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Association between pineal gland volume and probable rapid eye movement sleep behavior disorder in cognitively normal elderly individuals and Alzheimer's disease patients

인지정상 노인군 및 알츠하이머병 환자군에서 송과체 용적과 유력 렘수면행동장애 사이의 관계에 대한 연구

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박 정 빈

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이 논문을 이학박사 학위논문으로 제출함

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Abstract

Association between pineal gland volume and probable rapid eye movement sleep behavior disorder in cognitively normal elderly individuals and Alzheimer's disease patients

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Background and Objectives: Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by loss of normal skeletal muscle atonia during REM sleep and dream-enacting behavior. RBD can occur in association with (secondary RBD) or without (idiopathic RBD) a neurodegenerative disorder, and idiopathic RBD accounts for up to 60% at the diagnosis. Although secondary RBD is strongly related to synucleinopathies, it has also been reported in the Alzheimer's disease (AD) and the prevalence of RBD in AD is estimated to be around 10%. Although melatonin has been reported to improve the symptoms of RBD, the association of pineal gland with RBD has never been investigated in cognitively normal (CN) elderly individuals as well as in AD patients. In the current study, we first investigated the association between pineal gland volume and RBD symptoms in both CN elderly individuals and AD patients.

Methods: We enrolled 245 community-dwelling CN elderly individuals without major psychiatric or neurological disorders and 296 community-dwelling probable AD patients who did not meet the diagnostic criteria for possible or probable dementia with Lewy bodies. Among the

AD participants, 93 were Aβ-positive on ^{18}F-Florbetaben amyloid positron emission tomography. We assessed RBD symptoms using the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) and defined probable RBD (pRBD) as achieving a score of 5 or higher in the RBDSQ. We manually segmented the pineal gland on 3T T1-weighted brain magnetic resonance imaging and estimated its volume.

Results: Smaller pineal parenchyma volume was associated with more severe RBD symptoms in both CN elderly individuals ($p < 0.001$) and AD patients ($p < 0.001$). The participants with pRBD showed smaller pineal parenchyma volume than those without pRBD in both CN ($p < 0.001$) and AD ($p < 0.001$) groups. The larger pineal parenchyma volume was associated with lower risk of prevalent pRBD in both CN (OR = 0.939, 95% CI = 0.912–0.966, $p < 0.001$) and AD (OR = 0.909, 95% CI = 0.878–0.942, $p < 0.001$) patients. The pineal parenchyma volume showed good diagnostic accuracy for prevalent pRBD in both CN (AUC = 0.82, 95% CI = 0.762–0.863, $p < 0.0001$) and AD (AUC = 0.80, 95% CI = 0.750–0.844, $p < 0.0001$) patients. These results were not changed when we analyzed the 93 participants with Aβ-positive AD separately.

Conclusion: Our findings suggest that smaller pineal parenchyma volume is associated with more symptoms of RBD and the risk of prevalent pRBD in both CN elderly individuals and AD patients.

Keywords: Pineal gland, Rapid eye movement sleep behavior disorder, Alzheimer's disease, Aging, Magnetic resonance imaging, Amyloid positron emission tomography

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List of Abbreviations

3D	Three-Dimensioanl
AANAT	aralkylamine N-acetyltransferase
AD	Alzheimer's Disease
AUC	Area Under the Receiver Operator Characteristic Curve
Aβ	Amyloid beta
CDR	Clinical Dementia Rating
CERAD-K	Korean version of the Consortium to Establish a Registry for Alzheimer's Disease Assessment Packet Clinical Assessment Battery
CERAD-K-N	CERAD-K Neuropsychological Assessment Battery
CHRLSq	Cambridge-Hopkins Restless Leg Syndrome questionnaire
CI	Confidence Interval
CIRS	Cumulative Illness Rating Scale
CN	Cognitively Normal
DLB	Dementia with Lewy Bodies
GDS	Geriatric Depression Scale
ICC	Intraclass Correlation Coefficient
ICV	Intracranial Volume
KLOSCAD	Korean Longitudinal Study on Cognitive Aging and Dementia
KLOSHA	Korean Longitudinal Study on Health and Aging
LBD	Lewy Body Disease
LC	Locus Coeruleus
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging

OR	Odds Ratio
OSA	Obstructive Sleep Apnea
PET	Positron Emission Tomography
pRBD	probable Rapid Eye Movement Sleep Behavior Disorder
PSG	Polysomnography
RBD	Rapid Eye Movement Sleep Behavior Disorder
RBDSQ	Rapid Eye Movement Sleep Behavior Disorder Screening Questionnaire
RBDSQ-6	Item-6 score of the RBDSQ
RBDSQ-T	RBDSQ Total score
REM	Rapid Eye Movement
RLS	Restless Legs Syndrome
ROC	Receiver Operating Characteristic
SD	Standard Deviation
SNRI	Serotonin Norepinephrine Reuptake Inhibitor
SNUBH	Seoul National University Bundang Hospital
SPM	Statistical Parametric Mapping
SSRI	Selective Serotonin Reuptake Inhibitor
SU	Standard Units
VPC	Volume of Pineal Cysts
VPG	Volume of Pineal Gland
VPP	Volume of Pineal Parenchyma

1. Introduction

1.1. Study Background

Rapid eye movement (REM) sleep behavior disorder (RBD) is defined as a parasomnia characterized by the loss of normal skeletal muscle atonia during REM sleep and dream-enacting behaviors [1]. Its' prevalence was estimated to be 1–2% in the general elderly populations [2,3]. RBD can occur in association with (secondary RBD) or without (idiopathic RBD) a neurodegenerative disorder [1], and idiopathic RBD accounts for up to 60% at the diagnosis [4]. However, more than 80% of idiopathic RBD eventually developed a neurodegenerative disorder such as Parkinson's disease and Lewy body disease (LBD) in 12–14 years [5,6], which implies that a majority of idiopathic RBD may be a prodromal phase of α-synucleinopathies [1,4].

Although RBD associated with neurodegenerative disorder is strongly related to synucleinopathies, several cross-sectional [7-10] and longitudinal [11-16] studies have reported that RBD was found to be prevalent and incident in Alzheimer's disease (AD) patients. The prevalence of RBD was approximately 10% in AD patients [17-19]. The loss of central cholinergic activity is cited as one of the factors that can explain the high prevalence of RBD in AD, as acetylcholine is involved in the induction of REM sleep atonia [17]. Boeve et al. reported that RBD can rarely be associated with a non-synucleinopathy disorder based on their clinicopathological study. They claimed that the presence of RBD should at least raise suspicion of primary or coexisting LBD even in the typical AD cases [20]. However, as they pointed out, their study sample may not represent overall RBD and may also have a sampling bias [20]. Furthermore, it is an over-extended interpretation that RBD can rarely be associated with a non-synucleinopathy disorder based on their observation since RBD is also prevalent in cognitively normal (CN) older adults [4].

