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RESEARCH ARTICLE

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Network-based meta-analysis and the candidate gene association studies reveal novel ethnicity-specific variants in *MFSD3* and *MRPL43* associated with dementia with Lewy bodies

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Abstract

Dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia in elderly people, following Alzheimer's disease. Only three genes, *SNCA* (α-synuclein), *APOE* (apolipoprotein E), and *GBA* (glucosylceramidase), have been convincingly demonstrated to be associated with DLB. Here, we applied whole-genome sequencing to blood samples from 61 DLB patients and 45 cognitively normal controls. We used accumulation of candidate mutations to detect novel DLB-associated genes. Subsequent single nucleotide polymorphism (SNP) genotyping and association studies in a large number of samples from Japanese individuals revealed novel heterozygous variants in *MFSD3* (rs143475431, c.888T>A: p.C296*; $n = 5{,}421$, $p = 0.00063$) and *MRPL43* (chr10:102746730, c.241A>C: p.N81H; $n = 4{,}782$, $p = 0.0029$). We further found that the *MFSD3* variant increased plasma levels of butyrylcholinesterase ($n = 1{,}206$, $p = 0.029$). We believe that our findings will contribute to the understanding of DLB and provide insight into its pathogenic mechanism for future studies.

Abbreviations: AD, Alzheimer's disease; Ach, Acetylcholine; AchE, Acetylcholinesterase; BC, Betweenness centrality; BuChE, Butyrylcholinesterase; CADD, Combined Annotation Dependent Depletion; DLB, Dementia with Lewy bodies; MAF, Minor allele frequency; MIBG, Metaiodobenzylguanidine; NCGG, National Center for Geriatrics and Gerontology; PD, Parkinson's disease; PPI, Protein–protein interaction; SNP, Single nucleotide polymorphism; SNV, Single-nucleotide variant; SPECT, Single-photon-emission computed tomography; WES, Whole-exome sequencing; WGS, Whole-genome sequencing.

Daichi Shigemizu and Kouichi Ozaki contributed equally to this study.

KEYWORDS
candidate gene association studies, dementia with Lewy bodies, network-based meta-analysis, whole-genome sequencing

1 | INTRODUCTION

Dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia in elderly people, following Alzheimer's disease (AD) (Mueller, Ballard, Corbett, & Aarsland, 2017). DLB is clinically characterized by cognitive impairment with parkinsonism, recurrent visual hallucinations, cognitive fluctuations, and rapid-eye-movement sleep behavior disorder (McKeith et al., 2017), as well as by indicative biomarkers, such as reduced dopamine transporter uptake in the basal ganglia on nuclear medicine imaging (Shimizu et al., 2018). Previous studies have reported that patients with DLB have a higher mortality rate (Mueller et al., 2019; Price et al., 2017) and poorer quality of life (Bostrom, Jonsson, Minthon, & Londos, 2007) than those with AD. DLB accounts for about 4.6% of all prevalent dementia cases (Kane et al., 2018), and there are no curative treatments for patients who already have DLB.

The majority of DLB cases are sporadic, but there is a strong genetic component. The proportion of variance in the phenotype explained by common genetic variants (SNP-based heritability) has been estimated to be 36% (Guerreiro et al., 2018). However, to date, only three genes, *SNCA* (α-synuclein), *APOE* (apolipoprotein E), and *GBA* (glucosylceramidase), have been convincingly demonstrated to be associated with DLB (Bras et al., 2014; Guerreiro et al., 2018). Further understanding of the genetics underlying DLB has remained largely elusive.

The development of massively parallel DNA-sequencing technologies (Shendure & Ji, 2008) enables us to detect potential rare pathogenic variants for a variety of diseases. Whole-exome sequencing (WES) and whole-genome sequencing (WGS) have been used to successfully identify causal mutations of Mendelian diseases (Choi et al., 2009; Ng et al., 2010) and driver mutations in tumors (Agrawal et al., 2011; Varela et al., 2011; Wei et al., 2011). However, genetic analyses in DLB cases have examined only putative pathogenic mutations in genes already associated with the risk of neurodegenerative diseases (Geiger et al., 2016; Keogh et al., 2016), and pathogenic variants in DLB cases have not been comprehensively analyzed.

Although the cost of WGS has been decreasing more rapidly than that of WES, it is still less cost-effective than WES. However, WGS is able to detect more coding variants than WES owing to the limitations of WES capture methods and the advantages of the broader coverage by WGS (Belkadi et al., 2015). Here, we applied WGS to samples from patients with probable DLB as determined by clinical symptoms and imaging (dopamine transporter uptake on single-photon-emission computed tomography [SPECT] imaging or abnormal metaiodobenzylguanidine [MIBG] myocardial scintigraphy) and from clinically characterized, cognitively normal controls more than 60 years old (CN), and comprehensively examined pathogenic variants associated with DLB. In addition to identifying a missense mutation in *GBA* previously associated with DLB, we discovered novel ethnicity-specific heterozygous variants in the *MFSD3* and *MRPL43* genes through further SNP genotyping and association studies in a large number of samples from Japanese patients. Furthermore, we found that the *MFSD3* variants increases plasma levels of butyrylcholinesterase (BuChE). Given that even the largest DLB cohorts have generally been small (Geiger et al., 2016; Keogh et al., 2016), this study represents the largest genetic study of DLB in a Japanese cohort to date.

