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The protocol for an observational Australian cohort study of CADASIL: The AusCADASIL study

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ABSTRACT

Introduction: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is a rare genetic condition with a broad phenotypic presentation. This study aims to establish the first Australian cohort of individuals affected by CADASIL (AusCADASIL) and examine its clinical features and longitudinal course, and to investigate neuroimaging and blood biomarkers to assist in early diagnosis and identify disease progression.

Methods: Participants will be recruited from six study centres across Australia for an observational study of CADASIL. We aim to recruit 150 participants with diagnosed CADASIL, family history of CADASIL or suspected CADASIL symptoms, and 150 cognitively normal *NOTCH3* negative individuals as controls. Participants will complete: 1) online questionnaires on medical and family history, mental health, and wellbeing; 2) neuropsychological evaluation; 3) neurological examination and brain MRI; 4) ocular examination and 5) blood sample

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donation. Participants will have annual follow-up for 4 years to assess their progression and will be asked to invite a study partner to corroborate their self-reported cognitive and functional abilities.
Primary outcomes include cognitive function and neuroimaging abnormalities. Secondary outcomes include investigation of genetics and blood and ocular biomarkers. Data from the cohort will contribute to an international consortium, and cohort participants will be invited to access future treatment/health intervention trials.
Discussion: AusCADASIL will be the first study of an Australian cohort of individuals with CADASIL. The study will identify common pathogenic variants in this cohort, and characterise the pattern of clinical presentation and longitudinal progression, including imaging features, blood and ocular biomarkers and cognitive profile.

Background

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) describes a rare hereditary condition characterised by cerebral small vessel disease (cSVD) and recurrent stroke. Although rare, it is the most common monogenic form of cSVD, with regional studies suggesting an estimated prevalence of approximately 4 individuals per 100 000 [1,2]. Based on these estimates, there may be at least 500–1250 individuals with CADASIL in Australia. However, given that there has never been an Australian cohort study of CADASIL, the actual prevalence remains unknown and may be underestimated due to challenges around diagnosis, and the genotypic and phenotypic diversity of the disease. CADASIL is caused by variants in the *NOTCH3* gene [3], and large-scale database studies of *NOTCH3* gene variants suggest a greater frequency of pathogenic variants resulting in mild or asymptomatic disease [4]. Based on UK Biobank data, an estimated 0.2 % of the general population carry a *NOTCH3* variant, which is associated with increased risk of incident ischemic stroke and vascular dementia [5]. More research is needed to understand the presentation, progression, and clinical impact of CADASIL in an Australian context to inform treatment, including the potential for prevention strategies.

CADASIL is characterised by progressive damage within the microvasculature with severe consequences arising from affected vessels of the brain, producing a range of largely neurological symptoms [6]. CADASIL typically presents with multiple small strokes and complex migraines in individuals aged 30–55 years, leading to disturbances in motor and speech function, mood changes, dementia, and often incontinence [7]. Large-scale studies have indicated the genetic characteristics, and heterogeneity within the clinical features and severity of symptoms of CADASIL [8–11]. The clinical features, severity and prognosis of CADASIL may be influenced by the individual's ethnicity [12], the position of the pathogenic variant within the *NOTCH3* gene [13], or the presence of disease-modifying variants within the genome [14,15]. The clinical profile of CADASIL and distribution of pathogenic variants appears to cluster geographically and according to ethnicity [12,16]. Genotype-phenotype investigations in AusCADASIL will explore the interplay between pathogenic variant and clinical presentation in an Australian cohort.

At present, there are no disease-modifying therapies for CADASIL. Treatment and management are therefore symptom-driven, with a key goal being to improve cardiovascular health and lower stroke risk. There are also no comprehensive, evidence-based guidelines for the management of CADASIL, though there is a recent scientific statement aimed at providing recommendation for prevention and management considerations[17]. AusCADASIL will provide an understanding of the clinical presentation and progression in an Australian context. Studies of imaging [18] and molecular biomarkers[19] of CADASIL will indicate whether these biomarkers, either alone or in combination, have potential in the diagnosis and monitoring of CADASIL, and how they can be translated into clinical application. Insights from this observational study may inform existing empirical treatment regimes and the selection of therapies for future interventional trials. In addition, it will serve as a healthcare resource for Australians living with CADASIL by galvanising the expertise of multidisciplinary teams from several clinics around the country.

