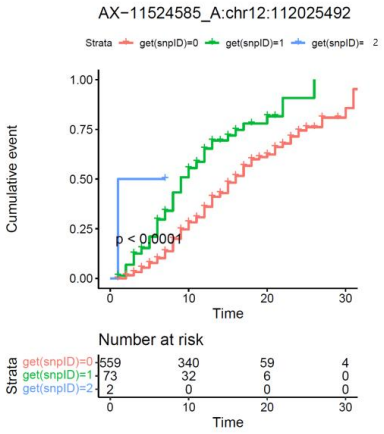
MUL1



ZHX2



ERP29



SLC11A1

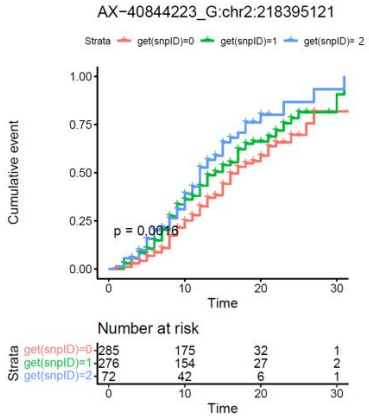
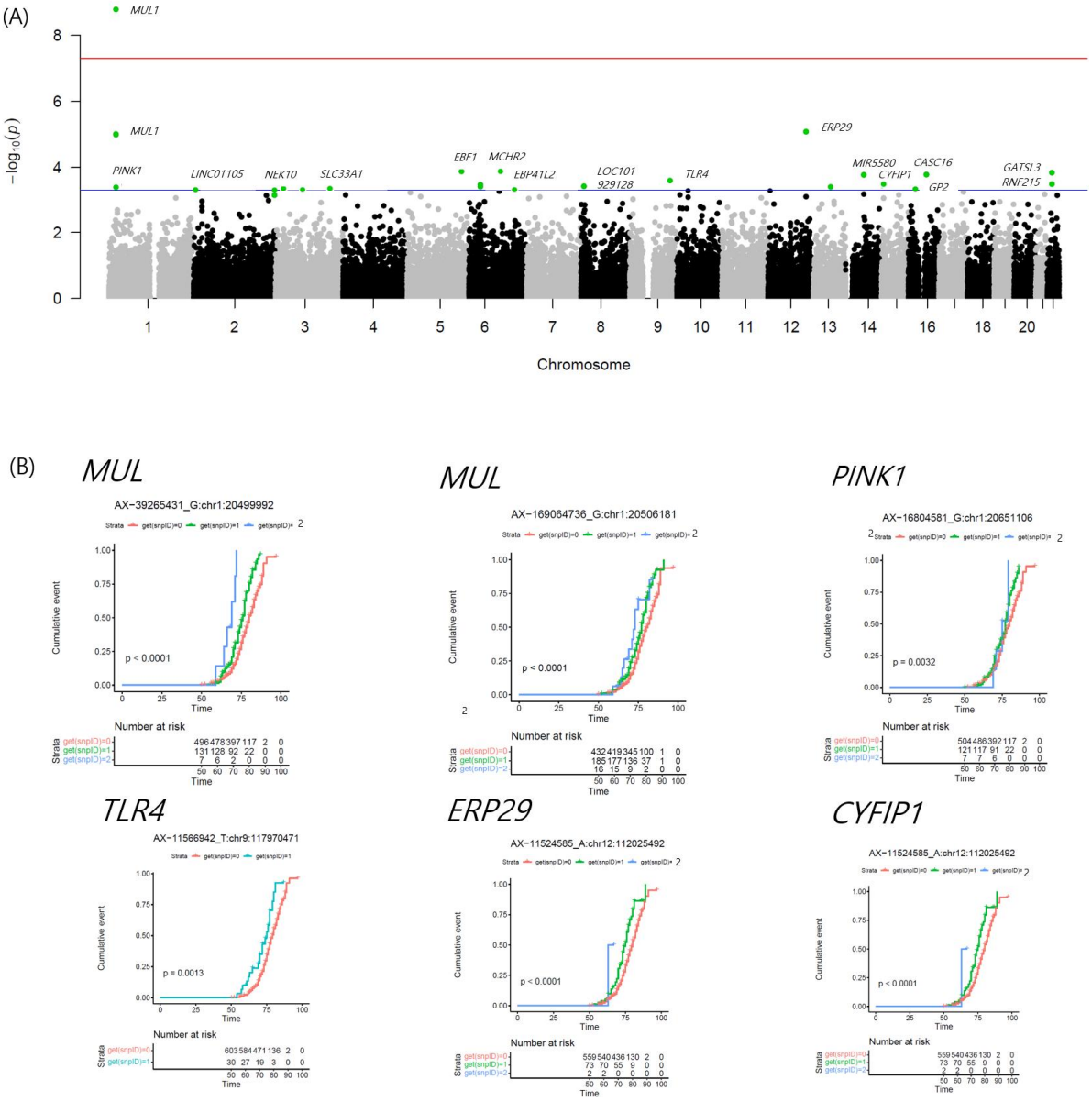