2 | METHODS

2.1 | Clinical samples

All 8,501 blood samples and the associated clinical data were obtained from the National Center for Geriatrics and Gerontology (NCGG) Biobank. Of these, 79 were from patients with DLB, 1,967 from patients with AD, 1,099 from patients with mild cognitive impairment (MCI), and 5,356 from CN subjects. All of the subjects were older than 60 years of age. The diagnosis of all subjects was conducted based on medical history, physical examination and diagnostic tests, neurological examination, neuropsychological tests, and brain imaging with magnetic resonance imaging or computerized tomography by experts including neurologists, psychiatrists, geriatricians, or neurosurgeons, all of whom are experts in dementia and familiar with its diagnostic criteria. DLB was diagnosed based on the criteria of the fourth report of the DLB Consortium (McKeith et al., 2017). Reduced dopamine transporter uptake on SPECT imaging or abnormal MIBG myocardial scintigraphy was confirmed in all subjects with DLB included in this study. Of the 79 DLB samples, 28 were confirmed by the SPECT imaging and the remaining 51 were confirmed by MIBG myocardial scintigraphy. The AD and MCI subjects were diagnosed using the criteria of the National Institute on Aging Alzheimer's Association workgroups (Albert et al., 2011; McKhann et al., 2011). Only patients with probable AD were included as AD subjects in this study. The CN subjects had subjective cognitive complaints but normal cognition on a neuropsychological assessment with a comprehensive neuropsychological test and a Mini-Mental State Examination score of more than 25. Of the 8,501 samples, 61 from DLB subjects and 45 from CN subjects were used for WGS analyses, and the remaining 8,395 samples were used as an independent replication cohort.

This study was approved by the ethics committee of the NCGG. The design and performance of the current study involving human subjects were clearly described in a research protocol. Participation was voluntary, and all participants provided informed consent in writing before being registered in the NCGG Biobank. Written informed

consent was obtained from all participants. All methods were performed in accordance with relevant guidelines and regulations. Consent for publication was obtained from all participants.

2.2 | WGS data analysis

DNA concentration was measured by using Picogreen, and fragmentation of DNA was assessed by agarose gel electrophoresis. Good-quality DNA was used for construction of the DNA library. The library was constructed using the TruSeq DNA PCR-Free Library Preparation Kit in accordance with the manufacturer's instructions. WGS conducted at Macrogen Japan Corporation, Takara Bio Inc, and GENEWIZ Inc. DNA was sequenced using the Illumina HiSeq X Ten and NovaSeq 6000 platforms with paired-end reads of 151 bp in accordance with the manufacturer's instructions.

2.3 | Variant calling of WGS data

All WGS data were downloaded from the NCGG Biobank database. Read sequences were mapped to the human reference genome (GRCh37) with Burrows-Wheeler Alignment-MEM (version 0.7.15) (Li & Durbin, 2009). Duplicate polymerase chain reaction (PCR) reads were identified and removed using Picard (version 2.21.4) (Li et al., 2009). Variant calling was conducted using Genome Analysis Toolkit (GATK, version 4.1.0.0) (DePristo et al., 2011). Individual variant calling was performed using GATK HaplotypeCaller. The multi-sample individual variants were jointly called with in-house data (WGS = 729) using GenotypeGVCFs. Variant quality score recalibration was applied according to the GATK Best Practice recommendations (Van der Auwera et al., 2013). We filtered out single nucleotide variants (SNVs) with Depth (DP) < 10, GenotypeQuality (GQ) < 20, Quality by Depth (QD) < 2, Quality score (QUAL) < 30, StrandOddsRatio (SOR) > 4, FisherStrand (FS) > 60, RMSMappingQuality (MQ) < 40, MappingQualityRankSumTest (MQRankSum) < −12.5, and ReadPosRankSumTest (ReadPosRankSum) < −8 and Insertions and Deletions (InDels) with DP < 10, GQ < 20, QD < 2, QUAL < 30, SOR > 10, FS > 200, and ReadPosRankSum < −20.

2.4 | Annotation of variants called

Functional annotations of the variants called in the WGS data were performed by using ANNOVAR (version 2019 Oct, 24). Variant frequency data were found in public databases: the Exome Aggregation Consortium (ExAC) (Lek et al., 2016), the Genome Aggregation Database (gnomAD) (Karczewski et al., 2020), the Exome Sequencing Project Exome Variant Server at the National Heart, Lung, and Blood Institute (ESP6500) (Fu et al., 2013), the 1,000 Genomes Project Phase 3 (1KG) (Via, Gignoux, & Burchard, 2010), UK10K Project (Consortium et al., 2015), and the Tohoku Medical Megabank Organization (ToMMo 8.3KJPN) (Tadaka et al., 2018). We used variants with frameshift insertions/deletions, nonframeshift insertions/deletions, stop-gains/-losses, nonsynonymous SNVs, synonymous SNVs, and splicing variants. We further evaluated the call rate of each variant and removed variants with a low genotype call rate (<0.90).

2.5 | Accumulation of pathogenic variants in genes

Candidate pathogenic variants were selected from variants with an allele frequency of 0.001 or less in the public databases (ExAC [Lek et al., 2016], gnomAD [Karczewski et al., 2020], ESP6500 [Fu et al., 2013], 1KG [Via et al., 2010], UK10K Project [Consortium et al., 2015], and ToMMo 8.3KJPN [Tadaka et al., 2018]) and a Combined Annotation Dependent Depletion (CADD) Phred-scaled score (Kircher et al., 2014) 30 or more (represents top 0.1% of the predicted deleteriousness). The candidates were excluded if they appeared in the CN samples used in the WGS. The number of expected candidates in each gene was estimated from the total number of candidates divided by the total number of all variants. Treating the number of expected candidates as a Poisson process allowed us to calculate a p value for the number of candidates actually observed in a gene by using the R expression 1−*ppois*(observed, expected). Candidate pathogenic genes were those with a significance level set at a Bonferroni-adjusted p-value of less than 0.05.

