PICALM mRNA Expression in the Blood of Patients with Neurodegenerative Diseases and Geriatric Depression

Hiroshi Kumon[a], Yuta Yoshino[a], Yu Funahashi[a], Hiroaki Mori[a], Mariko Ueno[a], Yuki Ozaki[a],
Kiyohiro Yamazaki[a], Shinichiro Ochi[a], Takaaki Mori[a], Jun-ichi Iga[a,*], Masahiro Nagai[b] and
Masahiro Nomoto[b] and Shu-ichi Ueno[1]
[a]*Department of Neuropsychiatry, Molecules and Function, Graduate School of Medicine, Ehime University,
Toon, Ehime, Japan*
[b]*Department of Neurology and Clinical Pharmacology, Graduate School of Medicine, Ehime University,
Toon, Ehime, Japan*

Abstract.
Background: Phosphatidylinositol-binding clathrin assembly protein (PICALM) is a validated genetic risk factor for late-onset Alzheimer's disease (AD) and is associated with other neurodegenerative diseases. However, *PICALM* expression in the blood of neurodegenerative diseases remains elusive.
Objective: This study aimed to assess the usefulness of PICALM expression levels in the blood of patients with AD, Parkinson's disease (PD), dementia with Lewy bodies (DLB), and geriatric major depressive disorder (MDD) as a diagnostic biomarker.
Methods: In total, 45, 20, 21, and 19 patients with AD, PD, DLB, and geriatric MDD, respectively, and 54 healthy controls (HCs) were enrolled in the study. Expression data from Gene Expression Omnibus database (GSE97760), (GSE133347) and (GSE98793), (GSE48350), and (GSE144459) were used to validate the ability of biomarkers in the blood of patients with AD, PD, geriatric MDD, and a postmortem human AD brain and animal model of AD (3xTg-AD mouse), respectively.
Results: *PICALM* mRNA expression in human blood was significantly increased in patients with AD compared with that in HCs. *PICALM* mRNA expression and age were negatively correlated only in patients with AD. *PICALM* mRNA expression in human blood was significantly lower in patients with PD than in HCs. No changes in *PICALM* mRNA expression were found in patients with DLB and geriatric MDD.
Conclusion: *PICALM* mRNA expression in blood was higher in patients with AD, but lower in patients with PD, which suggests that *PICALM* mRNA expression in human blood may be a useful biomarker for differentiating neurodegenerative diseases and geriatric MDD.

Keywords: Alzheimer's disease, blood, gene expression, major depressive disorder, phosphatidylinositol-binding clathrin assembly protein

INTRODUCTION

About 50 million people worldwide are living with dementia, and this number is predicted to increase to 152 million by 2050; therefore, dementia prevention, precise interventions, and person-centered care are expected to receive increasing attention in the years to come [1]. Most cases of Alzheimer's

*Correspondence to: Jun-ichi Iga, Department of Neuropsychiatry, Molecules and Function, Ehime University Graduate School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan. Tel.: +81 89 960 5315; Fax: +81 89 960 5317; E-mail: igajunichi@hotmail.com. ORCID ID: https://orcid.org/0000-0003-4409-3096.

disease (AD) (>95%) are sporadic and have a late onset (80–90 years of age) [2]. Many genetic risk factors for sporadic AD have been consistently identified in large-scale genome-wide association studies, and pathway analysis has implicated immunity, lipid metabolism, tau-binding proteins, and amyloid-β protein precursor metabolism [3]. Growing evidence suggests an overlap between AD and Parkinson's disease (PD) pathophysiology in a subset of patients. A recent study demonstrated common genetic variants in genome-wide association with AD as predictors of concomitant AD pathology in the brains of people with a primary clinicopathological diagnosis of PD or dementia with Lewy bodies (DLB) [4]. Furthermore, the shared genetic etiology underlying AD and major depressive disorder (MDD) showed common pathways related to immune response and the regulation of endocytosis [5]. These neurodegenerative diseases and geriatric MDD may have overlapping signatures underpinning common phenotypic manifestations such as cognitive impairment and depressed mood.