A series of clinical trials found that the symptoms of RBD were improved by N-acetyl-5-methoxytryptamine (melatonin). In RBD patients, dream-enacting behaviors were reduced and REM sleep muscle atonia was restored by the administration of melatonin [21-25] but relapsed by

discontinuation of melatonin [22]. Melatonin is a multifunctioning indoleamine produced by the pineal gland [26]. The pineal gland is a small secretory neuroendocrine organ that is derived from the embryonic forebrain, and it is the major part of the epithalamus, along with the habenular nuclei [27]. It is located in the midline of the brain, outside the blood brain barrier and attached to the roof of the third ventricle. Its size is individually variable (approximately 5–9 mm in length and 1–5 mm in width) and the average weight of pineal gland in human is around 150 mg [28]. In humans, the pineal gland has been postulated to have diverse physiological functions through the melatonin, including regulating sleep and circadian rhythms, clearing free radicals, improving immunity, protecting from the oxidative stress, and inhibiting neurodegeneration [29,30]. It is also known to play a role in the regulation of the sexual maturation, thermoregulation, bone metabolism, and glucose homeostasis [30]. The major function of the pineal gland is to receive and convey information about the light-dark cycle from the environment and, consequently regulate sleep and circadian rhythm through the synthesis and secretion of melatonin [26,31]. In order to produce melatonin, the transcription of aralkylamine N-acetyltransferase (AANAT) and phosphorylation of AANAT are controlled on a daily basis by the pineal gland, and its activity is modulated by photoperiod seasonal change. Furthermore, the phosphorylation of AANAT by protein kinase A is mediated by the stimulation of pinealocytes, and ultimately, contributes to the production of melatonin [32]. In humans, roughly 80% of the pineal gland comprises melatonin-producing pinealocytes [31], and the volume of pineal gland (VPG) is proportional to the levels of melatonin in plasma, urine or saliva [33-35]. Although the pineal gland is reported to fully develop after the first year of life and does not change in size or weight later in life [26,36,37], recent studies have found that VPG could be changed by lifestyle such as coffee consumption or pathological conditions that may change melatonin production [28,38,39]. Previous human studies have confirmed that AD patients show decreased endogenous melatonin levels [40] and have a smaller VPG compared to healthy controls [41].

1.2. Purpose of Research

Given the effects of melatonin on RBD symptoms and the association of melatonin with VPG [33-35], we may assume that VPG may differ based on the presence of RBD in both idiopathic and secondary RBD. However, the association between pineal gland and RBD has never been investigated in CN individuals as well as in AD patients. In the current study, we first investigated the association of VPG with RBD symptoms in CN elderly individuals without neurological or psychiatric disorders. We then investigated the association between VPG and RBD symptoms in AD patients, which may be another underlying mechanism of RBD in elderly adults with AD in addition to coexisting or missed synucleinopathies.

2. Methods

2.1. Study participants

2.1.1. Participants with normal cognition

We enrolled 245 CN elderly individuals comprised 157 and 88 subjects from the participants of the Korean Longitudinal Study on Cognitive Aging and Dementia (KLOSCAD) [42] and the Korean Longitudinal Study on Health and Aging (KLOSHA) [43], respectively, which were conducted from November 2010 to October 2012. Both the KLOSCAD and KLOSHA were population-based prospective elderly cohort studies. The KLOSCAD study randomly sampled 30 villages and towns from 13 specific districts across South Korea, and randomly selected 10% and 20% of the elderly adults from urban and rural areas, respectively, using resident rosters and data on residents aged ≥ 60 years. The KLOSHA study randomly selected community-dwelling Korean elderly adults aged ≥ 65 years from the resident roster of Seongnam, one of the largest satellite cities of Seoul.

We excluded the following conditions: cognitive disorders such as dementia and mild cognitive impairment; major psychiatric and/or neurologic disorders that could affect cognitive function; any history of brain tumors, substance abuse or dependence, and use of clonazepam, antidepressants (selective serotonin reuptake inhibitor [SSRI], serotonin norepinephrine reuptake inhibitor [SNRI], and others), or exogenous melatonin over the past 6 weeks; any serious medical conditions that could affect the structure and/or function of the pineal gland or abnormalities in pineal gland morphology such as neoplastic lesions or extremely large cystic gland (diameter greater than 15.0 mm) [44]; and conditions that could mimic the symptoms of RBD such as restless legs syndrome (RLS) and obstructive sleep apnea (OSA). We diagnosed RLS using the Cambridge-Hopkins Restless Leg Syndrome questionnaire (CHRLSq) [45] and defined OSA as a STOPBANG questionnaire [46] score of ≥ 5 points. All participants were fully informed with the protocol of this study, and provided written informed consents signed by themselves or their legal

guardians. This study was approved by the Institutional Review Board of the SNUBH.

2.1.2. Participants with Alzheimer's disease

We enrolled 296 community-dwelling probable AD [47] who did not meet the diagnostic criteria for possible or probable Dementia with Lewy Bodies (DLB) [48] and who visited the Dementia Clinic of the SNUBH. Among them, 104 participants underwent a ^{18}F-Florbetaben amyloid brain positron emission tomography (PET) and 93 participants were found to be amyloid beta (Aβ)-positive.

We excluded the following conditions: any major psychiatric and/or neurological disorders including Parkinsonism that could affect cognitive function other than AD; any history of brain tumors, substance abuse or dependence, and use of clonazepam or exogenous melatonin over the past 6 weeks; any serious medical conditions that could affect the structure and/or function of the pineal gland or abnormalities in pineal gland morphology such as neoplastic lesions or extremely large cystic gland (diameter greater than 15.0 mm) [44]; and those with high risk of RLS (positive on CHRLSq [45]) and OSA (STOPBANG questionnaire score of ≥ 5 points [46]) all of which could mimic symptoms of RBD [49,50]. All participants were fully informed with the protocol of this study, and provided written informed consents signed by themselves or their legal guardians. This study was approved by the Institutional Review Board of the SNUBH.

2.2. Diagnostic assessments

Geriatric psychiatrists with expertise in dementia research conducted face-to-face standardized diagnostic interviews, detail medical histories, laboratory tests, and physical and neurological examinations using the Korean version of the Consortium to Establish a Registry for Alzheimer's Disease Assessment Packet Clinical Assessment Battery (CERAD-K) [51] and the Korean version of the Mini International Neuropsychiatric Interview [52]. In addition, research neuropsychologists

administered the Digit Span Test [53], Frontal Assessment Battery [54], Geriatric Depression Scale (GDS) [55], and CERAD-K Neuropsychological Assessment Battery (CERAD-K-N) [51,56]. Global severity of dementia was determined according to the Clinical Dementia Rating (CDR) [57]. All participants performed -1.0 standard deviation [SD] of the age-, gender-, and education-adjusted norms of elderly Koreans on the Mini-Mental State Examination [58].

Using a study-specific standard interview, trained research nurses collected data on age, sex, years of education, duration of AD (months), intracranial volume (ICV), history of head injury, amount of smoking (packs/day), and alcohol drinking (standard units/week) over the past twelve months period, and use of drugs influencing sleep or motor activity, including cholinesterase inhibitors (donepezil, rivastigmine, and galantamine), antidepressants (SSRI, SNRI, and others), carbamazepine, triazolam, zopiclone, quetiapine, clozapine, and sodium oxybate to each participants.

We diagnosed dementia according to the Fourth Edition of the Diagnostic and Statistical Manual of Mental Disorders Text Revision criteria [59]. We determined probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association diagnostic criteria [47], and probable or possible DLB according to the diagnostic criteria proposed by McKeith et al [48]. We defined CN as functioning independently in the community and showing no evidence of cognitive impairment in objective neuropsychological tests.

