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Title Page

Magnetic resonance imaging in CADASIL and small vessel disease

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February 2024

1 Abstract

Introduction

CADASIL is the most common monogenic cause of small vessel disease affecting the brain. CADASIL results in subcortical infarcts, dementia, and disability. Magnetic resonance imaging reveals often extensive white matter hyperintensities, multiple lacunes, cerebral microbleeds and enlarged perivascular spaces. These generally occur at a younger age and in the absence of conventional vascular risk factors when compared to sporadic small vessel disease. The location of the cysteine altering NOTCH3 variant on the EGF domain is recognised as significant in determining disease progression and extent of imaging abnormalities. Other factors, that are not completely delineated, influence the rate of disease progression, and must account for the significant variation and unpredictable nature of this within CADASIL.

Methods

We used conventional MRI and ultra-high field intensity MRI to further investigate the relationships between clinical and imaging features in CADASIL and make comparisons with sporadic cerebral small vessel disease. We updated the population data for CADASIL in Scotland to estimate the current disease prevalence. We used 7-Tesla MRI in people who have recently had a lacunar infarct to detect the susceptibility vessel sign on susceptibility-weighted imaging and correlated this with time-of-flight magnetic resonance imaging to confirm small vessel thrombotic occlusion. We quantified differences between 1.5, 3 and 7-Tesla MRI in this setting for detection of lacunes, cerebral microbleeds and enlarged perivascular spaces. We used 7-Tesla MRI in a human volunteer study to establish measurement of flow velocity in the lenticulostriate branches of the middle cerebral arteries as a surrogate measure of blood flow to the basal ganglia region.

Main results

We estimated a disease prevalence of 2.76 per 100,000 based on clinical attendance at a national specialist clinic. The majority of people had a proximal genetic mutation with 59.7% mutations found at exon 4. The distribution of white matter hyperintensities, lacunes and cerebral microbleeds differed between CADASIL and sporadic small vessel disease. For white matter hyperintensities the anterior temporal pole and parietal lobe were

significantly more commonly affected in CADASIL. Lacunes were more likely to be present in the temporal and frontal lobes, and cerebral microbleeds were more likely to be found in the thalamus and brainstem in CADASIL and there was a suggestion of more microbleeds in the internal capsule. MRI in CADASIL displays significantly larger volumes of white matter hyperintensities, and larger numbers of lacunes and cerebral microbleeds than in sporadic small vessel disease even when compared to an older cohort with more extensive co-morbidities. Our cohort continues the previous genetic pattern of proximal genetic mutations (EGFR 1 to 6) displaying more extensive white matter hyperintensities, lacunes and cerebral microbleeds and suffering from earlier onset of stroke, as compared to those with distal mutations (EGFR 7 to 34) who are more likely hypertensive.

7-Tesla MRI provides detailed images of cerebral microbleeds and may identify susceptibility vessel sign on relevant sequences. We have not been able to corroborate this by visualising an associated occluded vessel on time-of flight magnetic resonance angiography in any of the cases. We used these images to discover that 7-Tesla allows visualisation of higher numbers of enlarged perivascular spaces compared to 1.5 and 3-Tesla. We were able to use phase-contrast imaging at 7-Tesla to assess the flow velocity of lenticulostriate vessels which may serve as a correlate to cerebral blood flow measured using alternative methods.

Conclusions

CADASIL is a rare small vessel disease with distinct imaging features. The extensive imaging abnormalities do not consistently correlate with extent of disability and symptom burden. Although there is increasing recognition of the genetic and clinical factors that influence outcome in CADASIL, there is a need to better understand the reasons behind and potential protective factors that cause such variable outcome. Further investigation of small vessel dysfunction and blood flow using ultra-high field strength will hopefully allow us to better understand these mechanisms, appreciate differences between genetic and sporadic small vessel disease and provide utility in investigating therapeutic interventions.