2.6 | Network-based meta-analysis

Network-based analysis was performed using NetworkAnalyst (Santiago & Potashkin, 2015) with the DifferentNet database (Basha, Shpringer, Argov, & Yeger-Lotem, 2018) that provides comprehensive information about interactions between proteins in human tissues. The Protein-Protein Interaction (PPI) network was graphically generated using Cytoscape v3.8.0 (Bader & Hogue, 2003).

2.7 | Replication study

Candidate pathogenic variants were genotyped in a large number of samples from our Japanese replication cohort. SNP genotyping was performed with the multiplex PCR-based Invader assay (Third Wave Technologies, Madison, WI; Ohnishi et al., 2001). Association analysis in the replication cohort was conducted with a logistic regression model adjusted for sex and age using PLINK software (--logistic) (Purcell et al., 2007).

2.8 | Acetyl-CoA measurement and association studies

Acetyl-CoA was measured by using an acetyl-CoA ELISA kit in accordance with the manufacturer's instructions (Shanghai Shuangying Co., Ltd., China). The BuChE concentration was obtained from routine

TABLE 1 Characteristics of patients whose samples were used for whole-genome sequencing

	Number of samples	Age (mean ± 1 *SD*)	Male: female	*APOE* genotype			
				E4/E4	E4/E3	E3/E3	E3/E2
DLB	61	78.12 ± 6.51	1:1.18	5	18	36	2
CN	45	75.60 ± 3.59	1:0.88	1	8	28	8

Abbreviations: CN, cognitively normal controls; DLB, dementia with Lewy bodies.

blood tests. Association analyses between the *MFSD3* variant and plasma levels of acetyl-CoA and between the *MFSD3* variant and plasma levels of BuChE were both conducted using a linear regression model with adjustment for sex and age in the R package *lm*.

3 | RESULTS

3.1 | WGS of Japanese individuals

A total of 106 samples from Japanese individuals older than 60 years of age were obtained from the NCGG Biobank. The samples came from 61 individuals with probable DLB and 45 CN individuals. The characteristics of the sample donors, including average age, sex ratio, and *APOE* genotype distribution, are summarized in Table 1. We performed WGS analysis of the 106 samples using the Illumina HiSeq X Ten and NovaSeq 6000 platforms. On average, 361 million read pairs were obtained from the analysis. Of these, 99.0% were mapped to the human reference genome (GRCh37), and 8.8% were removed as duplicate PCR read pairs (Table S1). The variant calling was performed using the GATK (DePristo et al., 2011). We evaluated the call rate of each variant and removed variants with a genotype call rate of less than 0.90. A total of 12,299,863 variants were obtained, of which 93,648 were found in protein-coding regions: 763 frameshift deletions; 401 frameshift insertions; 743 nonframeshift deletions; 313 nonframeshift insertions; 684 splicing variants; 734 stop-gains; 51 stop-losses; 44,176 synonymous SNVs; and 45,783 nonsynonymous SNVs.

3.2 | Mutation search within previously reported DLB pathogenic genes

To identify mutations in genes previously associated with DLB (*SNCA*, *APOE*, and *GBA*), we examined pathogenic variants with a minor allele frequency (MAF) of 0.001 or less in public databases and a CADD Phred-scaled score (Kircher et al., 2014) of at least 30 (see Section 2). Nongenic, intronic, and synonymous variants other than those occurring at canonical splice sites and nonsynonymous variants were then excluded. From the remaining variants, a missense mutation of the *GBA* gene was identified in a DLB patient (rs374306700, NM_001171811:c.841C>T:p.R281C, CADD Phred-scaled score = 31) that was not observed in any of the CN samples. The MAF of the mutation was 2.5×10^{-5} in ExAC (Lek et al., 2016), 1.2×10^{-5} in gnomAD (Karczewski et al., 2020), and 1.5×10^{-5} in ESP6500 (Fu et al., 2013). This rare mutation had not been observed in data from 1KG (Via et al., 2010) or ToMMo 8.3KJPN (Tadaka et al., 2018).

3.3 | Novel DLB pathogenic genes

Genome-wide association studies with small sample size rarely detect variants with statistical significance because they lack study power after Bonferroni correction for multiple testing. We therefore examined the accumulation of candidate pathogenic variants in genes to detect novel disease-associated genes. These variants were selected from variants with an allele frequency of 0.001 or less in public databases and a CADD Phred-scaled score of at least 30. We found 16 genes showing statistically significant accumulation of the candidates in DLB samples (see Section 2, Bonferroni-adjusted $p < .05$, Table 2). The most statistically significant gene was *MFSD3*, where a pathogenic variant (heterozygous stop-gain variant) was observed in six DLB samples (rs143475431, NM_138431:c.888T>A:p.C296*, Figure 1a). All of the *MFSD3* variants in WGS were validated by using Sanger sequencing. Because *MFSD3* encodes a putative acetyl-CoA transporter (Perland et al., 2017), we predicted the location of transmembrane segments using MEMSAT-SVM (Nugent & Jones, 2009) and found that the pathogenic variant is located on the transmembrane segments (Figure 1b).

3.4 | Assessment of the *MFSD3* variant using a large number of samples

To assess the pathogenicity of the *MFSD3* variant, we genotyped the variant in our cohort of 7,380 samples from Japanese individuals (79 DLB, 1,959 AD, and 5,342 CN), including the samples subjected to WGS. The MAF of the variant was 0.044 in DLB cases, 0.013 in CN samples, and 0.015 in AD samples (Table 3). When assessed with a logistic regression model adjusting for age and sex, the variant showed statistically significant differences between DLB and CN samples ($n = 5{,}421$, $p = .00063$, odds ratio = 4.32, 95% CI = 1.87 to 10.01), and between DLB and AD samples ($n = 2{,}038$, $p = .0096$, odds ratio = 2.96, 95% CI = 1.30 to 6.73). These results demonstrate that this *MFSD3* variant could be a pathogenic variant in DLB.