2.3. Assessment of RBD symptoms

We evaluated the behavioral features of RBD using the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) [60]. The RBDSQ is a self-reported screening instrument used to diagnose RBD and comprises 10 items assessing the most prominent clinical features of RBD: items 1 to 4, the frequency and content of dreams and their relationship to nocturnal movements and behavior; item 5, self-injuries and injuries to the bed partner; item 6, four subsections specifically

assessing nocturnal motor behavior, e.g. questions about nocturnal vocalization (6.1), sudden limb movements (6.2), complex movements (6.3) or bedside items that fall down (6.4); items 7 and 8, nocturnal awakenings; item 9, disturbed sleep in general; and item 10, the presence of any neurological disorder. Each item could be answered as ‘‘yes’’ or ‘‘no’’. The RBDSQ score ranges from 0 to 13 points, with higher scores indicating more features associated with RBD. We defined pRBD individuals as having a total score of 5 or higher on the RBDSQ [60]. The questionnaire was completed by the subjects with aid from their partners if needed.

2.4. Assessment of pineal gland volume

We performed brain magnetic resonance imaging (MRI) using a Philips 3.0 Tesla Achieva scanners (Philips Medical Systems; Eindohovenm, the Netherlands) within 3 months of the clinical assessments. We obtained 3D structural T1-weighted spoiled gradient echo sequences with the following parameters: acquisition voxel size = 1.0 × 0.5 × 0.5 mm; 1.0 mm sagittal slice thickness with no inter-slice gap; repetition time = 4.61 ms; echo time = 8.15 ms; number of excitations = 1; flip angle = 8°; field of view = 240 × 240 mm; and acquisition matrix size = 175 × 256 × 256 mm in the x-, y-, and z-dimensions. We implemented bias field correction to remove the signal intensity inhomogeneity artifacts of MR images using Statistical Parametric Mapping software (version 8, SPM8; Wellcome Trust Centre for Neuroimaging, London; http://www.fil.ion.ucl.ac.uk/spm) in MATLAB R2014a (MathWorks Inc., Natick, MA, USA). We resliced the MR images into an isotropic voxel size of 1.0 × 1.0 × 1.0 mm^3. We measured ICV using FreeSurfer software (version 5.3.0; http://surfer.nmr.mgh.harvard.edu) to adjust for inter-individual variabilities in brain volume.

For each participant, trained researchers who were blinded to the demographics and clinical characteristics constructed a 3D mask of each pineal gland by manually segmenting the pineal gland slice-by-slice on the resliced T1-weighted MR images using the ITK-SNAP (version

3.4.0; http://www.itksnap.org) volumetric imaging software. We segmented the pineal glands primarily on the sagittal planes and corroborated the results on the axial and coronal planes. We identified the pineal gland using the following structures as guides: the quadrigeminal cisterna, posterior portion of the third ventricle, superior colliculus, and habenula. Except for the portion connected to the habenula, defining of the boundaries of the pineal gland was straightforward as it is surrounded by the cerebrospinal fluid [31]. We carefully differentiated the pineal gland from the adjacent vascular structures, specifically the vein of Galen and the paired internal cerebral veins. We defined a pineal cyst as an area of homogenous intensity that was isointense to the cerebrospinal fluid in T1 sequence images with a diameter of 2.0 mm or greater [61]. We measured the VPG and volume of pineal cysts (VPC) and estimated the volume of pineal parenchyma (VPP) by subtracting VPC from VPG (**Figure 1**).

We assessed both intra-and inter-rater reliability to validate our manual segmentation approach using intraclass correlation coefficient (ICC). One evaluator blind to the clinical data assessed the VPP in all participants. To determine intra-rater reliability, the same evaluator without knowledge of the subjects' identities reassessed the VPP in a subset of 30 randomly selected subjects with a time gap of 2 months. To determine inter-rater reliability, two evaluators without knowledge of the subjects' identities independently assessed VPP in another subset of 20 randomly selected subjects on the same day with blinded to one another's readings. The ICC for intra-rater testing was 0.969 (95% confidence interval [CI] = 0.907–0.990, $p < 0.001$) and ICC for inter-rater testing was 0.934 (CI = 0.828–0.974, $p < 0.001$)

2.5. Assessment of brain amyloid deposition

We performed ^{18}F-Florbetaben amyloid brain PET imaging using a Discovery VCT scanners (General Electric Medical Systems; Milwaukee, WI, USA) in 3D acquisition mode. The participants were injected with 8.1 mCi (300 MBq) of ^{18}F-Florbetaben (Neuraceq) as a slow single intravenous bolus (6 sec/mL) in a total volume of up to 10 mL. After a 90-minute uptake period,

we obtained a 20-minute PET images comprising four 5-minute dynamic frames. Trained radiologists with expertise in nuclear medicine evaluated whether the participants were Aβ-positive or not. The determination was based on the visual interpretation of tracer uptake in the gray matter of the following four brain regions: the temporal lobes, the frontal lobes, the posterior cingulate cortex/precuneus, and the parietal lobes. Participants were considered Aβ-positive if smaller area(s) of tracer uptake were equal to or higher than those present in the white matter extending beyond the white matter rim to the outer cortical margin involving the majority of the slices within at least one of the four brain regions ("moderate" Aβ deposition), or a large confluent area of tracer uptake (i.e., signal intensity) was equal to or higher than that present in the white matter extending beyond the white matter rim to the outer cortical margin and involving the entire region including the majority of slices within at least one of the four brain regions ("pronounced" Aβ deposition). Participants were considered Aβ-negative if tracer uptake in the gray matter is lower than that in the white matter in all four brain regions (no Aβ deposition).

2.6. Statistical analyses

2.6.1. Pineal gland volume and RBD in CN

We compared continuous variables using independent samples t-tests and categorical variables using chi-square tests between groups. We examined the association of VPP with RBDSQ total score (RBDSQ-T) using multiple linear regression model adjusted for age, sex, years of education, ICV, head injury, smoking, and alcohol drinking as covariates. To test the robustness of our observation, we also examined the association of VPP with the item-6 score of the RBDSQ (RBDSQ-6) using multiple linear regression model adjusted for the same covariates. In each of the linear regression models, VPP, RBDSQ-T and RBDSQ-6 were entered as continuous variables. We assessed multicollinearity using collinearity statistical tests (tolerance and variance inflation factor). We compared VPP between the participants with pRBD and those without pRBD using

analysis of covariance that adjusted for the covariates stated above. We examined the association of VPP with the risk of prevalent pRBD using binary logistic regression analyses that adjusted for the same covariates. We examined the diagnostic performance of the VPP for prevalent pRBD using the Receiver Operating Characteristic (ROC) analyses. We calculated the optimal cutoff value and area under the receiver operator characteristic curve (AUC) using Youden index maximum (sensitivity + specificity − 1) [62].