Figure 11. SNPs associated with the diagnosis of Parkinson's disease dementia at early age using Cox regression. Red line indicated P=10^{-3}, and blue line indicates P=0.05



Discussion

In this study, we identified genetic variants that were significantly associated with PDD, whose median disease duration was over 12 years. The *MUL1* SNP rs3738128 showed the most significant association with PDD, and it was associated with the early development of dementia in PD. *ZHX2* and *ERP29* also showed a correlation with PDD. On the other hand, SNPs associated with DLB were not statistically significant after Bonferroni correction. SNPs associated with PDD were distinct when compared with variants associated with DLB or AD.

SNPs associated with PDD: *MUL1*

MUL1 that was the most significantly associated with PDD suggests a potential biological plausibility of mitochondrial dysfunction in the development of PDD. One case-control study in China showed that *MUL1* SNP rs529974 was correlated with PD (58). *MUL1* encodes mitochondrial ubiquitin ligase 1 (MUL1), a mitochondrial E3 protein ligase, that regulates mitofusin. The mitochondria are involved in cellular energy production and cell survival, playing an important role in the neurodegenerative process in PD (59). Mitochondrial genes, such as *parkin, PINK1, DJ-1, LRRK2, ATP13A2*, and *VPS35*, are associated with PD (60). An experimental study showed that *MUL1* suppressed the mitochondrial phenotype in *PINK1/parkin* mutant dopaminergic neurons, and the knockdown of *MUL1* from *parkin* knockout mouse cortical neurons augmented mitochondrial damage (61). Therefore, mutants with *MUL1* and *parkin* mutations are used as PD animal models (62). Other experiments showed that *MUL1* overexpression reduced degeneration of dopaminergic neurons, and enhanced motor activity in neurons of flies fed with rotenone (63). *MUL1* dysfunction renders dopaminergic neurons susceptible to mitochondrial damage. The loss of *MUL1* function may be more prominent when other mitochondrial dysfunctions coexist, as a result of genetic variants or environmental toxins. The lack of correlation between *MUL1* and PD might be explained by the adjunctive role of *MUL1* in mitochondrial function.