We next examined the population differences of the variant using several public databases. In the Asian population, the MAF of the

TABLE 2 List of 16 candidate pathogenic genes

Gene symbol	Number of samples	Candidate pathogenic variants						Adjusted *p*
		Chr.	Position (hg19)	rs#	Transcript ID	cDNA level change	Protein level change	
MFSD3	6	8	145,736,038	rs143475431	NM_138431	c.888T>A	p.C296*	7.58×10^{-9}
MRPL43	3	10	102,746,730	–	NM_176792	c.241A>C	p.N81H	2.70×10^{-4}
RASSF1	3	3	50,378,008	rs778876938	NM_170714	c.229C>T	p.R77C	7.90×10^{-4}
GSC2	2	22	19,137,175	rs561346073	NM_005315	c.513+1G>A	–	1.37×10^{-3}
RPP25	2	15	75,248,657	rs970208386	NM_017793	c.268G>T	p.G90C	1.63×10^{-3}
BMP2	3	20	6,751,089	rs2273074	NM_001200	c.316G>A	p.A106T	7.82×10^{-3}
PLCH2	4	1	2,436,477	rs527838044	NM_014638	c.4076G>C	p.R1359P	9.35×10^{-3}
SLC8A1	2	2	40,342,493	rs187703961	NM_001112802	c.2714G>A	p.R905Q	0.013
CCDC3	1	10	13,043,302	rs1454330198	NM_031455	c.269T>G	p.M90R	0.013
	1	10	13,043,314	–	NM_031455	c.257C>A	p.A86E	
CCDC70	2	13	52,440,073	–	NM_001346075	c.526G>T	p.E176*	0.013
MAP9	3	4	156,269,027	rs137869187	NM_001039580	c.1852C>T	p.R618*	0.032
PEX11A	2	15	90,226,994	rs1378950011	NM_001271572	c.265C>T	p.R89*	0.043
ATP6V1FNB	2	7	128,506,549	rs140308992	NM_001195150	c.86G>A	p.W29*	0.043
NR1I3	2	1	161,200,970	rs144211465	NM_001077473	c.673C>T	p.R225*	0.043
OR5K3	2	3	98,109,652	rs776066487	NM_001005516	c.143T>A	p.L48*	0.043
MRO	3	18	48,325,725	rs141420496	NM_001127175	c.615C>G	p.Y205*	0.047

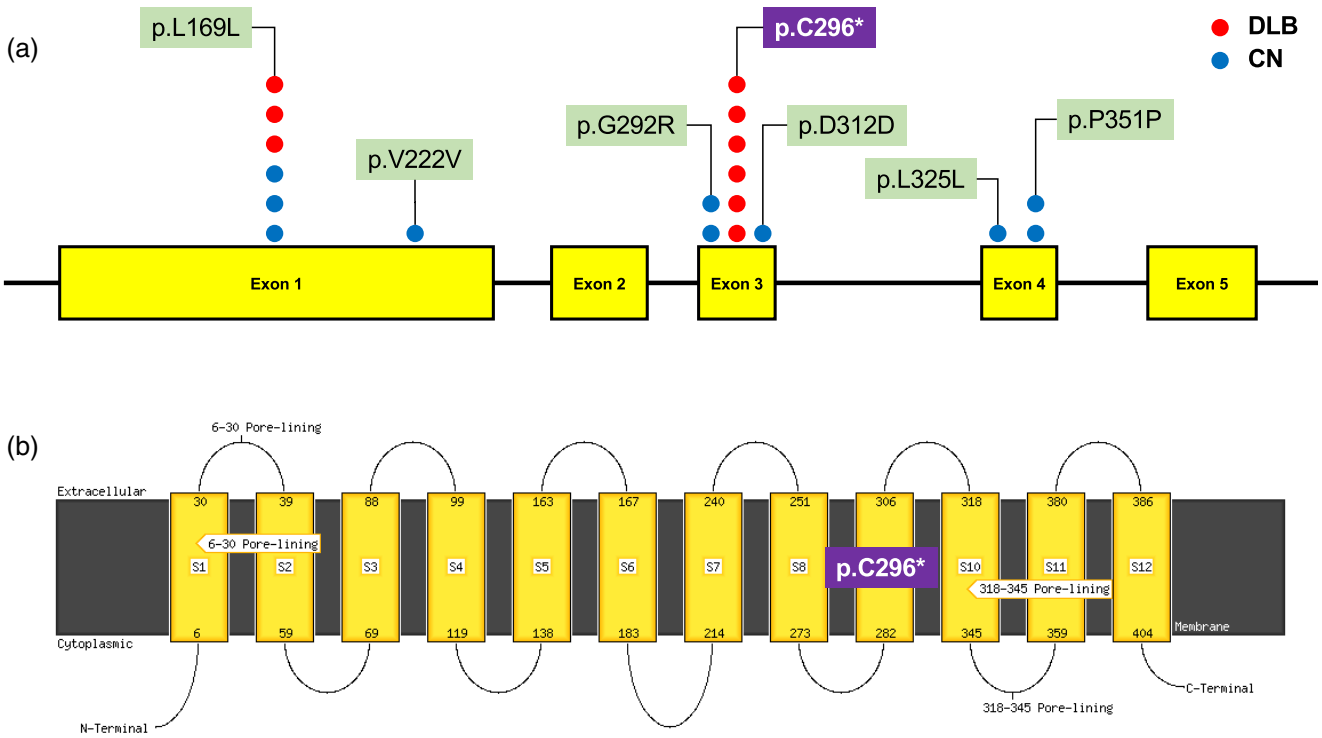

FIGURE 1 Distribution of *MFSD3* variants in DLB and CN samples. (a) Candidate pathogenic variant is shown in purple. (b) The variant was predicted in the location of transmembrane segments using MEMSAT-SVM.