Methods

Study design

AusCADASIL is an observational research study aimed at identifying clinical presentation, risk factors and genetic profiles of individuals with CADASIL. While some participants will be recruited from clinical settings and may be receiving ongoing clinical care, there are no interventions under evaluation in this study. Fig. 1 outlines the study processes.

AusCADASIL has begun recruitment. Details and updates can be followed at: https://cheba.unsw.edu.au/research-projects/vascular-contributions-dementia-centre-research-excellence/auscadasil.

Setting

Participants will complete assessments online and in-person at six centres across three Australian states: New South Wales, Queensland, and Victoria. Sites include hospitals, clinics, and universities. Online assessments include mood and quality of life questionnaires; in-person assessments include neuropsychological evaluation, brain MRI, ocular imaging, and blood collection.

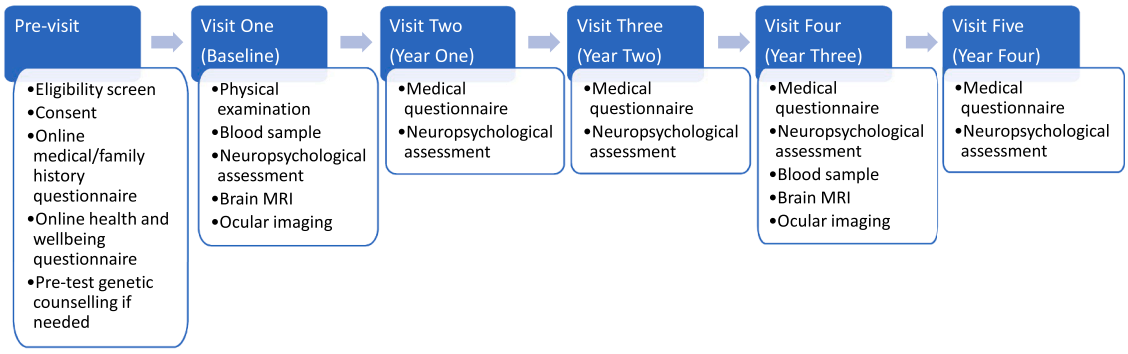

Fig. 1. Flowchart of AusCADASIL assessment steps at pre-visit, baseline (visit one), and follow-up (visits two-five).

Recruitment and participants

Individuals confirmed or suspected as having CADASIL ($n = 150$) will be recruited from online support groups, neurology clinics and genetic services. Family members, carers, previous study participants (those who have consented to being approached about future research studies), and the general population will be approached for recruitment to the variant-negative comparison group (controls, $n = 150$). The required target sample size is based on statistical power of 80% with an alpha of 0.05, and sufficient for analyses across five cognitive domains (executive function, memory and learning, language, complex attention, and perceptual-motor function) and a global cognitive score, and five primary neuroimaging markers of cSVD (white matter hyperintensities, microbleeds, lacunes, perivascular spaces, cerebral blood flow). This is in line with overseas studies focused on cognition in CADASIL [11], clinical markers of CADASIL [8], and longitudinal change in CADASIL [10].

Participants will be recruited by advertisement through multiple channels and venues. A purpose-designed leaflet containing study information and research team details, will direct potential participants to contact the team to express an interest in participating. A separate information leaflet directed to family members will be provided to participants who may voluntarily choose to share the details with them.

Eligibility

Participants will be assessed for eligibility to participate via a telephone screening call according to the following criteria:

Inclusion criteria:

1. Adults ≥18 years old
2. Ability to provide written informed consent
   - A large-print version is available for individuals with vision impairment
   - An easy-to-read version is available for individuals with cognitive difficulties who may require extra support
   - Support from a suitable interpreter, where available, for individuals from non-English speaking backgrounds who have limited ability to read, write, or otherwise communicate in English
3. Ability to attend a testing site
4. Ability to complete minimum dataset (medical examination and medical history questionnaire, blood test to determine genetic status and a short (20 min) neuropsychological assessment).
5. CADASIL participants according to one of the following categories:
   a) confirmed diagnosis via genetic testing (*NOTCH3* pathogenic variant), OR
   b) suspected diagnosis based on medical history and brain MRI, OR
   c) Relative of individual with CADASIL, who is positive for the *NOTCH3* pathogenic variant.

OR

6. Control participants:
   a) Relative of individual with CADASIL, who is negative for the *NOTCH3* pathogenic variants, OR
   b) Individual who has no known CADASIL relative, and no cognitive impairment.