Phosphatidylinositol-binding clathrin assembly protein (PICALM) is a highly validated genetic risk factor for late-onset AD [3]. PICALM is involved in trans-vascular Aβ clearance through the process of PICALM/clathrin-dependent endocytosis [6], and PICALM reductions in the brain endothelium of patients with AD correlate with AD neuropathology and cognitive impairment [7]. In addition to Aβ pathology, PICALM is associated with other neurodegenerative diseases with tau-mediated neuropathology, such as frontotemporal dementia and progressive supranuclear palsy [8, 9]. Although the relationship between *PICALM* expression and the reduction of endocytosis in the brain has been reported in several studies [8, 9], *PICALM* expression in the blood of neurodegenerative diseases remains elusive. Moreover, recent studies have suggested that both Aβ and tau pathologies may be associated with the pathophysiology of geriatric MDD [10–12], which is not only frequently comorbid, but also necessary to differentiate from AD [13], PD [14], and DLB [15].

In addition to the presence of amyloid plaques in the brain parenchyma and intraneuronal neurofibrillary tangles, recent evidence suggests additional AD pathophysiological pathways, such as innate immune responses, neuroinflammation, and vascular and cell membrane dysregulation [16–19]. Our previous studies suggest that the detection of transcriptome biomarkers related to cell stress and inflammation

in the blood has significant potential as a minimally invasive and inexpensive diagnostic tool for the diagnosis and early detection of developing AD [20–28].

Therefore, the purpose of this study was to assess the usefulness of *PICALM* expression levels in the blood of patients with AD and other neurodegenerative diseases (i.e., PD and DLB), as well as geriatric MDD, as a diagnostic biomarker.

MATERIALS AND METHODS

Participants

We enrolled 45 patients with AD, 20 with PD, 21 with DLB, 19 with geriatric MDD, and 54 healthy controls (HCs). All patients met the diagnostic criteria for AD, PD, DLB, and MDD according to the Aging/Alzheimer's Association [29], UK Parkinson's Disease Society Brain Bank [30], 2017 DLB clinical diagnostic criteria [31], and Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition [32], respectively. All patients with MDD were age >60 years. HCs had no current cognitive impairment, psychiatric symptoms, or history of mental disorders. The demographic characteristics of the patients and HCs are shown in Table 1. Cognitive function was evaluated using the Mini-Mental State Examination (MMSE) and psychiatric symptoms in patients with MDD were evaluated using the 21-item Hamilton Rating Scale for Depression (HAM-D21) (Table 1). All participants were unrelated and of Japanese origin and provided written informed consent using forms approved by the institutional ethics committees of Ehime University (Approval Number: 31-K8 and R2–4). This study was conducted in accordance with the Declaration of Helsinki.

Blood collection, RNA isolation, and cDNA synthesis

Total RNA was extracted from whole peripheral blood samples using PAXgene Blood RNA Tubes and the PAXgene Blood RNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol. RNA concentration and purity were determined by spectrophotometric analysis using a spectrophotometer (Nano Drop-1000; Thermo Fisher Scientific, Yokohama, Japan). A high-capacity cDNA reverse transcription kit (Applied Biosystems, Yokohama, Japan) was used for reverse transcription. RNA (1.0 μg) was reverse transcribed in a total reaction volume of 40 μL. We examined the RNA integrity of

Table 1
Characteristics of each group

	HCs	AD	PD	DLB	MDD	p
N	54	45	20	21	19	
Age (y)	77.0 ± 5.6	77.3 ± 4.4	75.8 ± 4.2	77.8 ± 7.5	$70.7 \pm 7.6^*$	$p < 0.001$[a]
Sex (male/female)	18/36	15/30	6/14	7/14	7/12	$p = 0.995$[b]
MMSE		18.2 ± 5.5	27.2 ± 3.3			
Duration (y)		4.2 ± 4.1				
NPI		13.8 ± 16.5				
MADRS		5.9 ± 4.9				
ADAS		19.5 ± 9.0				
CDR		1.5 ± 0.6				
L-dopa (mg/day)			415.4 ± 116.2			
UPDRS			27.9 ± 11.3			
HAM-D21					22.8 ± 10.1	

Data are shown as mean ± standard deviation. HCs, healthy controls; AD, Alzheimer's disease; PD, Parkinson's disease; DLB, dementia with Lewy bodies; MDD, major depressive disorder; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory; MADRS, Montgomery–Åsberg Depression Rating Scale; ADAS, Alzheimer's Disease Assessment Scale; CDR, Clinical Dementia Rating; L-dopa, L-3, 4-dihydroxyphenylalanine; UPDRS, Unified Parkinson's Disease Rating Scale; HAM-D21, 21-item Hamilton Depression Rating Scale. *Dunnett's test, $p < 0.001$ (HCs versus MDD). [a]One-way analysis of variance. [b]χ^2 test for independence.