variant was 0.0015 in 1KG (Via et al., 2010), 0.00061 in ExAC (Lek et al., 2016), 0.00040 in gnomAD (Karczewski et al., 2020), 0.0031 in the Korean Reference Genome Database (KRGDB) (Jung et al., 2020), and 0.0084 in ToMMo 8.3KJPN (Tadaka et al., 2018) (Table 4). In contrast, the variant has never been observed in European, American, and African populations included in these databases and DLB GWAS

Disease	Number of samples	#Allele 1	#Allele 2	Allele 1 frequency
DLB	79	7	151	0.044
CN	5,342	136	10,548	0.013
AD	1959	60	3,858	0.015
ALL	7,380	203	14,557	0.014

Abbreviations: AD, Alzheimer's disease; CN, cognitively normal controls; DLB, dementia with Lewy bodies.

TABLE 3 Minor allele frequency (MAF) of an *MFSD3* variant, rs143475431, among study groups

TABLE 4 Minor allele frequency (MAF) of rs143475431 in different populations

Population	Asian		European		American		African	
Database	Number of samples	MAF	Number of samples	MAF	Number of samples	MAF	Number of samples	MAF
1KG	993	0.0015	503	0	347	0	661	0
ExAC	9,024	0.00061	34,862	0	5,735	0	5,051	0
gnomAD	19,813	0.00040	57,710	0	14,758	0	7,870	0
ESP6500	0	0	0	0	6,503	0	0	0
KRGDB	1,461	0.0031	0	0	0	0	0	0
ToMMo	8,379	0.0084	0	0	0	0	0	0

Abbreviations: AD, Alzheimer's disease; CN, cognitively normal controls; DLB, dementia with Lewy bodies.

summary statistics from individuals of European ancestry (Chia et al., 2021), except for individuals of Asian ancestry. These results indicate that our finding is a novel Asian-specific DLB variant.

3.5 | Functional analysis of the *MFSD3* variant

Because an *MFSD3* variant could possibly cause a deficiency of acetyl-CoA, we examined the relationship between the variant and acetyl-CoA levels. The Human Protein Atlas database (Uhlen et al., 2015) shows that the *MFSD3* gene is expressed in several types of blood cells and in several brain regions (Figure 2a). We therefore examined the relationship between the *MFSD3* variant and acetyl-CoA by using 198 blood samples with and without the variant (A/T = 31, T/T = 167). A linear regression analysis was performed with adjustments for two covariates, age and sex, but there was no statistically significant association between the variant and the plasma levels of acetyl-CoA ($p = .23$, Figure 2b).

Acetyl-CoA is a precursor of the neurotransmitter acetylcholine (ACh), and ACh is hydrolyzed by two distinct enzymes: acetylcholinesterase (AChE) and BuChE. Clinical studies have shown that plasma levels of both AChE and BuChE are significantly higher in AD patients than in CN subjects (Mushtaq, Greig, Khan, & Kamal, 2014). Therefore, we examined the relationship between the variant and plasma levels of BuChE. A linear regression analysis of 1,206 samples was performed with adjustments for age and sex (A/T = 36, T/T = 1,170). A statistically significant association between the variant and plasma levels of BuChE was observed ($p = 0.029$, Figure 2c and Table S2). Although the plasma levels of BuChE decreased with increased age, the decrease was smaller in samples with the variant than in those without the variant (Figure 2d). These results indicate that the *MFSD3* variant could influence DLB pathogenesis through an increase in plasma levels of BuChE.

3.6 | Network-based meta-analysis using candidate pathogenic genes

PPI network analyses are effective for identifying functional modules and hub genes involved biological processes, such as the pathogenesis of DLB. Lewy bodies are observed in the anterior cingulate cortex (Brodmann area 24) early in DLB (Thal, Del Tredici, & Braak, 2004); we therefore performed a tissue-specific PPI network analysis based on the 16 candidate pathogenic genes detected above (Table 2) using NetworkAnalyst (Santiago & Potashkin, 2015) with the Differential-Net database (Basha et al., 2018). The most highly ranked hub genes were recognized in terms of the network topology measures of degree centrality (DC) and betweenness centrality (BC). Two top-ranked hub genes with a DC of 5 or more and a BC of 50 or more (*RASSF1*, DC = 31, BC = 615; *MRPL43*, DC = 5, BC = 134) were newly identified as strong candidates for pathogenic genes in DLB (Figure 3).

The candidate genes identified above had deleterious missense variants, with high CADD Phred-scaled scores in both *MRPL43* (chr10: 102746730, NM_176792:c.241A>C:p.N81H, CADD Phred-scaled score = 32) and *RASSF1* (rs778876938, NM_170714: c.229C>T:p.R77C, CADD Phred-scaled score = 34) (Figure 4). To evaluate the pathogenicity of the variants, we further genotyped them using our Japanese cohort, including those who contributed samples for the WGS (*MRPL43*, $n = 6{,}733$, DLB = 79, CN = 4,703, AD = 1,951; *RASSF1*, $n = 1{,}875$, DLB = 64, CN = 926, AD = 885). The MAF of the *MRPL43* candidate was 0.019 in DLB samples,