2.6.2. Pineal gland volume and RBD in AD

We compared continuous variables using independent samples t-tests and categorical variables using chi-square tests between groups. We compared VPP between the participants with pRBD and those without pRBD using analysis of covariance that adjusted for age, sex, years of education, ICV, head injury, smoking, alcohol drinking, and use of drugs influencing sleep or motor activity as covariates. We examined the association of VPP with the risk of prevalent pRBD using binary logistic regression analysis that was adjusted for the same covariates. We examined the diagnostic performance of the VPP for prevalent pRBD using the ROC analysis. We calculated the optimal cutoff values and AUC using Youden index maximum (sensitivity + specificity − 1) [62]. We examined the association of VPP with RBDSQ-T using multiple linear regression model adjusted for the covariates stated above. To test the robustness of our observation, we also examined the association of VPP with the RBDSQ-6 using multiple linear regression model adjusted for the identical covariates. We assessed multicollinearity using collinearity statistical tests (tolerance and variance inflation factor). We examined the correlations of VPP with MMSE score and CDR sum of boxes score using Pearson's correlation analysis. We examined whether the interaction effect between pRBD and AD on the VPP using two-way analysis of variance. To test the robustness of the effect of VPP on RBD symptoms, we performed a series of subgroup analysis on the 93 participants with positive amyloid PET imaging results.

For all analyses, we considered a two-tailed p-value less than 0.05 as statistically

significant, and we employed Bonferroni corrections to reduce type I error when multiple comparisons were conducted. We performed ROC analyses using MedCalc for Windows (version 18.11.3; MedCalc Software, Mariakerke, Belgium). We performed all the other statistical analyses using SPSS for Windows (version 20.0; International Business Machines Corporation, Armonk, NY).

3. Results

As summarized in **Table 1**, the participants with pRBD showed smaller VPP than those without pRBD in both CN and AD groups ($p < 0.001$). VPP was inversely associated with RBDSQ-T and RBDSQ-6 in both CN (standardized $\beta = -0.352$, $p < 0.001$ for RBDSQ-T; standardized $\beta = -0.239$, $p < 0.001$ for RBDSQ-6; **Figure 2A**) and AD (standardized $\beta = -0.410$, $p < 0.001$ for RBDSQ-T; standardized $\beta = -0.224$, $p < 0.001$ for RBDSQ-6; **Figure 2B**) participants, indicating that the individuals with smaller VPP may have more RBD symptoms compared to individuals with larger VPP. There was no evidence of multicollinearity in all regression models with the maximum variance inflation factor being 2.05. VPP was inversely associated with the risk of prevalent pRBD in both CN (odds ratio [OR] = 0.939, 95% CI = 0.912–0.966, $p < 0.001$) and AD participants (OR = 0.909, 95% CI = 0.878–0.942, $p < 0.001$), indicating that the individuals with larger VPP may have a lower risk of prevalent pRBD than the individuals with smaller VPP (**Table 2**). The diagnostic accuracy of the VPP for prevalent pRBD was good; AUC was 0.82 (95% CI = 0.762–0.863, $p < 0.0001$; **Figure 3A**) in CN and 0.80 (95% CI = 0.750–0.844, $p < 0.0001$; **Figure 3B**) in AD participants. The optimal cutoff values of the VPP for classifying pRBD was 70 mm^3 (sensitivity = 87.50%; specificity = 70.59%) and 62 mm^3 (sensitivity = 87.18%; specificity = 58.75%), respectively. VPP was positively correlated with MMSE score ($r = 0.135$, $p = 0.020$), but not correlated with CDR sum of boxes score ($r = -0.106$, $p = 0.068$).

These results were not changed when we analyzed the participants with Aβ-positive AD separately. Among the 93 participants with Aβ-positive AD, 11 (11.83%) had pRBD. The Aβ-positive AD patients with pRBD showed smaller VPP than those without pRBD ($p = 0.002$). VPP also showed significant inverse association with the RBDSQ-T (standardized $\beta = -0.491$, $p < 0.001$; **Figure 2C**) and the RBDSQ-6 (standardized $\beta = -0.276$, $p = 0.015$) in the Aβ-positive AD patients. VPP was inversely associated with the risk of pRBD in the Aβ-positive AD patients (OR = 0.901, 95% CI = 0.840–0.966, $p = 0.004$). VPP showed good diagnostic accuracy for pRBD in the Aβ-positive AD patients (AUC = 0.81, 95% CI = 0.710–0.880, $p < 0.0001$; **Figure 3C**). The

optimal cutoff value of the VPP for classifying pRBD was 60 mm^3 (sensitivity = 100%; specificity = 57.32%). VPP was not correlated with both MMSE score (r = 0.000, p = 0.999) and CDR sum of boxes score (r = 0.038, p = 0.719). The Aβ-positive AD patients with pRBD showed smaller VPP (n = 11, mean ± SD = 48.0 ± 9.2 mm^3) compared to the CN participants with pRBD (n = 24, mean ± SD = 58.4 ± 16.5 mm^3; p = 0.023). The Aβ-positive AD patients without pRBD also showed smaller VPP (n = 82, mean ± SD = 66.1 ± 19.6 mm^3) compared to the CN participants without pRBD (n = 221, mean ± SD = 87.6 ± 30.0 mm^3; p < 0.001).

As shown in **Table 3**, we found no significant pRBD × AD interaction on VPP, suggesting that the influence of pRBD does not depend on AD status (F = 3.04, p = 0.082). The pRBD showed larger effect size compared to the AD.

4. Discussion

In animals, nighttime REM sleep disturbances were induced by lesioning the pineal gland, which reduced pineal melatonin secretion [63]. However, the association between the pineal gland and RBD has never been investigated in humans. We directly showed that smaller pineal gland was associated with the more RBD symptoms in CN elderly individuals. Considering that the pineal gland volume was highly correlated with the endogenous melatonin level [33-35], our observation seems to be in line with previous studies on the effects of melatonin on RBD. Melatonin treatment decreased the frequency and severity of dream-enacting behaviors and the risk of falls in the elderly RBD patients [24], and the beneficial effects of melatonin (3–12 mg) lasted beyond a year in a series of RBD patients [23]. A couple of clinical trials on the polysomnography-diagnosed RBD patients found that the percentage of REM sleep without atonia and movement time in REM were reduced by administering melatonin before bedtime for 4–6 weeks [21,22] but relapsed by discontinuing melatonin [22]. Another open-label trial reported that the percentage of tonic REM activity was reduced from 16% to 6% by administering 3–9 mg of melatonin at night, especially in the elderly patients with low endogenous melatonin secretion [64]. A placebo-controlled trial also reported that the percentage of REM sleep increased from 14.7% to 17.8% and the clinical global impression and daytime dysfunction were improved by administering 3 mg melatonin before bedtime in the individuals with disturbed and reduced REM sleep duration [25]. It remains unknown how melatonin improves the RBD symptoms. Multiple actions of melatonin such as decreasing muscle tonicity during REM sleep, enhancing GABAergic inhibition, stabilizing circadian clock variability and desynchronization, protecting cytoskeletal structure through its antagonism of calmodulin, enhancing sleep efficiency and shortening sleep latency may underlie the its beneficial effect on RBD [1,24]. In a glycine/GABA-A receptor knockout transgenic mouse model of RBD, melatonin was efficacious in decreasing REM motor behaviors and restoring REM muscle atonia [65]. However, several recent randomized controlled clinical trials have reported that the administration of melatonin was not effective in treating RBD-related symptoms in idiopathic