Table of Contents

Contents

1	Abstract	ii
	Table of Contents.....	iv
	List of Tables	vii
	List of Figures	viii
	Acknowledgements.....	x
	Author's Declaration	xi
	Definitions/Abbreviations.....	xii
2	Introduction	1
2.1	History of CADASIL.....	1
2.2	CADASIL Epidemiology	1
2.3	Genetics in CADASIL.....	3
2.3.1	The NOTCH3 receptor.....	3
2.4	Clinical features of CADASIL	4
2.4.1	Migraine and migraine aura.....	4
2.4.2	Subcortical ischaemic events	5
2.4.3	Mood disorder.....	5
2.4.4	Cognitive impairment and dementia.....	6
2.4.5	Seizures.....	6
2.4.6	Blood pressure in CADASIL	6
2.4.7	Other clinical features.....	7
2.4.8	Prognosis in CADASIL	7
2.4.9	Disability measures in CADASIL	8
2.4.10	Correlation between genetics and prognosis	9
2.4.11	Magnetic resonance imaging in CADASIL	11
2.4.12	High field strength MRI for structural imaging in small vessel disease ...	17
2.5	Aims and hypotheses of this thesis.....	25
3	Neuroimaging correlates of disease progression and disability in CADASIL: a systematic review.....	3-26
3.1	Abstract	3-26
3.2	Introduction.....	3-27
3.3	Methods.....	3-29
3.4	Results	3-31
3.4.1	Imaging correlates of disability	3-32
3.4.2	Imaging correlates of disease progression longitudinally	3-35
3.5	Conclusions.....	3-37
	Appendix 1. Longitudinal studies.....	38
	Appendix 2. Cross-sectional studies	44

4	Population database description and epidemiology update CADASIL in Scotland and registry	4-58
4.1	Introduction	4-58
4.2	Methods	4-58
4.3	Results	4-59
4.4	Discussion	4-63
5	Anatomical descriptive study of small vessel disease biomarkers in CADASIL and sporadic small vessel disease.....	5-65
5.1	Introduction	5-65
5.2	Methods	5-65
5.3	Results	5-66
5.3.1	White matter hyperintensities	5-66
5.3.2	Lacunes	5-75
5.3.3	Cerebral Microbleeds	5-80
5.4	Discussion	5-83
6	Genotype-phenotype correlations in CADASIL and comparison with sporadic small vessel disease	6-85
6.1	Comparison of proximal versus distal mutations and low medium and high-risk mutations.....	6-85
6.2	Abstract	6-85
6.3	Introduction	6-86
6.4	Methods	6-86
6.4.1	Study Cohort	6-86
6.4.2	Clinical data	6-87
6.4.3	Brain imaging	6-87
6.4.4	Image processing	6-87
6.4.5	Statistical analysis	6-89
6.5	Results	6-90
6.5.1	Intra- and inter-rater reliability for WMH measures	6-90
6.5.2	Comparison of EGFr domains 1 to 6 with EGFr domains 7 to 34	6-92
6.5.3	Comparison of CADASIL with sporadic small vessel disease.....	6-93
6.5.4	Stroke free survival analysis comparison EGFr domains 1 to 6 and EGFr domains 7 to 34	6-93
6.5.5	Imaging analysis of high, medium and low risk CADASIL with matched cohort of spontaneous small vessel disease	6-94
6.6	Discussion	6-109
7	Rate of progression of imaging markers in CADASIL: a retrospective imaging analysis	7-112
7.1	Introduction	7-112
7.2	Methods	7-112
7.3	Results	7-113

7.4	Discussion	7-116
8	LACUNAr infarcts, Microvascular Arteriopathy: a 7-Tesla Analysis of Thrombosed Arteries (LACUNAR MATATA)	8-118
8.1	Introduction	8-118
8.2	Methods	8-119
8.3	Results	8-120
8.4	Discussion	8-123
9	Comparison of small vessel disease imaging markers at 7-Tesla with lower field strengths	9-124
9.1	Introduction	9-124
9.2	Methods	9-125
9.3	Results	9-126
9.4	Discussion	9-130
10	Imaging technical development study at 7-Tesla	10-131
10.1	Introduction	10-131
10.2	Methods	10-132
10.3	Results	10-135
10.4	Discussion	10-137
11	Discussion	11-139
11.1	Research problem and aims of thesis	11-139
11.2	Summary of key findings	11-139
11.3	Interpretation of results and their meaning	11-140
11.4	Limitations of studies	11-144
11.5	Future directions	11-145
	List of References	146