Considering that MUL1 pathway regulates mitochondrial damage in both dopaminergic neuron and cortical neuron (61, 64), defects in MUL1 pathway might affect cognitive decline in PD. But little is known about the association between *MUL1* and the cognitive decline in PD or other neurodegenerative diseases that cause dementia. Mitochondrial dysfunction causes energy deficiency, intracellular calcium imbalance, and oxidative stress, leading to synaptic dysfunction, and neuronal cell loss (65). This mechanism explains how mitochondrial dysfunction causes cognitive decline in neurodegenerative diseases such as Alzheimer's disease. Mitochondrial dysfunction is prominent in PD patients (66), and when *MUL1* function is reduced, both dopaminergic and cortical neurons might become more susceptible to the damage from mitochondrial dysfunction, leading to progression of cortical neuronal loss, synaptic dysfunction and cognitive decline. In addition, recent studies revealed that amyloid-beta and p-tau interact with mitochondrial proteins, resulting in increased mitochondrial fragmentation and reduced mitochondrial fusion in Alzheimer's disease (67). Similarly, pathogenic alpha-synuclein and amyloid-beta, found in the brain of patients with PDD (68, 69), might interact with MUL1, leading to mitochondrial dysfunction. Further studies are needed to clarify the exact pathogenic mechanisms of MUL1 in developing PDD.

SNPs associated with PDD: *ZHX2* and *ERP29*

Other genetic variants associated with PDD were located in the loci of *ZHX2* and *ERP29*. Zinc-finger and homeodomain protein 2 (ZHX2) regulate transcription and neuronal differentiation (70). Genetic variants in *ZHX2* were found in two affected members of familial corticobasal degeneration, mutation of which were predicted to damage protein function (71). However, the role of *ZHX2* in PD or cognitive decline is largely unknown. Both corticobasal degeneration and PD are neurodegenerative diseases showing damage to cortical neurons and cognitive decline. Since *ZHX2* is also associated with cortical neurogenesis (70), it may be associated with the progression to dementia. Endoplasmic reticulum protein of 29kDa (ERp29) is ubiquitously expressed in cells and regulates protein transport between the endoplasmic reticulum and Golgi (72). ERp29 is involved in protein misfolding and

mistrafficking (72, 73), which is one of the potent pathogenesis of PD and Alzheimer's disease (74). Considering that endoplasmic reticulum stress is related to Lewy body dementia (75), it is possible that ERp29 mutation also causes cortical neuronal damage and is linked to dementia progression in PD patients.

SNPs associated with DLB: *ATP7B and HIF1AN*

ATP7B and HIF1AN genes were associated with DLB, although the statistical significance did not pass Bonferroni correction. This might be explained by the small sample size. ATP7B is a monomer and functions as a copper-transporting ATPase, which regulates copper amounts. Mutations in *ATP7B* gene cause Wilson's disease. In PD, copper is associated with increased oxidative stress, alpha-synuclein oligomerization, and Lewy body formation (76). However, *ATP7B* was not associated with PD or PDD in previous studies. *HIF1AN* gene encodes hypoxia-inducible factor 1 subunit alpha inhibitor, which interacts with HIF1A. HIF1A changes the expression of PD-related genes, including *ATP13A2, DJ-1*, and *PINK1*. HIF1A variants rs11549465 were associated with increased risk for PD (77). Further studies with a larger study population are required to validate the importance of *ATP7B* and *HIF1AN* in DLB. Although the number of DLB patients was limited, this study suggests that PDD and DLB may have different genetic risk factors.

Previous studies dealing with genetic risk factors for LBD

A recent study dealing with a large population of LBD revealed that *APOE, SNCA*, and *GBA* were significantly associated with LBD.(39) *GBA* mutations were associated with pathologically confirmed LBD with or without AD pathology. (78) *GBA* polymorphism was associated with DLB in a GWAS,(38) and *GBA* mutations were associated with the early development of dementia in PD patients. In this study, *GBA* polymorphism (rs1509245) was not associated with PDD, when we compared PDD and PD without dementia (uncorrected P=0.058). This can be explained by several reasons; 1) the mean age at onset for *GBA* carriers were early 50s in previous studies, (79, 80) but the mean age at onset of this

study was 63.5. Therefore, the frequency of *GBA* carriers might have been low in this study. 2) Most of the previous studies were conducted in populations of European ancestry. (39) Asian GWAS for PD patients revealed that the genetic risk factors might be different between races.

