Supplementary Methods

Standard protocol approvals, registrations, and patient consent

This study was approved by the Ethics Committee of the National Taiwan University Hospital (NTUH-REC No. 202006173MINB), and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants and/or their relatives.

Study design and study population

This study comprised a cross-sectional analysis of an ongoing cohort study enrolling patients with CADASIL (Taiwan CADASIL registry).[1] Patients with symptomatic CADASIL who presented with either stroke, cognitive impairment, gait disturbance, psychiatric symptoms, or headache, and were genetically confirmed to have a *NOTCH3* mutation, were included. Presymptomatic *NOTCH3* mutation carriers were excluded from this study.

Patients with idiopathic Parkinson's disease (PD) were recruited from the movement disorder clinic of the same hospital for comparison. The diagnosis of PD was based on the clinical diagnostic criteria of the United Kingdom PD Society Brain Bank.[2] Patients were excluded if they had any contraindications to magnetic resonance imaging (MRI) examinations or severe medical illnesses that would predispose them to significant morbidity or mortality.

Clinical assessment

Demographic and clinical information of patients with CADASIL, including age, sex, medication history, vascular risk factors (hypertension, diabetes mellitus, dyslipidemia, and coronary artery disease), smoking history, and the number of first-degree relatives with stroke, were recorded.

Patients were evaluated for parkinsonism features, and assessed to see whether these features could be attributed to or disproportionate to post-stroke hemiparesis. We referred to the diagnostic criteria for vascular parkinsonism proposed by Zijlmans et al.[3] The age at onset, if available, was retrieved by reviewing the baseline CADASIL questionnaire or medical chart. The severity of parkinsonism motor symptoms was assessed using the Unified Parkinson's Disease Rating Scale part III (UPDRS-III) by two movement disorder specialists (YTC and CHL).[4] Items in the UPDRS-III were further categorized into four domains, namely tremor (items 3.15–3.18), rigidity (item 3.3), bradykinesia (items 3.1, 3.2, 3.4–3.8, and 3.14), and posture instability (items 3.9–3.13). The average scores for each domain were subsequently calculated. If stroke-related weakness prevented the proper assessment of UPDRS motor symptoms, the limb was not scored. Overall functional disability was assessed based on the modified Rankin Scale and Hoehn-Yahr (H-Y) stage.[5] Gait speed (m/s) was examined using a 4-meter walking test.[6] In our center, ^{99m}Tc-TRODAT-1 SPECT (single-photon emission computed tomography) was applied for dopaminergic transporter imaging. However, whether the patients underwent this imaging examination was optional.

Brain magnetic resonance imaging

All patients underwent at least one brain MRI examination at enrollment and follow-up examinations, usually every 2 years. We selected the MRI study closest to the parkinsonism evaluation, with an interval of less than six months.

Brain MRI was performed using a standard 1.5-T scanner. Sequences included high-resolution T1-weighted volumetric scan, T2, fluid-attenuated inversion recovery (FLAIR), and susceptibility-weighted imaging. Visual analyses of small vessel disease (SVD) markers, including white matter hyperintensity (WMH), lacunes, enlarged perivascular spaces (PVS), and cerebral microbleeds (CMB), were assessed according to the Standards for Reporting Vascular Changes on Neuroimaging 2 criteria.[7] The severity of WMH was scored using the Fazekas scale.[8] Enlarged PVS were scored using the Wardlaw scale.[9]

Quantitative analyses of MRI lesions included measurement of the WMH volume (mL) and mean cortical thickness (mm). WMH volumes were segmented on FLAIR imaging using a lesion growth algorithm implemented in the Lesion Segmentation Tool toolbox version 3.0.0[10] and confirmed later by visual screening. The mean cortical thickness was quantified on T1-weighted structural MRI scans using the FreeSurfer software version 7.2.0.[11]

Plasma biomarkers

Plasma α-synuclein levels in patients with CADASIL or PD were measured using the immunomagnetic reduction method (MF-ASC-0060; MagQu, Taiwan), as previously described.[12]

Statistical analysis

Continuous and categorical data are expressed as the median (interquartile range) and number (percentage), respectively. Differences between groups were compared using the Kruskal-Wallis one-way analysis of variance or Fisher's exact test. The first part of the analysis involved searching for factors associated with parkinsonism in patients with CADASIL. We subsequently compared clinical variables, UPDRS-III, H-Y stage, plasma α-synuclein, and MRI markers between patients with CADASIL with and without features of parkinsonism. We then applied univariate, age- and sex-adjusted, and multivariate logistic regression models to explore independent factors associated with the presence of par-

kinsonism in patients with CADASIL. The multivariate model was further adjusted for variables that reached statistical significance in the age- and sex-adjusted models. Additionally, in CADASIL patients with parkinsonism, we further conducted analyses using linear regression models for the associations between biomarkers (both plasma and neuroimaging) and UPDRS-III scores, as well as logistic regression models for associations with advanced H-Y stage (H-Y stage ≥3); these analyses were adjusted for age and sex. Plasma α-synuclein levels were log-transformed before entering the regression models.

The second part of the analysis aimed to compare the phenotypes and severity of parkinsonism between patients with CADASIL and those with PD. Only patients with CADASIL exhibiting parkinsonism features were included in this analysis, which involved comparison of total UPDRS-III scores and the average scores of the four domains, H-Y stage, plasma α-synuclein levels, and MRI markers. Finally, we investigated whether the burden of synucleinopathy contributed to parkinsonism features by analyzing the effects of plasma α-synuclein levels (log-transformed) on UPDRS-III scores and advanced motor stage (H-Y stage ≥3) in CADASIL patients with parkinsonism and PD patients, respectively. Given the exploratory nature of this study, multiple comparisons were not controlled for. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The level of statistical significance was set at $P<0.05$.

Supplementary References

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