List of Tables

Table 0.1 Longitudinal studies summary	38
Table 0.2 Cross-sectional study summary	44
Table 4.1 Estimated disease prevalence in Scottish regions and overall estimated mutation prevalence	4-60
Table 4.2 Distribution of genetic mutations in Scottish CADASIL cohort.	4-61
Table 5.1 Baseline characteristics for CADASIL and sporadic small vessel disease....	5-69
Table 5.2 Anatomical distribution of WMH and Fazekas score in CADASIL and sporadic small vessel disease.....	5-70
Table 5.3 Binary logistic regression model for location of WMH as independent variable and presence of CADASIL as dependent variable.....	5-74
Table 5.4 Differences in anatomical distribution of lacunes in CADASIL and sporadic small vessel disease	5-75
Table 5.5 Mann-Whitney U comparison of lacunes in CADASIL and sporadic small vessel disease	5-76
Table 5.6 Binary logistic regression with presence of lacune as independent variable and CADASIL as dependent.....	5-78
Table 5.7 Results of Mann-Whitney U test for differences in number of microbleeds between CADASIL and sporadic small vessel disease.	5-81
Table 5.8 Comparison between proportion of patients in each group affected by microbleeds.	5-82
Table 6.1 Summary characteristics of Scottish CADASIL population.....	6-95
Table 6.2 Imaging features of proximal and distal mutations in Scottish CADASIL cohort	6-96
Table 6.3 Comparison clinical characteristics CADASIL and sporadic small vessel disease cohort	6-97
Table 6.4 Imaging features of Scottish CADASIL cohort and sporadic small vessel disease	6-98
Table 6.5 Comparison characteristics CADASIL and sporadic small vessel disease cohort propensity score matching cohort	6-99
Table 6.6 Imaging features of Scottish CADASIL cohort and sporadic small vessel disease propensity score matching cohort	6-100
Table 6.7 Results of Kruskal-Wallis analysis of high, medium and low risk CADASIL against matched cohort of sporadic small vessel disease	6-101
Table 7.1 Baseline clinical characteristics proximal and distal mutations	7-114
Table 7.2 Differences in rate of progression each year of imaging markers in proximal and distal mutations.....	7-114
Table 7.3 Differences in clinical features between proximal and distal mutations	7-115
Table 8.1 Clinical data for study participants.	8-120
Table 9.1 Comparison of 1.5 / 3-Tesla MRI with 7-Tesla.....	9-126
Table 10.1 Flow velocity outputs in individual vessels shown in figure above ROIs from left to right.....	10-136