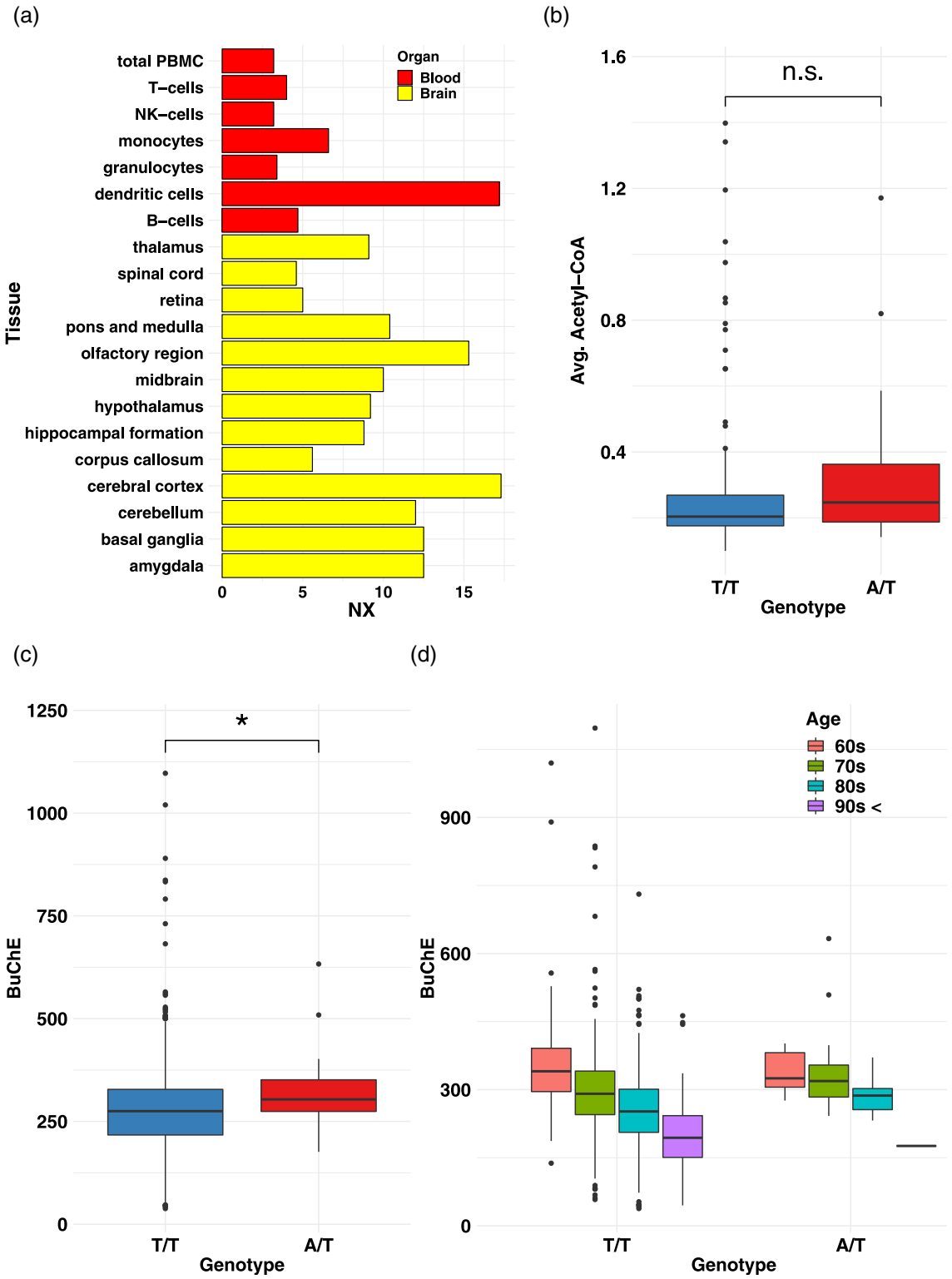

FIGURE 2 Functional analysis of the *MFSD3* variant. (a) The expression of the *MFSD3* gene in blood cells (red) and brain tissues (yellow) were checked in the Human Protein Atlas database. An *X*-axis represents the resulting transcript expression values, denoted normalized expression (NX), which were calculated for each gene in every sample. (b) Acetyl-CoA concentration was further examined using our 198 blood samples (AT = 31, T/T = 167). (c) BuChE concentration was examined using our 1,206 blood samples (AT = 36, T/T = 1,170). (d) BuChE concentration was further examined using the 1,206 blood samples by age. (b, c) *Statistically significant differences.