APOE is a well-known genetic risk factor for AD, as we found in this study. Preclinical studies showed that *APOE* ε4 regulates α-synuclein pathology and related toxicity, which is a pathogenic hallmark of PD.(81, 82) However, the clinical studies for *APOE* and PD showed inconsistent results. Some studies showed that *APOE* ε4 increased the risk for dementia in PD,(83-85) but other studies found no association. (86, 87) We also found that *APOE* genotype was associated with fast cognitive decline in the previous study. (88) In this study, *APOE* was associated with PD, when we compared PDD and PD without dementia (uncorrected P=0.03). However, the significance did not pass Bonferroni correction, and *APOE* was associated with AD than PD when we compared AD and PD.

SNPs associated with PD: *SNCA*

In our study, the *SNCA* SNP rs11931074 was the most significantly associated with the susceptibility to PD, which is consistent with previous results (29, 31-34, 89). Mutations in *SNCA* gene were first found in familial PD with autosomal dominant inheritance (90, 91), and several SNPs across the *SNCA* locus were also linked to the increased risk for sporadic PD in GWAS studies (29-34). The *SNCA* gene encodes alpha-synuclein, which is a main component of Lewy bodies, the pathologic hallmark of PD. Interestingly, *SNCA* SNP rs11931074 that had the most significant association with PD in this study had showed distinct relationship with PD according to races (89): *SNCA* SNP rs11931074 increased risk of PD in allele model, homozygote model, and recessive model for Asian population, while the association was true only in allele model for Caucasian population. This result supports the quality of PD sample in this study, and might emphasize the role of *SNCA* SNP rs11931074 in Asian population.

SNPs associated with PD: *SPHK1* and *FYN*

We found that *SPHK1* and *FYN* SNPs were associated with PD. *SPHK1* gene encodes sphingosine kinase 1 protein, which phosphorylates sphingosine into sphingosine-1-phosphatate (S1P). S1P synthesized by SPHK1 exert mitogenic and anti-apoptotic effects in an autocrine or paracrine manner (92). Sphingosine kinase 1 was downregulated in experimental models of PD, and inhibition of sphingosine kinase 1 decreased cell viability and enhanced the reactive oxygen species (93). *FYN* gene encodes Fyc protein, which is a tyrosine phosphor-transferase of the Src family nonreceptor kinase. Fyc has been suggested to regulate alpha-synuclein phosphorylation, oxidative stress-induced dopaminergic neuronal death, and enhanced neuroinflammation (94). Therefore, both sphingosine kinase 1 protein and Fyc were suggested as potential therapeutic targets for PD (92, 94), and our data supports the protective effects of *SPHK1* and *FYN* genes in PD.

SNPs associated with AD: *APOE, PVRL2, and TOMM40*

The *APOE, PVRL2*, and *TOMM40* SNPs were well-known genetic risk factor for AD (13, 95, 96). *APOE* is the major genetic risk factors for AD, and some studies suggested its association with PDD. Preclinical data suggest that the *APOE* ε4 regulates α-synuclein pathology and related toxicity, which is a pathogenic hallmark of PD, and some clinical studies identified the *APOE* ε4 as a risk factor for PDD (81, 88). However, we found no significant correlation between *APOE* and PDD or early development of dementia. *APOE* might contribute to the development of dementia through concomitant amyloid-beta pathology in PD, which might explain the development of dementia late stage of PD. The development of dementia at the early stage of PD might be associated with mechanisms other than amyloid pathology, such as synuclein deposition in the cerebral cortex. *PVRL2*, and *TOMM40* are located near *APOE* gene, and showed moderate to strong linkage disequilibrium.(97) *PVRL2* encodes a membrane glycoprotein which could work as an entrance for certain viral strains and is involved in the cell-to-cell transmission of viruses.(98) *TOMM40* encodes a subunit of the mitochondrial outer membrane protein translocator.(97)

By comparing patients with PDD and patients with AD, we tried to identify characteristic genes. The most significant SNPs according to *P* value were AD-related genes. Also, we found that PDD and AD have different genetic risk factors even if they have the same dementia symptoms.