List of Figures

Figure 2.1 Representation of CADASIL EGFr domains divided into high, medium and low risk mutations along the NOTCH3 protein (81)	10
Figure 2.2 MRI imaging features of CADASIL (85)	12
Figure 2.3 MRI imaging features of small vessel disease (82)	16
Figure 2.4 (a) 1.5T MRI (b) Stroke at 7T (c) control at 7T (136)	19
Figure 3.1 Search strategy and process of study selection	3-31
Figure 5.1 Example of extensive white matter hyperintensities and lacunes in CADASIL.5-67	
Figure 5.2 Example of multiple lacunes in CADASIL.	5-67
Figure 5.3 Cerebral microbleeds in the thalamus in CADASIL.	5-68
Figure 5.4 Differences in Fazekas score between CADASIL (1) and sporadic small vessel disease (0)	5-71
Figure 5.5 Comparison of WMH anatomical distribution CADASIL and sporadic small vessel disease	5-72
Figure 5.6 Comparison of lacunes anatomical distribution in CADASIL and sporadic small vessel disease	5-72
Figure 5.7 Comparison of cerebral microbleed anatomical distribution in CADASIL and sporadic small vessel disease.....	5-73
Figure 5.8 Distribution of lacune number in CADASIL and sporadic small vessel disease.	5-79
Figure 5.9 Proportion of CADASIL cases in each group based on number of cerebral microbleeds.	5-80
Figure 6.1 Example image with white matter hyperintensities and intracranial cavity volume.	6-88
Figure 6.2 Correlation between calculated WMH volume and measured Fazekas score	6-90
Figure 6.3 Repeat measure of normalised WMH volume in CADASIL	6-91
Figure 6.4 Bland-Altman plot for repeat measures of WMH volume in CADASIL ...	6-91
Figure 6.5 Comparison of measured WMH volume readings in this study with official study data	6-92
Figure 6.6 Kaplan-Meier survival curve for age of first stroke in CADASIL	6-102
Figure 6.7 Comparison of WMH against age for CADASIL proximal and distal mutations and sporadic small vessel disease	6-103
Figure 6.8 Comparison of WMH against age for CADASIL proximal and distal mutations and propensity matched sporadic small vessel disease	6-103
Figure 6.9 Distribution of CADASIL cases across different EGFr domains	6-104
Figure 6.10 Comparison of WMH against age for CADASIL low, medium and high risk mutations and a propensity score matched sporadic small vessel disease	6-105
Figure 6.11 Age against normalised WMH divided into low medium and high-risk CADASIL genetic mutations.....	6-105
Figure 6.12 Comparison of Fazekas score distribution in CADASIL and sporadic small vessel disease.	6-106
Figure 6.13 Comparison of age versus WMH volume in CADASIL and sporadic small vessel disease	6-106
Figure 6.14 Age versus Fazekas score in CADASIL and sporadic small vessel disease ..	6-107
Figure 6.15 Age versus lacune count in CADASIL and sporadic small vessel disease	6-107
Figure 6.16 Age versus cerebral microbleed count in CADASIL and sporadic small vessel disease	6-108
Figure 6.17 Age versus enlarged perivascular spaces in CADASIL and sporadic small vessel disease	6-108

Figure 7.1 Correlation between rate of progression of WMH and lacunes in proximal and distal mutations	7-115
Figure 7.2 Boxplot with differences in rate of progression of WMH, lacunes and CMBs in proximal and distal mutations	7-116
Figure 8.1 (A-F). SWI with DWI sequences showing likely SVS and infarct locations ..	8-121
Figure 8.2 (A-F). SWI, with DWI sequences showing possible SVS and infarct locations.	8-122
Figure 8.3 Axial MRI sample distribution of lacunar infarct location in our study population.	8-122
Figure 9.1 3-Tesla (left) and 7-Tesla (right) SWI imaging of cerebral microbleeds...	9-128
Figure 9.2 7-Tesla (left) and 1.5-Tesla (right) SWI MRI.....	9-128
Figure 9.3 7-Tesla (left) and 3-Tesla (right) SWI imaging of cerebral microbleeds...	9-128
Figure 9.4 T2-TSE imaging two 3-Tesla images and one 7-Tesla image (right) for EPVS	9-129
Figure 9.5 7-Tesla (left) and 3-Tesla (right) T2-TSE MRI for EPVS	9-129
Figure 10.1 Image analysis processing pathway (161)	10-134
Figure 10.2 a to e Examples of output display of MCA and LSA with box ROI selecting vessels for flow velocity measurement. Boxes labelled 1 to 4 from left to right on each image.....	10-135
Figure 10.3 Example output from phase-contrast imaging at 7-Tesla	10-137

Acknowledgements

I would first like to sincerely thank my supervisor Prof Keith Muir, an expert in all things stroke and CADASIL. I've thoroughly enjoyed the time spent discussing the small vessels. Prof David Porter has also been very supportive and offered great guidance throughout.

I would like to thank other researchers involved in this thesis including Dr Natasha Fullerton for her neuroradiology expertise, Dr Rosemary Davidson for her input on CADASIL genetics, Prof Jesse Dawson, Dr Fiona Moreton and Dr Stephen Makin for allowing me access to valuable imaging data and David Dickie for advice on imaging analysis.

The 'brains of the operation' Pauline Hall-Barrientos, Graeme Keith, and Belinda Ding Yuan, thank you very much for your input through challenging circumstances. The research radiographers Rosie Woodward, Tracey Hopkins, Evonne McLennan and team, you've been great to work with. I would like to thank Shirley Mitchell for the extensive input into the study documents. Ammad, Viveka, Cameron, Laurence, Amith and William: thanks for the help and generally being a good laugh.