all samples using the Bioanalyzer DNA kit (Agilent Technologies Japan, Ltd., Tokyo, Japan). The mean (±standard deviation [SD]) RNA integrity numbers in each group were over 7.6 (HCs: 7.62 ± 0.52, AD: 7.78 ± 0.68, PD: 7.78 ± 0.61, DLB: 7.74 ± 0.63, MDD: 7.76 ± 0.68), and no significant differences were found (analysis of variance [ANOVA] $p = 0.84$). These results indicated that the RNA qualities were appropriate for real-time PCR experiments.

mRNA expression

We performed real-time qPCR to examine *PICALM* and *GAPDH* mRNA expression using the TaqMan method (Applied Biosystems). Specific TaqMan probes were Hs00200318_m1 for *PICALM* and Hs02758991_g1 for *GAPDH*, which was used as an internal standard. Because preceding studies, including ours, identified GAPDH as the most suitable reference gene for blood gene expression analysis using the PAXgene blood RNA system [33, 34], we have presented the results of gene expression analyses using GAPDH for future validation. Because some reports have indicated that GAPDH gene expression decreases with age [35, 36], we examined the relationship between age and GAPDH levels (threshold cycle value) and found no significant correlation between them (Pearson correlation test: $r = -0.095$, $p = 0.233$). Additionally, we selected *18S rRNA* as the second reference gene for validation. The specific TaqMan probe for 18S rRNA was Hs99999901_s1. RT-qPCR was performed with $2.0 \, \mu L$ cDNA in a reaction mixture containing TaqMan Gene Expression Master Mix (Applied Biosystems). The total

volume of each well was $20 \, \mu L$. RT-qPCR was run in 96-well reaction plates using a StepOnePlus real-time PCR system (Applied Biosystems). mRNA expression was determined in duplicate. Thermal cycling conditions included one cycle at $50^\circ C$ for 2 min and one cycle at $95^\circ C$ for 10 min, followed by 50 cycles of amplification at $95^\circ C$ for 15 s and $60^\circ C$ for 1 min. Relative mRNA levels were calculated via the $\Delta \Delta C_t$ method using StepOne software (Applied Biosystems).

Validation analyses using public functional genomics data

To validate the ability of biomarkers in the blood of patients with AD, PD, geriatric MDD, and a postmortem human AD brain and animal model of AD (3xTg-AD mouse), we used expression data from Gene Expression Omnibus database (GSE97760) [37], (GSE133347) and (GSE98793) [38], (GSE48350) [39], and (GSE144459), [40] respectively. From human blood AD data (GSE97760), old female HCs (N = 10, mean age ± SD: 72.1 ± 13.1 years) and female patients with advanced AD (N = 9, mean age ± SD: 79.3 ± 12.3 years) were examined. From human blood PD data (GSE133347), HCs (N = 5, mean age ± SD: 59.2 ± 3.5 years) and patients with PD (*N* = 5, mean age ± SD: 61.8 ± 3.3 years) were examined. From human blood MDD data (GSE98793), old HCs (N = 19, M/F = 2/17, mean age ± SD: 65.8 ± 3.8 years) and patients with geriatric MDD (N = 38, M/F = 4/34, mean age ± SD: 66.0 ± 4.1 years) were examined. From human hippocampal data (GSE48350), old HCs

(N = 21, M/F = 10/11, mean age ± SD: 84.7 ± 8.4 years) and patients with AD (N = 19, M/F = 9/10, mean age ± SD: 83.1 ± 8.5 years) were examined. From animal model blood and hippocampal data (GSE144459), wild-type male mice (*n* = 8 each, age 12 and 52 weeks) (named as C12bld, C52bld, C12 hip, and C52 hip) and 3xTg-AD male mice (*n* = 8 each, age 12 and 52 weeks) (named as AD12bld, AD52bld, AD12 hip, and AD52 hip) were examined.

Statistical analysis

SPSS software (version 22.0; IBM Japan, Tokyo, Japan) was used for the statistical analysis. After the normal distribution of age and mRNA expression data was analyzed using the Shapiro–Wilk test, expression data were examined using one-way ANOVA to compare age and *PICALM* mRNA expression between patients with AD, PD, DLB, geriatric MDD, and HCs. Sex differences were analyzed using Pearson's chi-squared test. The Spearman correlation test was used to determine correlations with clinical parameters. Comparisons of the expression data from the Gene Expression Omnibus database were analyzed using a *t*-test or one-way ANOVA with Tukey's honestly significant difference (HSD) test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