Rare variants associated with PDD: *AK5* and *PIK3CG*

GWAS investigate several hundred thousand to million SNPs in a large population, to investigate common complex diseases. GWAS generally targets common variants with a mutation frequency more than 5% within the population, considering 'common disease, common variant' hypothesis. Most common variants have small effect sizes. However, the genome-wide association findings could not fully explain the heritability. This is called missing heritability. (99) One of the explanations for the missing heritability includes rare variants, which have low frequency (less than 5% within the population) but have large effect sizes. We used SKAT-O to investigate rare genetic variants. (100)

Rare variants in *AK5,* and *PIK3CG* genes were also associated with PDD. *AK5* encodes adenylate kinase 5, which is a nucleoside monophosphate, and is expressed exclusively in the brain. Antibodies to AK5 contribute to the development of limbic encephalitis and the development of dementia. (101) *PIK3CG* encodes phosphoinositide 3-kinase γ, which functions in the migration of inflammatory cells, synaptic dysfunction, and cognitive deficit in AD mice model. (102) We can assume that the inflammatory process contributes to the development of PD in a subgroup of PD patients.

SNPs associated with the early development of PDD

In a genome-wide survival study conducted on 3,821 European PD patients, *RIMS2, TMEM108*, and *WWOX* were associated with cognitive progression. (42) In this study using a similar method, we found that MUL1 genotype was associated with the early development of dementia from the onset of PD diagnosis and development of dementia at a young age. The reason for showing different results can be attributed to racial differences.

The strength of this study includes the clinical diagnosis of dementia based on the long-term follow-up of patients with PD. The prevalence of dementia in patients with PD is 17% at 5 years after diagnosis, 46% at 10 years after diagnosis (9). Therefore, including PD patients with a short follow-up duration would misclassify them into PD without dementia. A previous GWAS study investigating the cognitive decline in PD included PD patients whose median follow-up duration was 4 years (43), and other GWAS study assessed cognition using cross-sectional MMSE score or MoCA scores (36).

This study has limitations. First, the sample size was relatively small, which may explain why genetic variants associated with PDD did not remain statistically significant after stringent Bonferroni correction. Also, the number of patients with DLB was too small to find the correlation. Second, the biological functions of the genetic variants were not validated. However, the experimental studies on *SPHK1*, *FYN*, *MUL1*, *ZHX2*, and *ERP29* genes, as discussed above, might support the biological plausibility of these genes. Therefore, future functional studies are required to confirm our results. Third, the diagnostic criteria for PDD, DLB, AD, and DLB were based on clinical criteria, which might be different from pathologic diagnosis. Especially for AD, the information on amyloid or tau deposition was limited. However, the accuracy rate of probable AD using NINCDS-ADRDA ranges between 65% and 92%,(103) and our GWAS results is consistent with previous studies.

In conclusion, we identified distinct genetic variants associated with PDD using a customized microarray chip. Variants in *MUL1* gene, which regulate mitochondrial function, showed the most significant association with PDD. Variants in genes for protein trafficking or inflammation, such as *ERP29, AK5, PIK3CG,* and *TLR4* were also associated with early development of PDD. *APOE* was strongly associated with AD, but not with PD or PDD. *ATP7B* and *HIF1AN* were associated with DLB, although limited by the small number of patients.

