Supplementary Methods

Participants

Study participants were recruited from a prospective Taiwan CADASIL Registry (TCR) cohort. The TCR study sites were the National Taiwan University Hospital (NTUH) and the Taipei Veterans General Hospital (VGH-TPE), which are university-affiliated tertiary medical centers. Individuals with clinical and neuroimaging features suggestive of cerebral small vessel disease (CSVD) were screened for cysteine-altered *NOTCH3* variants. The main clinical presentations include stroke, cognitive dysfunction, and gait disturbance, while neuroimaging features suggestive of CSVD include moderate-to-severe leukoaraiosis, multiple lacunes, and mixed locations of cerebral microbleeds (CMBs). Patients with genetically confirmed cysteine-altering *NOTCH3* variants were enrolled and their clinicopathological characteristics, including age, sex, vascular risk factors, history of stroke, cardiovascular medications, and type of blood pressure (BP)-lowering drugs, were documented. Informed consent was obtained from all patients. The research ethics committees of both hospitals approved this study (NTUH: No. 201807044RIND; VGH-TPE: No. 2019-02-025A).

Genetic analysis

In Taiwan, the p.R544C variant in exon 11 of *NOTCH3* accounts for more than 70% of cases of CADASIL.[1] For this reason, all enrolled patients were initially screened for the *NOTCH3* p.R544C variant. If it was not detected, the analysis of *NOTCH3* exons 2 through 24 was performed by Sanger sequencing.[2]

Blood pressure

From February 2019, each patient enrolled in the TCR was provided with a standardized automated, home-based sphygmomanometer (BP A2 Easy, Microlife AG, Widnau, Switzerland) to record their BP for at least 90 consecutive days. Instructions for performing standardized home BP measurements were provided at enrollment. Patients and their caregivers were asked to record their systolic BP (SBP) and diastolic BP (DBP) twice a day, once in the morning (sometimes between 8 AM and 12 PM) and again in the evening (6 PM to 10 PM). An empirical approach was adopted to allow patients to measure their BP only once at a time and on a flexible schedule, without an exact hour requirement. Pulse pressure (PP) was calculated by subtracting the SBP from the corresponding DBP. Average morning and evening BP parameters were used if both were recorded. Parameters used in the analysis included the mean and standard deviation (SD) of the SBP, DBP, and PP. We also applied the cut-off value based on the American College of Cardiology/American Heart Association (ACC/AHA) guidelines to define hypertension, that is, SBP >130 mm Hg for systolic hypertension and DBP >80 mm Hg for diastolic hypertension.[3] Patients recorded BP consecutively for 90 days, and those with available BP records <50% of days were excluded.

Neuroimaging analysis

All patients with TCR underwent 1.5-T brain magnetic resonance imaging (MRI) upon enrollment. Although patients were recruited and MRI was performed at two study sites, a harmonized common scanning protocol was defined that included a high-resolution T1-weighted volumetric scan, T2 and fluid-attenuated inversion recovery (FLAIR)-T2 scans for the evaluation of white matter lesions (WMLs) and detection of lacunes, and a susceptibility-weighted sequence for the detection of CMBs. Follow-up MRI was performed using the same protocol for each patient at intervals of 1–2 years. All images were sent to NTUH for visual ratings and quantitative analyses.