Exclusion criteria:

1. Significant cognitive impairment leading to an inability to provide informed consent.
2. Confounding comorbidities such as HIV/AIDS, multiple sclerosis, metastatic cancer, active autoimmune disease, and sarcoidosis. Requirement to exclude based on previous clinical investigations and discussion with treating clinician.

Measures and objectives

Table 1 outlines the study's measures, related objectives and assessments, with more details of the assessments outlined below.

The primary hypothesis of AusCADASIL is that there will be differences in the cognitive profile, proposed imaging markers and select blood proteins between those with CADASIL and the healthy control group. Secondary to this, we propose that these markers will be linked to clinical severity of CADASIL. Finally, we hypothesize that the patterns of features observed in the CADASIL cohort will be linked to the identified pathogenic variant characteristics.

Assessment

Assessments, performed over multiple visits, if necessary, comprise the below components, which will be completed within 3 months. Participants will return for annual follow-ups for a total of 4 years.

i. Consent

All participants will provide written informed consent. It is not necessary to consent to all components, only to collection of the minimum dataset. Participants can consent to having their assessment results provided to them and/or their referring clinician. This includes a general summary of assessment results as well as any

Table 1
AusCADASIL measures, objectives, and assessments.

Measures	Objectives	Assessments and timepoint
Clinical Profile: • Clinical features of CADASIL including motor deficits, migraine, apathy, fatigue, depression, anxiety • Medical and family history of CADASIL and vascular disease	To characterise the clinical features of the disease that contribute most to disability (e.g., motor deficits, migraine, cognitive impairment, apathy, fatigue, depression, anxiety). To describe the role of vascular risk factors (e.g., smoking, hypertension, diabetes) for stroke in CADASIL patients. To determine the longitudinal course of mood disturbances, cognitive impairment, and MRI changes in CADASIL.	Online questionnaires at baseline and annual follow-up Structured physical examination at baseline
Neuropsychological Profile: • Test battery assessing cognitive capacity/decline	To characterise the cognitive profile and pattern of progression, and to identify neuropsychological tests particularly sensitive to cognitive deficits in CADASIL.	Neuropsychological evaluation at baseline and annual follow-up
Biomarkers: Neuroimaging	To describe changes on MRI that are useful for the diagnosis of CADASIL.	MRI brain scan at baseline and after three years
Biomarkers: Blood	To determine blood marker changes that are associated with CADASIL. To develop a blood biobank for CADASIL to further develop biomarkers for vascular cognitive impairment and dementia and cSVD.	Blood sample donation at baseline and after three years
Biomarkers: Ocular	To describe retinal/ocular changes that are associated with CADASIL.	Ocular examination at baseline and after three years
Genetic Profile	To determine any correlation between different types of *NOTCH3* pathogenic variants and clinical presentation.	Genetic testing at baseline

secondary or incidental health findings that are identified from the study assessments.

ii. Online questionnaires

Participants will be asked to complete the questionnaires listed below, within a week of each other. The medical and family history questionnaire asks participants to provide details of their CADASIL as well as other systemic health concerns including diagnoses, symptoms, medications, and treatment regime. Participants will be asked to nominate a study partner, someone who knows them well (e.g., carer or spouse), to complete additional online questionnaires about the participant's mental and physical functioning.

Questionnaires will be administered using the Research Electronic Data Capture (RedCap) platform (https://projectredcap.org/), prior to the first clinic visit, with the option for participants to complete these in-person at their clinic visit if required.

a) Medical and family history questionnaire (developed in-house)
b) Hospital Anxiety and Depression Scale[20]
c) Patient Health Questionnaire-9[21]
d) Multidimensional Fatigue Inventory[22]
e) Quality of Life Scale (EQ-5D-5L)[23]
f) Apathy Evaluation Scale[24]
g) PROMIS Sleep Disturbance[25]
h) STOP-BANG Questionnaire for Sleep Apnoea[26]
i) COVID-19 questionnaire (developed in-house, based on the 45 and Up Study)[27]
j) Instrumental Activities of Daily Living Scale[28]
k) Neuropsychiatric Inventory - Questionnaire[29]

Questionnaire results will be compared between groups to identify any differences in health, wellbeing, and functioning. Results will also be compared within and between groups across the follow-up timepoints to assess any changes.