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국문 요약

제목: Lewy body dementia의 유전적 위험인자 발굴

연구 배경 및 목적: Lewy body dementia는 파킨슨병 치매 (Parkinson's disease dementia)와 루이소체 치매(dementia with Lewy bodies)를 포함하는 포괄적인 용어이다. 파킨슨병 치매와 루이소체 치매는 모두 치매와 파킨슨증의 임상증상을 공통적으로 보이며, 공통된 신경 병리소견을 보인다. 파킨슨병 치매와 루이소체 치매에서 인지저하는 다양한 예후를 보이며, 유전적 위험인자가 이러한 다양성을 설명할 수 있을 것이다. 파킨슨병에서 전장 유전체 연관분석 연구를 통해서 중요한 위험인자들이 밝혀졌지만, 파킨슨병 치매와 루이소체 치매에서 인지 저하와 연관된 유전자를 찾는 연구는 적었다. 본 연구에서는 파킨슨병 치매와 루이소체 치매에서 치매 발병과 관련된 유전적 돌연변이를 조사하였다. Lewy body dementia는 알츠하이머병 및 파킨슨병의 임상, 유전적 위험인자를 공유하기 때문에 건강인과 알츠하이머병 환자를 대조군으로 사용하였다. 이번 연구를 위해 이전의 유전 연구에서 확인된 유전체 변이체를 활용한 맞춤형 플랫폼을 기반으로 개발된 마이크로어레이 칩을 사용하였다.

연구 방법: 이번 연구에서는 치매가 있는 파킨슨병 환자 313명, 치매가 없는 파킨슨병

환자 321명, 알츠하이머병 환자 232명, 루이소체 치매 환자 11명, 건강한 대조군 635명을 전향적으로 모집하였다. 유전정보의 일차 분석은 연령과 성별을 보정한 다중 로지스틱 회귀 모델을 사용하여 전장유전체연관분석을 수행하였다. 또한 희귀 변이 분석을 수행하였고, 콕스 회귀 분석을 사용하여 치매 발병 연령과 관련된 유전자형을 분석하였다.

결과: *SNCA* 유전자의 단일 염기 다형성 rs11931074는 파킨슨병과 가장 유의하게 관련이 있었다. (교차비 = 0.66, 95% 신뢰 구간 = 0.56 – 0.78, $P = 7.75 \times 10^{-7}$). 파킨슨병 환자만을 대상으로 한 분석에서 *MUL1* 유전자의 단일 염기 다형성 rs3738128 가 파킨슨병 치매와 가장 유의하게 관련이 있었다 (교차비 = 2.52, 95% 신뢰 구간 = 1.68 – 3.79, $P = 8.75 \times 10^{-6}$). *ZHX2* 및 *ERP29* 유전자의 단일 염기 다형성도 파킨슨병 치매와 관련이 있었다. 루이소체 치매 환자에서는 *ATP7B* 유전자의 단일 염기 다형성 rs148399850가 유의하게 관련이 있었는데, 본페로니 교정 후 통계적 유의성은 사라졌다. (교차비 = 18.73, 95% 신뢰구간 = 4.64 – 75.70, $P = 3.92 \times 10^{-5}$) 파킨슨병 치매와 구분되는 알츠하이머병 관련 단일 염기 다형성은 *APOE* 유전자 (rs769449 및 rs75627662), *PVRL2* 유전자 (rs12972156, rs519113, rs3852860, 및 rs6859) 및 *TOMM40* 유전자 (rs59007384, rs405697)에서 관찰되었다. 희귀 변이 분석에서 *AK5* 및 *PIK3CG* 유전자의 희귀 변이체는 파킨슨병 치매와 관련이 있었다. 콕스 회귀 분석에서 *MUL1* SNP rs3738128의 유전자형

은 파킨슨병 환자에서 치매의 빠른 발병과 가장 유의하게 관련이 있었다 ($P = 1.63 \times 10^{-9}$).

고찰: 맞춤형으로 제작된 마이크로어레이칩 기반 유전형 분석을 통해서 파킨슨병 치매와 관련된 *MUL1*의 새로운 유전자좌를 확인했으며, 이는 파킨슨병 환자의 치매 발병에서 미토콘드리아 기능 장애의 중요한 역할을 시사한다.

중심단어: 전장유전체 연관분석, 파킨슨병, 치매, 루이소체 치매