RBD patients as well as RBD patients with PD [66,67]. Therefore, we cannot rule out the possibility that the presence of RBD symptoms may not be due to the decreased endogenous melatonin secretion caused by the degeneration of pineal gland itself. The suprachiasmatic nucleus (SCN), which acts as a central circadian clock in the brain, innervates the pineal gland through multisynaptic connections [68]. As long as the neuronal connections between SCN and the pineal gland are functionally active, the rhythmicity of the SCN determines the rhythm of melatonin formation and release [26]. A previous animal study has demonstrated that lesioning the SCN directly induced REM sleep deprivation with loss of circadian rhythms in rats [69]. Degeneration of serotonergic and noradrenergic innervation between SCN and pineal gland decreased pineal melatonin secretion in elderly individuals [70]. Regarding these observation, degeneration of the SCN and/or functional disconnection of SCN and pineal, which may contribute to degeneration of pineal gland, might also increase risk of RBD or worsen RBD symptoms, directly or indirectly. In the future, combining complementary imaging approaches with clinical measures in a multimodal approach are warranted.

VPP showed good diagnostic accuracy for prevalent pRBD in the current study. However, the reduced VPP of the elderly individuals with idiopathic pRBD or those who are destined to have idiopathic pRBD in the future may be associated with prodromal α-synucleinopathies rather than RBD itself. Indeed, Parkinson's disease patients showed diminished endogenous melatonin production compared to controls [71]. In addition, melatonin directly blocked the α-synuclein fibril formation, destabilized preformed α-synuclein fibrils, and decreased α-synuclein-induced cytotoxicity [72]. Nevertheless, the changes of pineal gland volume in the patients with α-synucleinopathies have not been directly investigated yet.

We found that that smaller pineal gland volume was associated with more severe RBD symptoms in AD patients, which is in line with the results of CN participants. To the best of our knowledge, there have been no previous studies assessing the association between the pineal gland volume and RBD in AD patients. It is now well established that RBD is a strong predictor

of neurodegeneration, in particular α-synucleinopathies [1]. According to a previous clinicopathological study, 94% of the polysomnography (PSG)-confirmed pRBD patients were found to have synucleinopathies at autopy [20], suggesting that the presence of RBD in patient with dementia may favor the diagnosis of DLB [73]. However, not all RBD patients progressed to neurodegenerative syndrome with synucleinopathies. The overall conversion rate from idiopathic RBD to an overt neurodegenerative syndrome was 6.3% per year in the elderly adults aged 66.3 ± 8.4 years on average [74]. Previous polysomnographic studies have reported that RBD may also occur in AD [7,8] and 3–11% of RBD patients developed AD according to previous prospective cohort studies [11-16]. In a recent study, RBD was observed in 24.6% of amyloid PET-proven AD patients [10]. RBD can occur alone without any neurological conditions (idiopathic form) and large clinical series have reported that the idiopathic form of RBD accounts for up to 60% of the cases [4]. Therefore, we should be more cautious in confirming that all dementia with RBD is a synucleinopathy or at least a neurodegenerative disease having synucleinopathies as a secondary pathology. Although synucleinopathies may be a common sufficient condition for RBD, it is not a necessary condition for RBD.

Some authors have argued that an imbalance of acetylcholine transmission, a hallmark of AD, could explain the occurrence of RBD in a small portion of AD patients [8]. This is based on the findings that acetylcholine may be involved in the induction of REM sleep atonia [17], considering that an injection of cholinergic agonists induced muscle atonia in dogs [75] and the administration of cholinesterase inhibitors augmented the amount of REM sleep [76]. However, the plausibility of this hypothesis has little support from direct evidence [17]. We showed that AD patients with RBD had smaller pineal gland than those with RBD, suggesting that reduced endogenous melatonin production may contribute to the development of RBD in AD patients. Compared to healthy controls, AD patients showed disrupted circadian melatonin rhythm with lower melatonin levels in the cerebrospinal fluid, and serum and postmortem pineal gland [40], and had smaller pineal parenchyma [41]. These were observed in the AD mouse models [77]. Since the

pineal gland is a circumventricular organ surrounded by the cerebrospinal fluid [31], it can be easily targeted by soluble Aβ peptides [78]. A previous *in vitro* study of isolated rat pineal glands confirmed that Aβ directly inhibited pineal melatonin synthesis and impaired melatonergic systems, leading to a neuroinflammatory response within the gland [78]. Therefore, atrophy of the pineal gland due to enduring insults of Aβ may reduce pineal melatonin production and increase the risk of RBD in AD patients. Additionally, under physiological conditions, melatonin *in vivo* protects central cholinergic neurons against Aβ-mediated toxicity via its antioxidant and anti-amyloidogenic properties [29]. Melatonin not only inhibits Aβ generation but also arrests the formation of amyloid fibrils by a structure-dependent interaction with Aβ [29]. Therefore, reduced melatonin production due to pineal atrophy may also increase the risk of RBD or worsen RBD symptoms in AD patients indirectly via reduced protection of the cholinergic system from amyloid toxicity. Nevertheless, brainstem regions have been considered in RBD pathophysiology based on lesion studies in animals, especially involving pontine nuclei including the noradrenergic locus coeruleus (LC), cholinergic pedunculopontine nucleus, and laterodorsal tegmental nucleus [1]. The medullary magnocellular reticular formation plays a role in motor neuron inhibition, and forebrain structures have been tied into these circuits: substantia nigra, hypothalamus, thalamus, basal forebrain, and frontal cortex [1]. It has been reported that lesions in the LC cause REM sleep without atonia, and the size of the lesion determines whether simple or complex behaviors are exhibited [79]. The LC is prone to early neurodegeneration [80], and it was previously demonstrated that the loss of LC neurons by up to 70% in AD brains [81]. Therefore, atrophy of the LC with impaired noradrenergic systems could be related to the presence of RBD symptoms in AD patients. Pathophysiology of RBD in AD could be multifactorial, in which more research is necessary. Considering that roughly 40–50% of AD patients have been reported to have α-synuclein positive Lewy bodies [82,83], we cannot completely rule out the possibility that the presence of RBD symptoms may be attributable to the shared Lewy body pathology in some AD patients. However, even in synucelinopathies, the pineal gland may be associated with the risk of RBD because

melatonin also played a protective role against synucleinopathies [84].

This study has a couple of strengths. First, we excluded the VPC when we estimated the pineal parenchyma volume because pineal cysts do not contain pinealocytes [39,61], and exclude the subjects with extremely large cystic glands from the current study. It has been reported that pineal parenchyma volume (i.e., non-cystic volume) better reflected the levels of endogenous melatonin secretion than total pineal gland volume in adult individuals [33,34]. Although we did not directly investigate the association between VPC and RBD, this result may suggest that the presence of the pineal cysts itself may not affect RBD symptoms. Second, we confirmed our findings by a subgroup analysis for patients with Aβ-positive AD using brain PET imaging. It had been shown that the ^{18}F-Florbetaben binding acquired from this method was well correlated with Aβ plaque density of human autopsy brain tissue [85]. Third, we accounted diverse medications that could affect the proportion of REM sleep [86] or symptoms of RBD [87] in our analysis.