We examined *PICALM* mRNA expression in the blood of patients with AD, PD, DLB, geriatric MDD, and HCs. Each group except geriatric MDD showed no significant differences in age or sex (Table 1). The geriatric MDD group was significantly younger than the other groups (one-way ANOVA $p < 0.001$, Tukey's HSD $p < 0.001$ [HCs versus MDD]). The mean ± SD *PICALM* mRNA expression in patients with AD was significantly higher than that in HCs (AD: 0.98 ± 0.19; HCs: 0.84 ± 0.23, Tukey's HSD $p < 0.01$), while that in patients with PD was significantly lower than that in HCs (PD: 0.60 ± 0.17, HCs: 0.84 ± 0.23, Tukey's HSD $p < 0.001$). No significant differences were found between patients with DLB, geriatric MDD, and HCs (DLB: 0.89 ± 0.22; geriatric MDD: 0.84 ± 0.25, HCs: 0.84 ± 0.23) (Fig. 1). Validation experiments using *18S rRNA* as the second reference gene showed similar expressional changes (Supplementary Figure 2).

We also analyzed the correlations between *PICALM* mRNA expression and clinical parameters. A significant negative correlation between *PICALM*
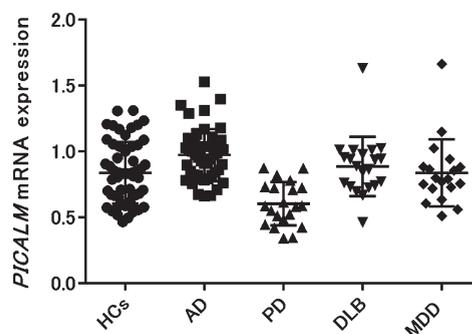

Fig. 1. *PICALM* mRNA expression was compared among patients with several neuropsychiatric diseases (AD, PD, DLB, and geriatric MDD) and HCs. *PICALM* mRNA expression was significantly higher in patients with AD, but significantly lower in patients with PD compared with HCs (AD versus HCs: $p = 0.004$, PD versus HCs: $p < 0.001$). AD, Alzheimer's disease; PD, Parkinson's disease; DLB, dementia with Lewy bodies; MDD, major depressive disorder; HCs, healthy controls.

mRNA expression and age was observed only in patients with AD ($p = 0.013$, $r = -0.37$) (Fig. 2), whereas no significant correlations were found with other clinical parameters in patients with AD (MMSE): $p = 0.29$, $r = 0.16$, duration: $p = 0.24$, $r = 0.19$, Neuropsychiatric Inventory: $p = 0.49$, $r = 0.11$, Montgomery–Åsberg Depression Rating Scale: $p = 0.14$, $r = -0.27$, Alzheimer's Disease Assessment Scale: $p = 0.61$, $r = 0.09$, Clinical Dementia Rating: $p = 0.19$, $r = -0.21$). In patients with PD, no significant correlation was found between mRNA expression and age. In addition, no correlation was seen between *PICALM* mRNA expression and other clinical parameters in patients with PD (L-3,4-dihydroxyphenylalanine: $p = 0.91$, $r = -0.038$, MMSE: $p = 0.98$, $r = -0.009$, Unified Parkinson's Disease Rating Scale: $p = 0.3$, $r = 0.31$) or geriatric MDD (HAM-D21): $p = 0.08$, $r = 0.42$).

Validation analyses using public functional genomics data showed significantly higher *PICALM* mRNA expression levels in the blood of patients with AD ($p < 0.001$ logFC: 1.46) (Supplementary Figure 1A), in that of 3xTg-AD mice (one-way ANOVA $p < 0.001$, Tukey's HSD $p < 0.001$ [C12 bld versus AD12 bld and C12 bld versus AD52 bld]) (Supplementary Figure 1E), and in the hippocampus of 3xTg-AD mice (one-way ANOVA $p < 0.001$, Tukey's HSD $p < 0.001$ [C12 hip versus AD52 hip]) (Supplementary Figure 1F). No changes were seen in *PICALM* mRNA expression in the blood of patients with PD or geriatric MDD (Supplementary Figure 1B and C) or in the hippocampus of patients with AD (Supplementary Figure 1D).
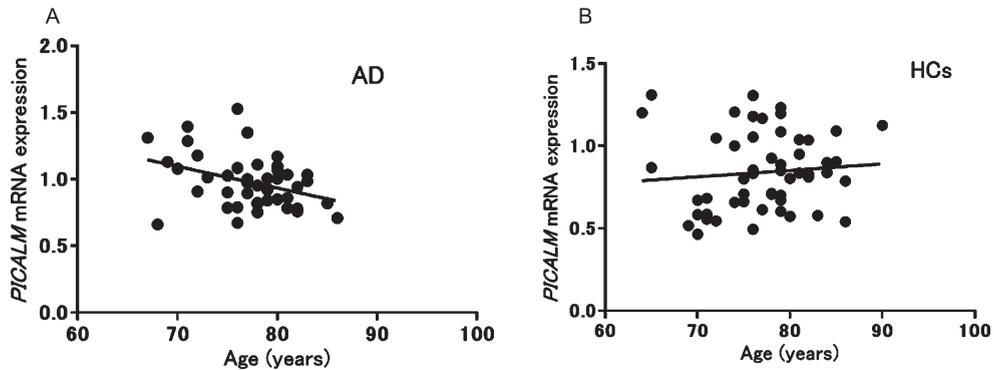
Fig. 2. Correlation between *PICALM* mRNA expression and age in patients with Alzheimer's disease (AD; A: $r = -0.37$, $p = 0.013$) and healthy controls (HCs; B: $r = 0.10$, $p = 0.48$).