iii. Neuropsychological Assessment

Participants will be invited to complete an in-person neuropsychological assessment (phone option available if required). The battery (Table 2) has been curated to assess all cognitive domains with a focus on those previously identified to be affected in CADASIL, including executive function and attention [30]. For participants affected by disability or significant impairment, only the minimum dataset (20 min) will be required to be completed. Individuals not fluent in English will be administered the minimum dataset tests, with an interpreter using published translated test versions, where available. Years of education, age, sex, and handedness will also be recorded. Published norms (age- and education-adjusted [where available]) will be used to aid in clinical interpretation and analysis. Analysis will examine cognitive domains and a global index of cognitive functioning, compared between groups, and over time.

iv. Structured physical examination

The physical and neurological exam will be performed by a clinician or trained researcher. This examination will follow a guided protocol including the National Institutes of Health Stroke Scale [36], the Modified Rankin Scale [37], as well as measures of blood pressure, height, weight, waist and hip circumference, speed, stability and grip strength. These measures will be used to give an indication of disease severity in combination with the other assessment results.

v. Blood sample

A blood sample (volume of approximately 45 mL) will be collected at clinics or affiliated pathology services, by professionals trained in venipuncture. The measures include:

1) blood chemistry (Fasting glucose, HbA1c, lipids, C-reactive protein, creatinine, liver function tests and vitamin D).
2) blood archived for genomics, proteomics, lipidomics, metabolomics, epigenomics and further blood biochemistry.
3) all participants with suggestive medical history and supportive neuroimaging findings will be tested for *NOTCH3* variants, unless the results of a previous accredited test are available.

Following extensive literature review, a panel of approximately 15–20 plasma biomarkers will be selected relevant to various pathways thought to be involved in the pathogenesis of CADASIL including endothelial dysfunction, blood brain barrier breakdown, neurovascular unit dysfunction, neuroinflammation, cardiac dysfunction, coagulation, and thrombosis [19,38,39]. We will also investigate correlations between plasma biomarkers, neuroimaging markers and cognitive features. Analytes will be multiplexed using Invitrogen ProcartaPlex assay (Thermo Fisher, USA)[40] using the Bioplex 200 instrument platform (Bio-rad, California, USA) which employs a bead-based multiplex assay to allow simultaneous detection of multiple analytes within a single sample. It involves coupling capture antibodies of target analytes to fluorescent beads, incubating them with the plasma samples, and then detecting bound proteins using fluorescently labelled detection antibodies. The instrument quantifies the fluorescence signals emitted by the beads, allowing simultaneous measurement of multiple biomarkers. Standard curves generated from known concentrations of reference proteins are used to determine the concentrations of the protein biomarkers in the samples.

Table 2
Neuropsychological battery for baseline and annual follow-up.

MINIMUM DATASET (20 min)
Montreal Cognitive Assessment (MoCA)-Full [31]*
Trail Making Test Part A [32]^
Trail Making Test Part B [32]^
Category Fluency (Animals) [33]*
Digit Span Backwards (WAIS-IV) [34] *
SHORT BATTERY (50 min) - includes these additional computerised tests [35]
NIHCTB: Flanker
NIHCTB: Pattern Comparison Processing Speed
NIHCTB: Picture Sequence Memory
NIHCTB: Dimensional Change Card Sort
FULL BATTERY (65 min) – includes these additional tests
Letter Fluency (FAS) [33]*
Rey Auditory Verbal Learning Test [33] *

NIHCTB: National Institute of Health Cognition Toolbox.
^ For patients with motor issues, e.g., hemiplegia, Trails A/B will be replaced with Oral Symbol-Digit Modalities test.
* Phone-only neuropsychological battery excludes Trails A/B and the NIHCTB, all other tests retained (MoCA-Blind replaces MoCA-Full).

vi. Brain MRI scan

A comprehensive set of non-contrast MRI sequences (approximately 1 hour) will be administered to all consenting AusCADASIL participants, except those who have contraindications to MRI scanning (including implanted devices, pacemakers and/or claustrophobia), for whom recent neuroimaging records will be obtained instead, where possible.

The MRI will be focused on investigating common features in CADASIL including white matter hyperintensities, dilated perivascular spaces, lacunes, microbleeds, atrophy, and cerebral blood flow. The core MRI sequences will be harmonised across sites where possible, to include bandwidth-matched structural imaging, T2-weighted fluid-attenuated inversion recovery, susceptibility-weighted MRI, diffusion-weighted MRI, pseudo-continuous arterial spin labelling, and resting-state blood oxygenation level dependent imaging. Global oxygen extraction fraction mapping and myelin water imaging sequences may also be implemented, depending on site-availability. More detailed MRI protocol information is discussed in the supplementary material.

vii. Ocular assessment

A comprehensive vision and retinal assessment (approximately 90 min) will be obtained when feasible. Equipment and protocols will be harmonised where possible but may vary at individual testing sites. This will include a structured ocular history and symptoms questionnaire, distance high contrast visual acuity, near acuity, contrast sensitivity, stereopsis, tonometry, perimetry, slit lamp biomicroscopy, pupil dilation (tropicamide 0.5 % or 1.0 %), colour fundus photography (45°) of the posterior pole and/or ultra-widefield retinal imaging where available.