This study also has several limitations. First, we used a questionnaire to determine if a participant was at a high risk of RBD whereas video polysomnography (vPSG) is required to establish the definitive diagnosis of RBD [1]. This could be a substantial problem when the participants have significant cognitive impairment such as AD leading to a recall bias and misclassification bias. However, considering that the previous reports have suggested that the prevalence of vPSG-confirmed RBD in AD subjects ranges from 5% (mean age [SD], 70.5 [9.4]; mean disease duration of AD, 16.1 [7.1] months) [8] to 27% (mean age, 70.2 [5.6] with global deterioration scale score of 3 or 4) [7], our results with the prevalence of vPSG-confirmed RBD 13% (mean age, 77.3 [7.4]; mean disease duration of AD, 37.8 [27.6] months) seems to be in a reasonable extent. In addition, we tried to reduce the risk of misclassification bias by confirming the association of VPP with the RBDSQ item 6 score as well as RBDSQ total score. The RBDSQ item 6 queries the core symptoms of RBD with a good specificity [88]. Moreover, we obtained the RBDSQ data with the corroboration from the participant's partners which could increase their validity. Second, we cannot completely rule out the possibility that non-RBD symptoms might

have influence the RBDSQ scores. For example, some patients with RLS or severe OSA showed the behaviors mimicking RBD during sleep [49,50]. Although we exclude the participants with OSA by excluding the participants who got 5 points or higher in STOPBANG, the participants with pRBD showed modestly higher STOPBANG score than those without pRBD at baseline. Third, we could not adjust for the volume of pineal calcification because we did not perform additional brain computed tomography or high-resolution T2-weighted MRI. Although the effects of pineal calcification on melatonin production or human REM sleep remain unclear, a couple of previous studies argue that pineal calcification may inhibit the capacity for pineal melatonin synthesis and be associated with polysomnographic sleep parameters in humans [28,89]. Furthermore, calcifications can appear hypointense on T1-weighted images, which may lead underestimation of the VPP. Fourth, although we excluded individuals who took exogenous melatonin over the past 6 weeks, we did not directly quantify the endogenous nocturnal melatonin levels in the blood. Fifths, although we strictly excluded the probable AD patients who simultaneously met the diagnostic criteria for possible or probable DLB, it is still possible that our study samples could have mixed neuropathological findings such as Lewy bodies variant of AD because of the overlapping clinical features between AD and DLB [90]. Sixths, due to the relatively small sample size, the accuracy of cutoff value of VPP for classifying pRBD may need to be further validated in future studies with a larger sample size. Finally, it was impossible to determine the direction of causality between the pineal gland volume and pRBD in CN elderly individuals as well as AD patients because of the cross-sectional study design used in this study. Future longitudinal follow-up studies are warranted to further confirm the involvement of the pineal gland in RBD.

5. Conclusion

In conclusion, we found that the smaller pineal gland was associated with the more severe RBD symptoms and the higher risk of prevalent pRBD in both CN elderly individuals and AD patients. All patients with dementia showing RBD may not represent DLB and may be a pure AD, which may be associated with reduced volume of pineal gland. We hope that the results of current thesis improve our understanding on the possible roles of pineal gland in the development of RBD later in life, as well as provide a lead for more studies of pineal gland to other diseases.

Table 1. Characteristics of the participants with normal cognition and Alzheimer's disease

	Normal cognition			Alzheimer's disease		
	Without pRBD (n = 221)	With pRBD (n = 24)	p	Without pRBD (n = 257)	With pRBD (n = 39)	p
Age (years, mean ± SD)	71.8 ± 6.2	72.5 ± 6.2	0.594[a]	77.4 ± 7.4	76.8 ± 7.4	0.634 [a]
Sex (women, %)	51.6	45.8	0.592[a]	69.3	79.5	0.191 [a]
Education (years, mean ± SD)	11.4 ± 4.9	10.4 ± 5.0	0.341[a]	9.9 ± 5.6	8.1 ± 5.5	0.065 [a]
CDR (points, mean ± SD)	0 ± 0	0 ± 0	-	0.7 ± 0.4	0.9 ± 0.5	0.903 [a]
Duration of AD (months, mean ± SD)	-	-	-	36.9 ± 25.7	44.0 ± 37.8	0.265 [a]
Presence of cohabitants, (present, %)	86.4	95.8	0.188[a]	80.5	74.4	0.371 [a]
Alcohol drinking (SU/week, mean ± SD)	3.0 ± 7.7	6.0 ± 11.2	0.215[a]	1.8 ± 7.1	0.7 ± 3.4	0.375 [a]
Smoking (packs/day, mean ± SD)	0.03 ± 0.2	0.02 ± 0.1	0.797[a]	0.1 ± 0.6	0.0 ± 0.2	0.750 [a]
Drugs influencing sleep or motor activity (users, %)	-	-	-	29.2	38.5	0.240 [a]
History of head injury (present, %)	5.0	4.2	0.861[a]	9.0	10.3	0.792 [a]
MMSE (points, mean ± SD)	27.4 ± 2.1	27.2 ± 2.8	0.616[a]	18.5 ± 5.2	17.5 ± 5.0	0.799 [a]
GDS (points, mean ± SD)	7.7 ± 5.6	9.9 ± 7.7	0.199[a]	12.2 ± 6.9	16.5 ± 6.7	< 0.001 [a]
STOPBANG (points, mean ± SD)	2.5 ± 0.9	3.0 ± 0.8	0.037[a]	2.3 ± 0.9	2.6 ± 1.0	0.041 [a]
RBDSQ (points, mean ± SD)						
Total score	1.5 ± 1.3	5.8 ± 1.2	< 0.001[a]	1.4 ± 1.2	6.1 ± 1.4	< 0.001 [a]
Item 6 score	0.1 ± 0.4	1.2 ± 1.1	< 0.001[a]	0.2 ± 0.5	1.2 ± 1.2	< 0.001 [a]
Intracranial volume (cm^3, mean ± SD)	1565.4 ± 160.9	1553.1 ± 170.7	0.725[a]	1515.5 ± 147.7	1509.1 ± 154.1	0.805 [a]
VPP (mm^3, mean ± SD)	87.6 ± 30.0	58.42 ± 16.5	< 0.001[b]	69.5 ± 18.5	51.7 ± 10.8	< 0.001 [c]
Cerebral amyloid deposition (present, %)	-	-	-	31.9	28.2	0.643 [a]

Abbreviations: pRBD = probable REM sleep behavior disorder; SD = standard deviation; AD, Alzheimer's disease; SU = standard units; MMSE = Mini-Mental State Examination; GDS = Geriatric Depression Scale; CDR = Clinical Dementia Rating; RBDSQ = REM Sleep Behavior Disorder Screening Questionnaire; VPP = pineal parenchyma volume

[a] Independent sample t-test for continuous variables and Chi-square test for categorical variables

[b] Analysis of covariance adjusted for age, sex, years of education, intracranial volume, head injury, amount of smoking, and amount of alcohol drinking

[c] Analysis of covariance adjusted for age, sex, years of education, intracranial volume, head injury, amount of smoking, amount of alcohol drinking, and use of drugs influencing sleep or motor activity

Table 2. Association of the pineal parenchyma volume (mm^3) with the prevalent probable REM sleep behavior disorder in the participants with normal cognition and Alzheimer's disease