I am very grateful to the people who have very generously funded parts of the research including the Scottish Neurological Research fund, the Medical Research Council, Confidence in Concept Award, and the Academy of medical sciences.

Personally, I really appreciate the support from my parents Mags and Dave who have always encouraged me to study for as many degrees as possible.

Finally thank you to my wife, Deepa for being so patient, supportive, kind and encouraging. Been a great thesis journey.

Author's Declaration

All analyses are the work of the author unless otherwise stated.

The COVID-19 pandemic had an impact on the research included within this thesis. Recruitment to our prospective imaging study was postponed for several months in different stages during the early stages of the pandemic.

Definitions/Abbreviations

ADAS-cog	Alzheimer's disease assessment scale
ADC	Apparent diffusion coefficient
ASL	Arterial spin labelling
BET	Brain extraction tool
BG	Basal ganglia
BMET	Brief Memory and Executive Test
BOLD	Blood oxygen level-dependent
BOMBS	Brain Observer Microbleed Scale
BPF	Brain parenchymal fraction
CAA	Cerebral amyloid angiopathy
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CAMCOG	Cambridge Cognitive Examination
CBF	Cerebral blood flow
CBV	Cerebral brain volume
CCA	Common carotid artery
CI	Confidence interval
CMBs	Cerebral microbleeds
CSF	Cerebrospinal fluid
CSO	Centrum semiovale
DTI	Diffusion tensor imaging
DTI-ALPS	Diffusion tensor imaging analysis along the perivascular space
DWI	Diffusion weighted imaging
EGFr	Epidermal growth factor-like repeat
EPVS	Enlarged perivascular space
FA	Flip angle
FLAIR	Fluid attenuation inversion recovery
GCA	Global cortical atrophy
GE	Gradient-echo
GOM	granular osmiophilic material
ICA	Internal carotid artery
IQR	Interquartile range
LSA	lenticulostriate arteries
MARS	Microbleed Anatomical rating scale
MC	Mutation carrier
MCA	Middle cerebral artery
MD	Mean diffusivity
MDRS	Mattis dementia rating scale
MIP	Maximum intensity projection
MMSE	Mini-mental state examination
MOCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
mRS	Modified Rankin scale
NHS	National health service
NIHSS	National Institutes of Health Stroke Scale
OR	Odds ratio
PACS	Picture Archiving Communications System
PD	Proton density
QOL	Quality of life
ROI	Region of interest
SD	Standard deviation

SDMT	Symbol Digit Modalities Test
SIDAM	Structured interview for the diagnosis of Dementia of the Alzheimer type
SVD	Small vessel disease
SVS	Susceptibility vessel sign
SWAN	Susceptibility-weighted angiography
SWI	Susceptibility-weighted imaging
T	Tesla
TCD	Trans-cranial Doppler
TE	Echo time
TMT	Trail Making Test
TOF	Time-of-flight
TR	Repetition time
TSE	Turbo spin echo
VADAS-cog	Vascular Dementia Assessment Scale cognitive subscale
VENC	encoding velocity
VRS	Virchow-Robin space
WIP	work-in-progress
WMH	White matter hyperintensities
WMS	Wechsler Memory Scale

2 Introduction

2.1 History of CADASIL

The earliest likely description of CADASIL was reported in 1955 by Otto Binswanger, a German neuropathologist. He described two normotensive siblings who developed a progressive insidious onset condition including weakness, personality change and cognitive impairment (1). An entity called hereditary multi-infarct dementia was being studied in the 1970s. A family was studied with multiple generations suffering from stroke and disability and pathological confirmation of small infarcts in central grey and white matter (2). Observation of the family tree confirmed both sexes were affected and that transmission was apparently autosomal dominant in fashion (2). Chronic familial vascular encephalopathy had also been proposed as a description of what was presumed to be the same novel clinical condition described separately elsewhere (3).