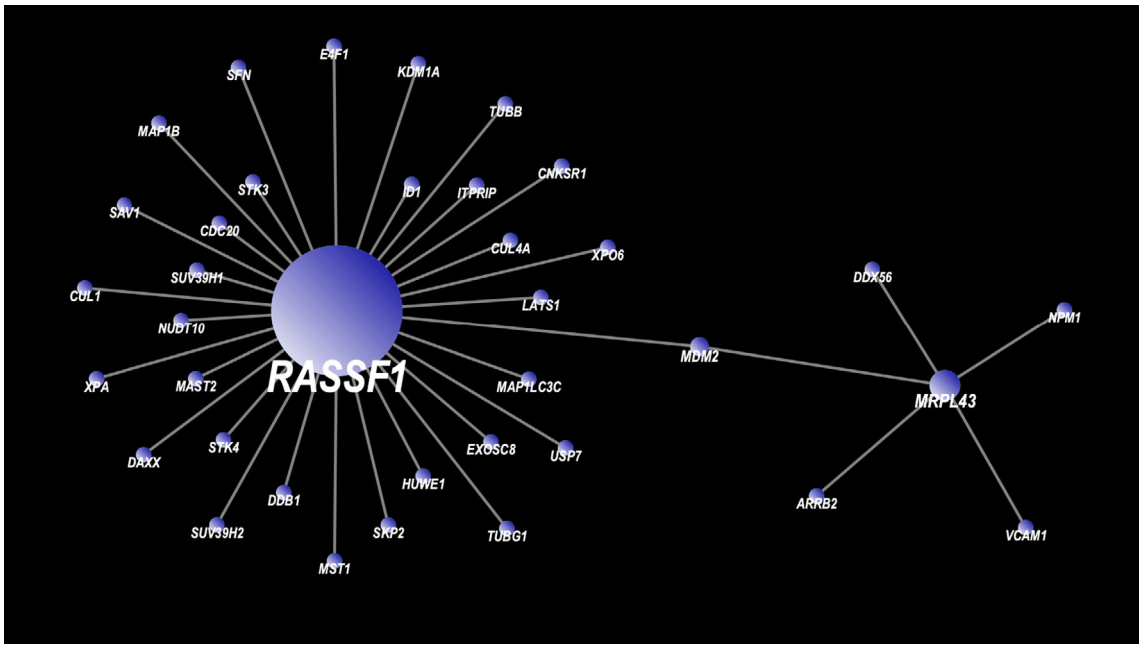

FIGURE 3 Network-based meta-analysis using candidate pathogenic genes

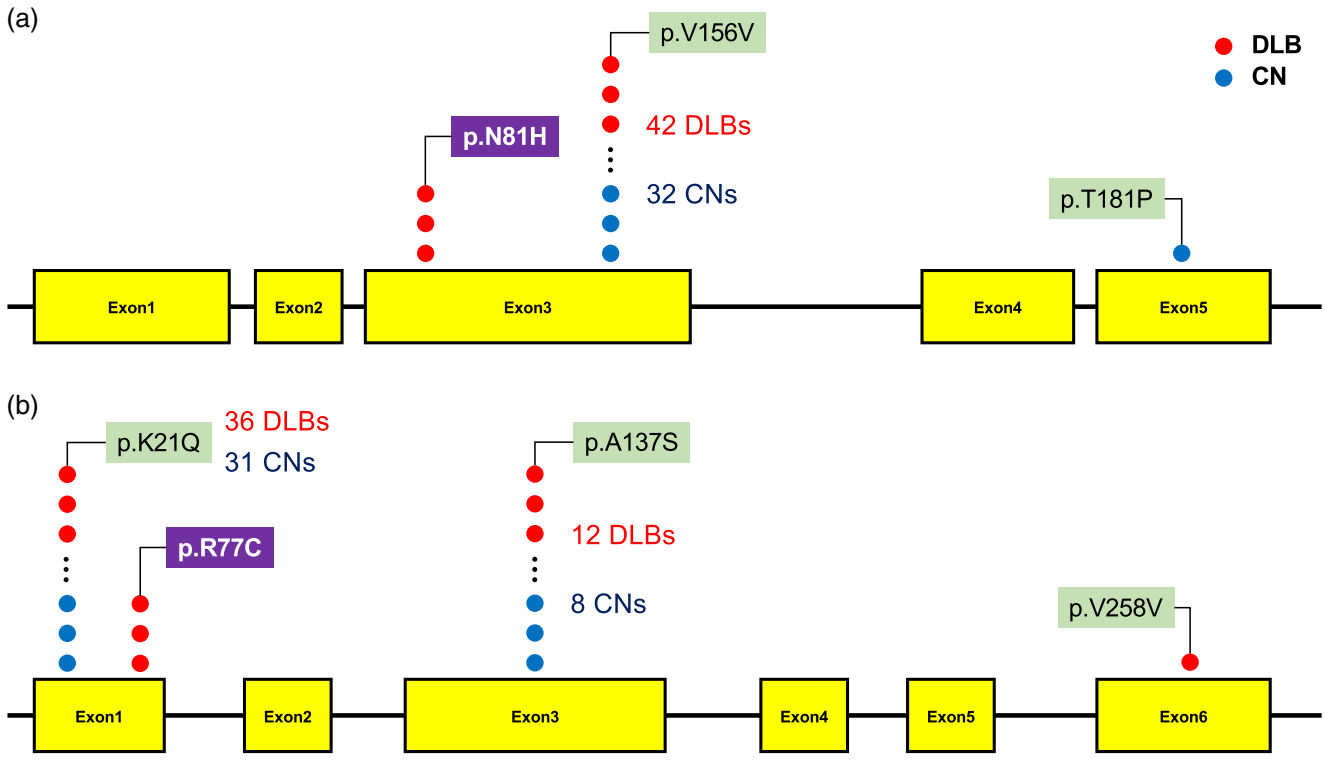

FIGURE 4 Distribution of *MRPL43* and *RASSF1* variants in DLB and CN samples. Candidate pathogenic variants are shown in purple. (a) *MRPL43*. (b) *RASSF1*.

0.0033 in CN samples, and 0.0013 in AD samples, and that of the *RASSF1* candidate was 0.023 in DLB samples, 0.0086 in CN samples, and 0.012 in AD samples (Table 5). In *RASSF1*, there were no statistically significant differences between DLB and CN samples ($p = .11$) or between DLB and AD samples ($p = .32$) when assessed with logistic regression models adjusting for age and sex. In contrast, the candidate variant of *MRPL43* showed statistically significant differences between the DLB and CN samples ($p = .0029$, odds ratio = 7.12, 95% CI = 1.96–25.9) and between DLB and AD samples ($p = .00024$, odds ratio = 15.84, 95% CI = 3.63–69.07). All of the *MRPL43* variants in

TABLE 5 Minor allele frequencies (MAFs) of *MRPL43* and *RASSF1* variants in the study groups

Gene	Disease	Number of samples	#Allele 1	#Allele 2	Allele 1 frequency
MRPL43	DLB	79	3	155	0.019
	CN	4,703	31	9,375	0.0033
	AD	1,951	5	3,897	0.0013
RASSF1	DLB	64	3	125	0.023
	CN	926	16	1,836	0.0086
	AD	885	22	1,748	0.012

Abbreviations: AD, Alzheimer's disease; CN, cognitively normal controls; DLB, dementia with Lewy bodies.