DISCUSSION

This study had four major findings. First, *PICALM* mRNA expression in blood was significantly increased in patients with AD compared with that in HCs. Inconsistent with our results, a previous study using postmortem human brain and AD model mice showed reduced expression of PICALM in AD endothelium correlated with amyloid-β (Aβ) pathology and cognitive impairment [7]. This inconsistency may be due to tissue differences. Another study reported a decrease in PICALM gene methylation in the blood of patients with AD [41]. As DNA methylation levels were generally inversely correlated with RNA expression levels, this result may be consistent with our result. Although PICALM gene methylation in the same study was positively associated with MMSE scores, *PICALM* mRNA expression in our study was not associated with MMSE scores, probably because of the small sample size. Interestingly, validation analyses using public functional genomics data in the blood of patients with AD (Supplementary Figure 1A) and in the blood and hippocampus of 3xTg-AD mice (Supplementary Figure 1E and F) could replicate the increased *PICALM* mRNA expression in the blood of patients with AD. Although public functional genomics data in the hippocampus of patients with AD (Supplementary Figure 1D) did not show a significant change, *PICALM* mRNA expression in the hippocampus of 3xTg-AD mice showed a significant increase (Supplementary Figure 1F). Further analyses of *PICALM* mRNA expression in the human postmortem brain are therefore warranted.

Second, we observed a negative correlation between *PICALM* mRNA expression and age only in patients with AD (Fig. 2); no association was seen in HCs. In a previous study, PICALM was identified as having gene-based genome-wide significance with larger effects at a younger age in AD [42]. Therefore, the negative correlation observed between expression and age in patients with AD may suggest that the risk of PICALM for AD is modified by age.

Third, *PICALM* mRNA expression in human blood was significantly lower in patients with PD compared with that in HCs. In a previous study, an association was reported between PICALM rs3851179 and cognitive impairment in patients with PD [43]. Regarding the relationship between PICALM polymorphisms and PD, significant differences have been reported in the genotypes and allele frequencies of an SNP PICALM rs3851179 A allele among patients with AD, PD, and HCs [44]; however, it has also been reported that PICALM rs3851179 polymorphism is not related to PD [45, 46]. Although we could not confirm the same result in our validation analysis (Supplementary Figure 1B), PICALM may also be involved in the pathophysiology of PD.

Fourth, we did not find any changes in *PICALM* mRNA expression in patients with DLB and geriatric MDD. Public functional genomics data also showed that *PICALM* mRNA expression in the blood of patients with geriatric MDD had not changed (Supplementary Figure 1C). Because geriatric MDD is frequently comorbid with neurodegenerative diseases such as AD, *PICALM* mRNA expression may be useful as a differential biomarker for treating geriatric diseases that show psychiatric symptoms.

This study had several limitations. As few studies have reported finding relationships between *PICALM* and neurodegenerative diseases, patients should be recruited in various stages and the effects of medication should be considered in future studies. Moreover,

this study had a small sample size. Due to the limited power, the negative findings in this study cannot be interpreted as showing no associations.

CONCLUSIONS

We demonstrated that *PICALM* mRNA expression in blood was higher in patients with AD, but lower in patients with PD. Therefore, *PICALM* mRNA expression in human blood may be a useful biomarker for differentiating neurodegenerative diseases and geriatric MDD. Because *PICALM* mRNA expression using another reference gene (18S rRNA) was not changed in PD and decreased in geriatric MDD, these results need further validations.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-201046.

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