The acronym CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) was established through a clinical and imaging study of an affected family who had recurrent subcortical type infarcts and extensive white matter changes in both symptomatic and asymptomatic family member (4). Genetic analysis of two separate families positioned the disease causal mutation on chromosome 19q12 (5). Further study of seven affected families allowed more detailed characterisation of the clinical features and imaging findings in CADASIL. This suggested a disease with complete penetrance and mean age of symptomatic presentation of 45 years. The pattern of clinical features included recurrent subcortical ischaemic events leading to dementia, pseudobulbar palsy, migraine with aura and mood disturbance (6).

A clinical syndrome was emerging that encompassed an autosomal dominant inheritance pattern, subcortical infarcts and vascular dementia and the brain appeared uniquely targeted.

2.2 CADASIL Epidemiology

Since the initial studies that included analysis of seven families from within France (6), there have been many studies analysing and estimating population prevalence of CADASIL in different geographical regions. Within the United Kingdom studies have estimated an initial prevalence of 2 per 100,000 adults and extrapolated this to estimate

probable mutation prevalence of 4 per 100,000 (7). These figures were updated to account for additional presentations and increasing disease recognition to a more recent prevalence of 4.6 per 100,000 adults with estimated mutation prevalence of 10.7 per 100,000 (8). A combination of definite, probable and possible cases of CADASIL also yielded a combined prevalence of 4.1 per 100,000 in the North of England (9), the same prevalence as reported in a retrospective study within Central Italy at minimum 4.1 per 100,000 (10). A separate group interrogated the large dataset of the Genome Aggregation Database (Gnomad) (11) and reviewed influence of race and geographical variation on Notch 3 prevalence and found highest prevalence of NOTCH 3 abnormality in East Asian and in South Asian populations at 1.1 and 1.2 % respectively (12). A Korean genetic database review has found a high rate of 1.2 per 1000 NOTCH 3 abnormalities detected and noted an even higher rate in the relatively isolated population in Jeju (13). A national CADASIL review in Japan showed a minimum prevalence of 1.2 per 100,000 with the assumptions made that the questionnaire methodology used might lead to a significant under-estimate (14).

Accepting the limitations of the methodologies and the geographical variation clinically diagnosed CADASIL had been considered a rare disease for more than the first two decades following its conception. This viewpoint was challenged with the marked contrast between disease prevalence estimates and the results of a population genetic study looking for NOTCH 3 genetic abnormalities distinctive for CADASIL in a larger population database of more than 60,000 people (15). This study discovered a prevalence of 3.4 per 1000 of the aberrant NOTCH 3 mutations, a surprise result at almost 100 times the frequency of the upper estimates of previous CADASIL disease prevalence (15). The UK biobank was also interrogated for NOTCH 3 abnormalities and findings confirmed a similarly high prevalence of 2.2 per 1000. This cohort also suggested that those with NOTCH 3 but no CADASIL diagnosis had higher white matter hyperintensity (WMH) load than controls and lower than people with already confirmed CADASIL diagnosis (16). These results posed a significant challenge for clinicians and epidemiologists involved in CADASIL both for clinical discussions and for research. Further study is needed to define CADASIL and understand differences between a clinical diagnosis of CADASIL, a genetic abnormality consistent with CADASIL and imaging features of CADASIL and people with NOTCH 3 abnormalities who appear to be asymptomatic carriers.