Visual rating analyses of CSVD markers were performed in accordance with the STandards for ReportIng Vascular changes on nEuroimaging (i.e., STRIVE) criteria.[4] The severity of WMLs in the periventricular and deep white matter was evaluated on FLAIR and graded against the Fazekas scale.[5] The presence and number of lacunes were evaluated using T1, T2, and FLAIR images. Enlarged perivascular spaces (EPVS) were visualized on T2-weighted images, and the severity of EPVS in the basal ganglia and centrum semiovale were assessed on a 4-point rating scale.[6] The numbers and distribution of CMBs were evaluated on susceptibility-weighted imaging, documented using the Microbleed Anatomical Rating Scale framework, and classified into lobar and deep regions.[7] All visual rating analyses were performed by CHC and YWC, and any inconsistency was solved with consensus reading. Quantitative analyses of the MRI lesions included mean cortical thickness, brain parenchymal fraction, and WML volume. The mean cortical thickness and estimated total intracranial volume (eTIV) were quantified on T1-weighted structural MRI scans using the pipeline and the output of the FreeSurfer software version 7.2.0.[8] The brain parenchymal fraction, which represents the overall volume of the brain, was calculated by dividing the brain segmentation volume by the eTIV. WMLs were segmented using a lesion growth algorithm implemented in the Lesion Segmentation Tool (LST) toolbox version 3.0.0 (www.statistical-modeling.de/lst.html) for Statistical Parametric Mapping.[9] A FLAIR sequence was used for lesion segmentation, and a T1 image was used as a reference for registration. The final segmented lesions from the output of the LST were visually screened for accuracy, and the WML volumes were expressed in milliliters. To control for variations in head size, WML volumes were adjusted for eTIV

and expressed as the WML proportion (% of eTIV). To account for the possibility of large infarct or hemorrhagic stroke effects, we visually checked the FreeSurfer output. If severe distortion was observed due to a large infarct or intracerebral hemorrhage (ICH), we used only the data from the unaffected hemisphere. For the WML volume, the LST output was visually checked. The gliosis caused by the previous infarct or ICH was manually removed and the WML volume was recalculated.

Outcomes

The clinical outcome was stroke incidence. All enrolled patients with CADASIL were regularly followed up in the outpatient service until November 30, 2022, at a loss to follow-up, or death. Any incident stroke event was documented and defined as an acute episode of focal neurological dysfunction lasting more than 24 hours with corresponding neuroimaging evidence of cerebral infarction or hemorrhage.

Neuroimaging outcome was the progression of MRI markers between baseline and follow-up scans. Because the WML and EPVS scores on the visual rating scale rarely changed over 1–2 years, these two markers were excluded. The number of incident lacunes and CMB detected on follow-up MRI was divided by the interval between scans and expressed as the annual change (n/year). Similarly, annual changes in the proportion of WML, mean cortical thickness, and brain parenchymal fraction were calculated. Because the median annual increases in the number of lacunes and CMBs were 0 and 1, respectively, any incident lacunes or increased CMB numbers ≥2 per year were defined as meaningful neuroimaging outcomes.

Statistical analyses

Descriptive analyses of clinical demographic characteristics, BP parameters, and neuroimaging features were performed. A Cox regression model was used to test the influence of BP parameters on incident stroke and was adjusted for age, sex, hypertension, and history of stroke. Logistic regression models were applied to test the association between the BP parameters and any incident lacune or incident CMB ≥2 per year, with covariates of age, sex, hypertension, and baseline lacune or CMB numbers. In the above models, the mean and SD of SBP, DBP, and PP, as well as systolic hypertension (90-day mean SBP >130 mm Hg) and diastolic hypertension (90-day mean DBP >80 mm Hg) were individually tested as independent variables. For the sensitivity analysis, we included patients who were initially excluded because there was no follow-up MRI to test the effects of BP on incident stroke (clinical outcome).

Furthermore, correlations between BP parameters and annual changes in neuroimaging markers were plotted as scatterplots and tested using unadjusted simple linear regression. A linear mixed model for repeated measures was used to test the associations between the change in WML proportion or mean cortical thickness (dependent variable) and each of the BP parameters (independent variable), adjusted for age, sex, hypertension, study site, and MRI intervals. Due to the skewed distribution and many zero values for the numbers of lacunes and CMBs, a Poisson mixed-effect model (Poisson generalized linear mixed model) was applied to test the associations between the incident lacunes or CMB number and BP parameters and adjusted for age, sex, hypertension, study site, and MRI intervals. The significance level was set at $P<0.05$. No adjustments were made for multiple comparisons because this was an exploratory analysis. All analyses were performed with SAS, Version 9.4 (SAS Institute Inc., Cary, NC, USA).

Supplementary References

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