Multimodal imaging will include near infrared reflectance, fundus autofluorescence, optical coherence tomography (OCT) and OCT angiography of the posterior pole and optic nerve head.

High quality image features will be compared with primary outcomes, accounting for age and sex, to identify associations of ocular manifestations with known CADASIL features. Additionally, change in retinal health and visual functioning will be compared between groups.

viii. Genetic Testing/Counselling:

All participants will have received genetic testing prior to study enrolment unless they have suspected CADASIL (from clinical investigation) or are a control participant with no known relative with CADASIL.

Genetic counselling will be offered through a clinical genetics service as Telehealth or in-person, and structured according to the Human Genetics Society of Australasia guidelines [41].

Participants who have already had prior clinical investigations as per standard care indicating suspected CADASIL will undergo targeted diagnostic *NOTCH3* genetic testing, using a pre-established 15 gene panel at the Queensland University of Technology. This panel contains *NOTCH3*, alongside other genes known to cause conditions with overlapping symptoms and potential differential diagnoses. These individuals will receive genetic counselling both prior to and following blood test results.

Untested control participants (no known relative with CADASIL) will be provided with pre-test education material on CADASIL and genetic testing. In the unlikely event that a positive *NOTCH3* finding occurs in control individuals recruited from the general population, the participant will be offered referral to a genetic service for the option of clinically validated genetic testing to confirm a diagnosis.

All participants will have the option of follow-up genetic counselling appointments at their request, as per standard care.

Feedback will be provided to participants and general practitioner/treating physician in accordance with individualised consent. This will include assessment feedback and/or any secondary or incidental health findings.

Safety and risk assessment

All assessments are safe and pose minimal risk to participants. There is a risk of psychological harm from genetic testing for CADASIL (testing positive for a *NOTCH3* pathogenic variant) and/or discovering neuroimaging markers of cSVD or other neuropathology. Participants will have the option to not be told if clinical indication for CADASIL is discovered (option provided on consent form). However, if they choose to know research results, participants will be referred for genetic counselling (available through the AusCADASIL study), and/or to a neurologist for further discussion. Only clinically accredited *NOTCH3* results will be provided to participants. Participants who prefer not to know their genetic status can decline to have a genetic test at the pre-test genetic counselling stage.

Privacy and confidentiality

All data collected in this study will be deidentified and stored securely using RedCap and the Monash Secure eResearch Platform. Physical data will be stored in locked cabinets. Biospecimens will be stored at the University of New South Wales in freezers only accessible to approved researchers.

Discussion

AusCADASIL is the first Australian cohort study of people affected by CADASIL. Australia is a multicultural society with extensive ethnic diversity. The predominant ancestry is reported to be from the United Kingdom and Europe (51–81 %)[42] and, therefore the clinical presentation and pedigree of CADASIL is hypothesized to be most similar to individuals from those regions [9,43]. However, there is also a growing Asian and South East Asian population [42], which may influence the range of clinical presentation of CADASIL[44,45] in Australia. This multi-ethnic distribution adds to the novelty of AusCADASIL in bringing together individuals from these previously studied regions. This study will represent a sample of individuals living with CADASIL in Australia and identify the genetic variation specificities in this cohort alongside any diversity in their clinical features.

The CADASIL clinical phenotype follows similar patterns, but there is reported diversity in the onset, severity, and combination of symptoms. Some variation may be accounted for by vascular risk factors, though ethnic pedigree and pathogenic variant may be other likely explanations [46]. The greatest population-based heterogeneity seems to be in some Far East pedigrees, where there is, typically, a lower prevalence of migraine and mood disturbance, later symptom onset, and higher incidence of cerebral microbleeds and haemorrhagic stroke, than typically reported in Western studies [12,47]. Given the rarity of CADASIL, many studies report data from relatively small cohorts. This may lead to underestimation of the heterogeneity and prevalence of the disease. Large-scale genome studies have suggested higher than expected prevalence of *NOTCH3* variants in the general population [4], with variation across ethnic groups [47,48].