WGS were validated by using Sanger sequencing. The *MRPL43* variant has not been observed in DLB GWAS summary statistics from individuals of European ancestry (Chia et al., 2021), but only in the Asian population (MAF = 0.0032 in ToMMo 8.3KJPN), showing that it may also be a novel Asian-specific pathogenic variant in DLB.

Finally, we genotyped the *MFSD3* and *MRPL43* variants we identified in patients with mild cognitive impairment in our NCGG Biobank, which were observed in 68 of 1,097 MCI patients (3.2%) and 7 of 1,080 MCI patients (0.32%), respectively. These patients should be studied to determine whether they convert to DLB at a higher rate than patients without the variants.

4 | DISCUSSION

In this study, we used WGS from patients with probable DLB and CN to investigate pathogenic variants associated with DLB. To ensure reliable diagnosis of DLB, we only included samples from patients whose diagnosis was confirmed with dopamine transporter–SPECT or MIBG scintigraphy, which have high accuracy for diagnosing the typical clinical syndrome in DLB (Nihashi, Ito, & Terasawa, 2020). Not only did we identify a mutation in *GBA*, one of three genes previously associated with DLB (Guerreiro et al., 2018) (Bras et al., 2014), but also we discovered several new candidate genes associated with the risk of DLB by considering the accumulation of pathogenic variants. Through subsequent replication studies using a large number of samples from Japanese individuals and PPI analyses, we narrowed our findings down to a heterozygous stop-gain variant in *MFSD3* and a missense variant in *MRPL43*. Interestingly, both variants have only been observed in patients of Asian origin, suggesting that these are ethnicity-specific DLB susceptibility loci. Population-based prevalence and incidence studies are essential for understanding the pathogenesis of DLB and planning for the range of healthcare services needed for these individuals. In a systematic review, Hogan et al reported that DLB incidence ranged from 0.5 to 1.6 per 1,000 person-years; prevalence varied more widely, from 0.02 to 33.3 per 1,000 persons (Hogan et al., 2016). Because therapeutic efficacy has also been reported to vary with ethnicity for some diseases (Chang, Yang, Zhang, & Liu, 2014; Izumi et al., 2020), understanding differences in the genetic variant profiles among ethnicities will be important for interpreting the results of clinical trials and individualizing treatment, as well as for understanding the pathogenic mechanisms.

The *MFSD3* gene has been reported to encode a putative acetyl-CoA transporter (Perland et al., 2017), and its variant could result in a deficient supply of acetyl-CoA. Therefore, we examined the relationship between the variant and acetyl-CoA levels in blood samples. *MFSD3* is expressed in blood cells as well as brain tissues (Uhlen et al., 2015). However, there was no statistically significant association found between *MFSD3* variant and acetyl-CoA concentration in blood cells. Nonetheless, the variant might be related to a decrease of acetyl-CoA levels in brain tissues. Acetyl-CoA is a precursor of the neurotransmitter ACh, and ACh can be hydrolyzed by two distinct enzymes: AChE and BuChE. Previous studies have reported that plasma levels of AChE and BuChE are significantly higher in AD patients than in CN subjects (Mushtaq et al., 2014). We found that the decrease of plasma levels of BuChE with increased age was smaller in samples with the variant (A/T) than in those without it (T/T). These results suggest that the *MFSD3* variant could influence DLB pathogenesis through an increase of the plasma levels of BuChE.

Although differences exist between DLB and Parkinson's disease (PD) in the timing and severity of motor symptoms, psychotic symptoms, and the presence of Alzheimer-type pathology, both of these diseases are neuropathologically characterized by the accumulation of aggregated α-synuclein protein in Lewy bodies (Outeiro et al., 2019), and no objective biomarkers have yet been found that can reliably differentiate them. The *MRPL43* gene identified in this study, a mitochondrial ribosomal protein, has been reported to be highly expressed in more than three brain regions and co-expressed with a PD gene, *PARK7* (Billingsley et al., 2019). Mitochondrial dysfunction is one of the major molecular pathologies of PD (Exner, Lutz, Haass, & Winklhofer, 2012). Furthermore, many of the DLB patients with *MRPL43* variant have parkinsonian symptoms (Table S3). These results suggest that this variant might have a key role as an effective biomarker to distinguish these two diseases.

The *MFSD3* and *MRPL43* variants we identified are present in 3.2% and 0.32%, respectively, of patients with MCI in our NCGG Biobank. These patients should be studied to determine whether they convert to DLB at a higher rate than patients without the variants. We believe that early cognitive intervention for these patients with high risk will contribute to a delay in conversion to DLB.

This study represents the largest genetic study of DLB conducted in Japanese subjects to date. Our findings, which contribute to an enhanced understanding of DLB, will provide insight into its pathogenic mechanisms in future investigations. Although our dataset is not large enough to comprehensively identify rare variants in DLB, an association study between DLB and CN using WGS data with a large sample size could lead to the identification of further novel DLB variants.

AUTHOR CONTRIBUTIONS

D.S. developed the method and performed the analyses; Y.A. performed the WGS and SNP genotyping experiments; S.A. and S.H. provided technical assistance; T.S. and K.I. contributed to data acquisition and analyses; D.S. wrote the manuscript; and D.S., S.N., and K.O. organized the work. All authors contributed to and approved the final manuscript.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

All datasets used or analyzed in the current study are available from the corresponding author on reasonable request.

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