	Total, n	pRBD, n	OR (95% CI)[a]	p
Normal cognition	245	24	0.939 (0.912 - 0.966)	< 0.001 [a]
Alzheimer's disease	296	39	0.909 (0.878 - 0.942)	< 0.001 [b]

Abbreviations: pRBD = probable REM sleep behavior disorder; OR = odds ratio; CI = confidence interval

[a] Binary logistic regression analysis adjusting age, sex, years of education, intracranial volume, head injury, amount of smoking, and amount of alcohol drinking as covariates

[b] Binary logistic regression analysis adjusted for age, sex, years of education, intracranial volume, head injury, amount of smoking, amount of alcohol drinking, and use of drugs influencing sleep or motor activity

Table 3. Association between probable REM sleep behavior disorder, Alzheimer's disease and its interaction on VPP

	F	p [a]	η^2 (effect size)
pRBD	52.73	< 0.001	0.089
AD	14.64	<0.001	0.027
pRBD × AD	3.04	0.082	0.006

Abbreviations: pRBD = probable REM sleep behavior disorder; AD, Alzheimer's disease

[a] Two-way analysis of variance

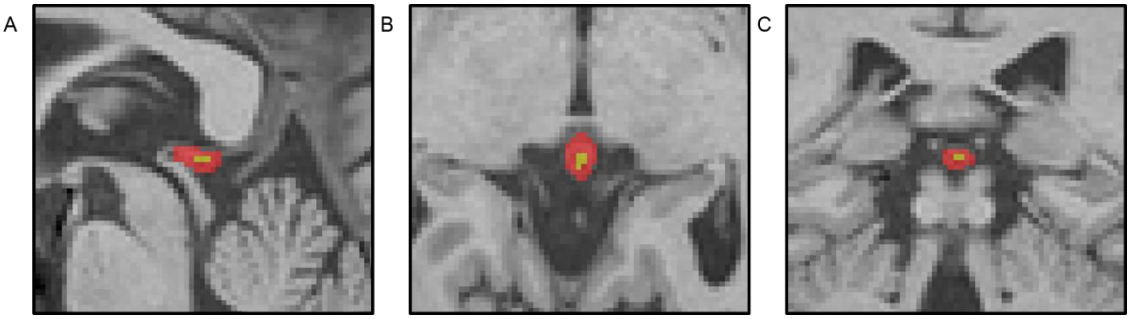

Figure 1. Segmentation of pineal gland volume on 3D T1-weighted brain magnetic resonance imaging

Sagittal (A), axial (B), and coronal (C) views of sample brain MRI of the pineal gland in subjects with pineal cysts (71 years old, men, normal cognition). The pineal gland (colored in red) and pineal cysts (colored in yellow) were identified, and then the pineal parenchyma was defined.

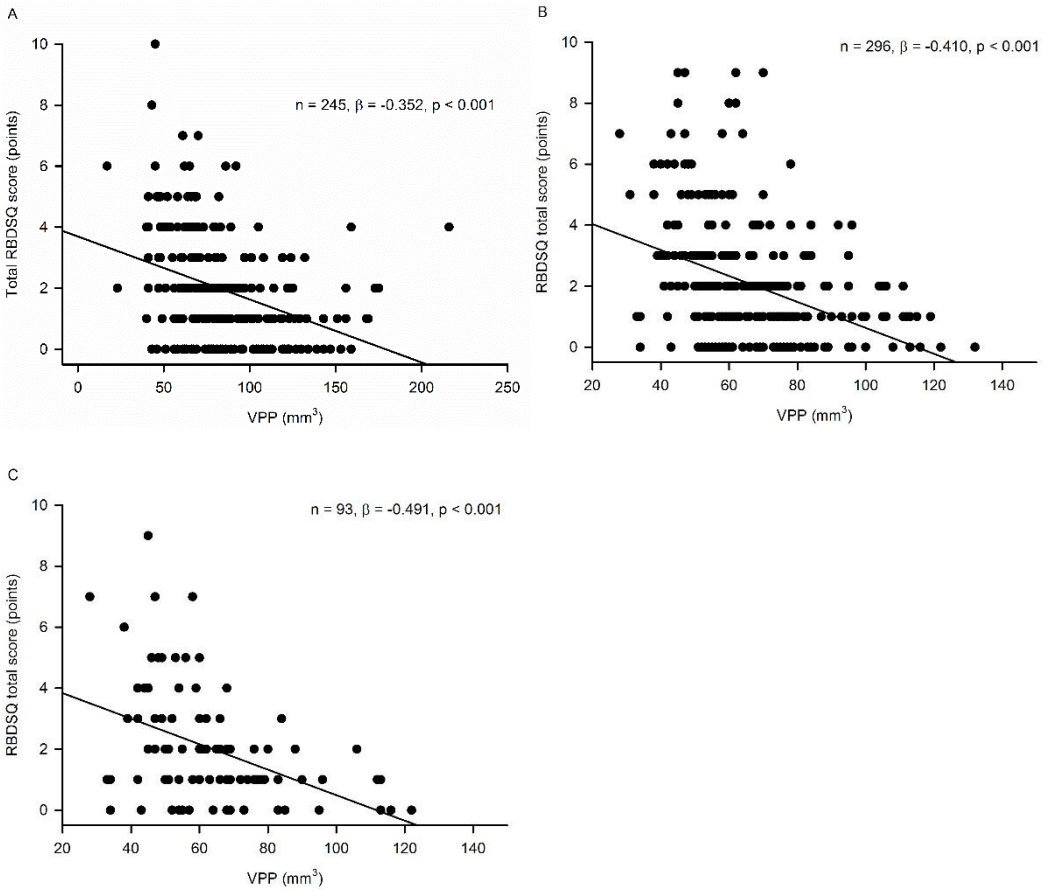

Figure 2. Association of pineal parenchyma volume (VPP, mm^3) with the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) total score in the (A) participants with normal cognition [a], (B) all participants with Alzheimer's disease [b], and (C) participants with Aβ-positive Alzheimer's disease [b].

[a] Multiple linear regression model adjusted for age, sex, years of education, intracranial volume, head injury, amount of smoking, and amount of alcohol drinking

[b] Multiple linear regression model adjusted for age, sex, years of education, intracranial volume, head injury, amount of smoking, amount of alcohol drinking, and use of drugs influencing sleep or motor activity