The *NOTCH3* gene has 33 exons, with most CADASIL-causing variants located in exons 2–24, which encode the 34 epidermal growth factor-like repeat (EGFr) domains. Advancements in genetic sequencing methods have enabled increased detection of variants, including additional cysteine variants in exons 11, 18 and 20, and a novel missense mutation [49]. The affected exon varies across populations and ethnicities [50]. Further, the prognosis seems to be dependent on the pathogenic variant. The classic CADASIL disease phenotype appears to be linked to the domain of the cysteine amino acid change, whereas cysteine-altering variants in the 1–6 EGFr domains have been associated with earlier stroke onset and encephalopathy [43], as well as more severe presentation and worse prognosis [13].

Through AusCADASIL we intend to build on previous studies to identify any risk factors that are common to this cohort, which may be

potentially modifiable, such as hypertension and smoking [46]. We will describe any longitudinal, clinical or neuropsychological features of CADASIL [7,11], as well as any predictors of worsening [51]. The combination of traditional pen-and-paper neuropsychological assessments and the computerized NIH Cognition toolbox which has been validated in clinical samples including stroke [52,53], is designed to provide AusCADASIL with a comprehensive understanding of the cognitive profile and progression of cognitive functioning in CADASIL, whilst providing a novel opportunity to recognise the use of computerised tools for this cohort. This study will use neuroimaging outcomes to characterise disease[54,55] and identify the sensitivity of longitudinal MRI markers of cSVD to disease progression [10]. AusCADASIL aims to expand on current knowledge of neuroimaging features by including anatomical and vascular sequences as well as more novel approaches to investigate brain oxygen extraction and myelination. There have been some promising reports of blood biomarkers which we intend to validate and expand upon including serum neurofilament light [56], and plasma endostatin and the Notch 3 extracellular domain [57]. We also intend to investigate the purported association between CADASIL severity and ocular markers such as reduced retinal vessel density and retinal thickness [58–60]. Most previous ocular literature in CADASIL is limited to one modality and small sample size [59]. We aim to include multiple ocular imaging technologies and functional assessment to better encapsulate the potential range of ocular manifestations in this cohort. Should AusCADASIL be successful in identifying biomarkers of CADASIL, these may serve in disease management and/or as clinical trial outcomes. AusCADASIL has initiated discussion with international researchers of CADASIL to develop the Global CADASIL Consortium. This will facilitate the harmonisation of CADASIL protocols as well as the sharing of data and biospecimens, enabling potential validation of biomarkers identified in AusCADASIL across different cohorts. This has the advantage of analysis in a larger sample size and with increased cohort diversity.

AusCADASIL will work in conjunction with online support groups and patient advocacy initiatives to provide resources and support to Australians who live with this rare disease. We aim to build on the global understanding of the clinical presentation and progression of CADASIL and hope to be a key contributor to international collaborative efforts in CADASIL.

Declarations

Funding

This study is supported by a National Health and Medical Research Council (NHMRC) grant for a Centre of Research Excellence in Vascular Contributions to Dementia (Grant: 2006765).

Informed consent

Written informed consent will be obtained from all subjects before the study.

Ethical approval

Ethical approval for this study was obtained from the Hunter New England Human Research Ethics Committee (2023/ETH01132), all sites have been approved by the main ethics. All study procedures will adhere to the tenets of the Declaration of Helsinki.

Trial registration

AusCADASIL has been registered on ClinicalTrials.gov (identifier: NCT06148051).

Guarantor

PSS

Contributorship

PSS, MP, AB, CL, JCK, MO, and KB conceived the study and obtained funding. All authors were involved in overall protocol development. DGS, BB, VSC, RM and AP were involved in gaining ethical approval. JJ, CMH, and WW developed the MRI protocol. JH-L, LN-S and PvW developed the ocular examination protocol. KAM, LRG, RAS informed genetic testing and analyses. AS and PJ developed the genetic counselling protocol. ACB and NAK developed the neuropsychological protocol. TJ, AP, GKH, SH and TJH developed the biomarker protocol. AT and RC will inform statistical analyses. DGS, SH, RJC, BB, and AP will be involved in testing. DGS operationalised the protocol, compiled the testing manual and data management protocol, and wrote the first draft of the manuscript. All authors reviewed and edited the manuscript, and approved the final version.

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Declaration of competing interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cccb.2024.100225.

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