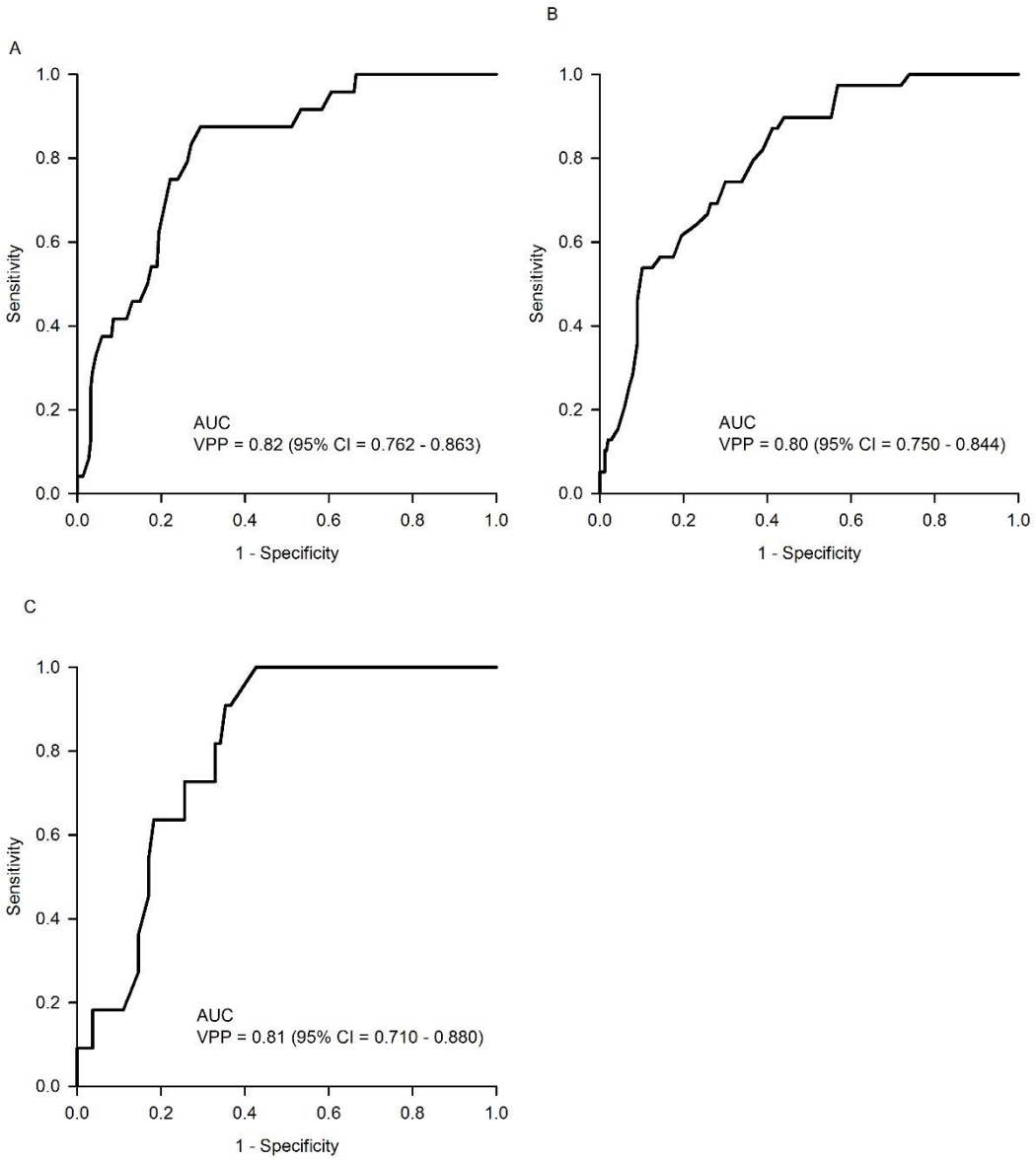

Figure 3. Diagnostic accuracy for prevalent probable REM sleep behavior disorder of the pineal parenchyma volume (VPP, mm^3) in the (A) participants with normal cognition, (B) all participants with Alzheimer's disease, and (C) participants with Aβ-positive Alzheimer's disease.

Abbreviations: Aβ = amyloid beta; AUC = area under the receiver operator characteristic curve; CI = confidence intervals

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국문초록

인지정상 노인군 및 알츠하이머병 환자군에서 송과체 용적과 유력 렘수면행동장애 사이의 관계에 대한 연구

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박정빈

연구배경 및 목적: 렘수면행동장애 (REM sleep behavior disorder, RBD)는 렘수면 동안 정상적으로 발생하는 골격근의 무긴장증이 소실되어 꿈의 내용과 관련된 움직임이 나타나는 것을 특징으로 하는 사건수면 중 하나이다. RBD는 다른 신경학적 질환이 동반되거나 (secondary RBD) 동반되지 않은 상태 (idiopathic RBD)에서 발병할 수 있으며, 진단 시 idiopathic RBD의 경우가 최대 60%를 차지한다. Secondary RBD는 시누클레인병증들과 밀접하게 연관되어 있으나, 알츠하이머병 (Alzheimer's disease, AD)환자에서도 보고되었으며 그 유병률은 약 10%로 추정된다. 비록 멜라토닌이 RBD 증상완화에 효과적이라고 보고되고 있지만, 인지정상 노인과 AD 환자에서 송과체와 RBD 사이의 관계성은 아직까지 조사된 바 없다. 본 연구에서는 인지정상 노인과 AD 환자를 대상으로 송과체 용적과 RBD 증상 간의 관계성을 조사하였다.

연구방법: 본 연구는 주요 정신학적 또는 신경학적 질환이 없는 245명의 지역사회 거주 인지정상 노인과, 가능 또는 유력 루이소체 치매 진단기준에 해당되지 않는

296명의 지역사회 거주 유력 알츠하이머병 환자를 대상으로 진행하였다. 알츠하이머병 환자 중 93명이 ^{18}F-Florbetaben 아밀로이드 양전자단층촬영을 통해 베타아밀로이드 양성소견을 보였다. RBD 증상은 렘수면행동장애 선별검사 설문지 (RBDSQ)를 사용하여 측정하였고, RBDSQ 점수 5점 이상을 유력 렘수면행동장애 (probable RBD, pRBD)로 정의하였다. 송과체를 T1-강조 뇌 자기공명영상에서 수동으로 구획화하여 용적을 측정하였다.

연구결과: 인지정상 노인군 ($p < 0.001$)과 알츠하이머병 환자군 ($p < 0.001$) 에서 송과체 실질 용적이 작아질수록 더 심한 RBD 증상을 나타냈다. 인지정상 노인군 ($p < 0.001$)과 알츠하이머병 환자군 ($p < 0.001$) 에서 pRBD 환자는 비환자군에 비해 더 작은 송과체 실질 용적을 가지고 있었다. 인지정상 노인군 (OR = 0.939, 95% CI = 0.912 – 0.966, $p < 0.001$)과 알츠하이머병 환자군 (OR = 0.909, 95% CI = 0.878 – 0.942, $p < 0.001$) 에서 송과체 실질 용적이 클수록 pRBD 유병 위험이 감소하였다. 인지정상 노인군 (AUC = 0.82, 95% CI = 0.762 – 0.863, $p < 0.0001$)과 알츠하이머병 환자군 (AUC = 0.80, 95% CI = 0.750 – 0.844, $p < 0.0001$) 에서 송과체 실질 용적은 pRBD 유병 위험에 대해 우수한 진단 정확도를 보여주었다. 이러한 결과는 아밀로이드 양성소견을 보인 93명의 AD 환자를 별도로 분석한 경우에도 변하지 않았다.

결론: 본 연구는 인지정상 노인과 AD 환자에서 작은 송과체 실질 용적이 더 심한 RBD 증상과 더 높은 pRBD 유병 위험성과 연관되어 있음을 제시한다.

주요어: 송과체, 렘수면행동장애, 알츠하이머병, 노화, 자기공명영상, 아밀로이드 양전자 방출 단층촬영

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