2.3 Genetics in CADASIL

2.3.1 The NOTCH3 receptor

CADASIL is caused by cysteine altering mutations in the NOTCH3 gene, previously mapped to chromosome 19p13 (17). NOTCH3 has involvement in differentiation and embryological development of vascular smooth muscle cells and integrity of the vessel wall structure (18). The NOTCH3 protein consists of an intracellular end, a transmembrane section and an extracellular end projecting from the edge of the cell. Signalling from the intracellular domain then leads to activation of transcription of genes that mediate the effects of NOTCH3 signalling (19). NOTCH3 is a receptor protein that attracts other cells called ligands which bind to the extracellular domain of the NOTCH3 protein. It has been reported that if NOTCH becomes inactive there is a tendency to develop epidermal precursors that convert normally functioning cells into neuroblasts (19). NOTCH3 has 33 exons and encodes a single-pass transmembrane receptor section essential for vascular smooth muscle function (20-22). Exons 2 to 24 of the NOTCH3 gene encode the 34 separate epidermal growth factor repeats (EGFr) of the extracellular domain. Each of these EGFRs contains six cysteine residues forming three disulfide bonds essential for ligand binding and stability (23). The ligands attach to the extracellular membrane and cause detachment of the intracellular domain. This NOTCH3 intracellular domain enters the cell nucleus and controls activity of other genes through transcription mechanisms. Accumulation of NOTCH3 in vascular smooth muscle of vessel walls is thought to result in deposition of granular osmiophilic material (GOM) first identified in 1993 (24). NOTCH3 is expressed throughout the body and is not unique to the brain, although clinically has specific relevance for blood vessels in the brain. An early study of CADASIL identified a more than 95% of the mutations were heterozygous missense mutations caused by single nucleotide exchange, with a few in-frame deletions (25) and splice site mutations (26). These causative mutations of the NOTCH3 gene in CADASIL, through gain or loss of a cysteine residue, result in an uneven number of cysteine residues in one of the 34 EGFr repeat domains. There have also been reports of cysteine-sparing missense mutation of the NOTCH3 resulting in typical clinical features of CADASIL (27). These variants include R61W (28), pR75P (29), pD80G (30), pR213K (31) and pG73A (32). By 2014 there was a list of more than 200 known individual cysteine altering missense mutations in NOTCH3 recognised as causative for CADASIL (33). Known mutations all occur in exons 2 to 24 which encode the 34 EGFr repeats of the extracellular domain, and this is reflected in current CADASIL genetic testing. New mutations continue

to be identified and repositories have been set up to record and share this data such as those stored within the Dutch registry (15), and the Human Gene Mutation Database run by clinical genetics in Cardiff (34).

2.4 Clinical features of CADASIL

2.4.1 Migraine and migraine aura

Migraine aura is very common in CADASIL. One study using questionnaire methodology found that more than half had a history of migraine attack (35). A majority (84%) of migraine episodes included migraine aura (35) and a further study in a different geographical CADASIL population reported 226 of 300 (75.3%) with migraine and nearly 90% of those with accompanying aura (36). Aura can be atypical in CADASIL for its prolonged nature or its very acute-onset (37). Symptoms can include hemiplegic weakness, basilar migraine aura symptoms, including dysarthria, ataxia, and vertigo (37). This degree of migraine aura is much more prevalent than within the general population with migraine. A large telephone survey of more than 4000 people carried out in the United Kingdom found migraine had occurred in around 1 in 7 respondents in the previous year, with a significant female preponderance (38) and there are systematic review papers that report similar prevalence (39). Just over a third affected by migraine or 5.8% of the population reported migraine aura (38). Despite the higher reported and suspected increased prevalence of migraine in CADASIL, the generally high frequency and common nature of migraine within the general population mean this is not specific to CADASIL.

Migraine encephalopathy is an extension of migraine aura that results in sometimes prolonged periods of fluctuating and reduced conscious level (40). This is frequent within the CADASIL population and cases lasting mean 8 days were reported in 11% of cases in one cross-sectional questionnaire study within the United Kingdom (36). Acute imaging during an episode of migraine encephalopathy using arterial spin labelling (ASL) brain perfusion has confirmed alterations to intracranial blood flow with hyperperfusion demonstrated on ASL MRI (41). MR imaging during acute episodes will usually show no DWI change but a small number demonstrate T2-weighted intensity changes of unilateral increased signal intensity and enlarged cortical gyri consistent with brain swelling (42). The nature of the connection between CADASIL and migraine is not completely understood although it could relate to dysfunctional vasoreactivity which has been

demonstrated to be abnormal in CADASIL population (43). Interestingly, migraine aura appears to confer some neuroprotection as evidenced by lower risk of stroke and less cognitive impairment and disability in a group with migraine aura compared to those unaffected (44).

2.4.2 Subcortical ischaemic events

Since early in the study of CADASIL, as the name suggests ischaemic events have been common in the population. Stroke or transient ischaemic attack (TIA) was the presenting symptom in 45 of 105 (43%) of an early CADASIL cohort with mean age of onset only 41 years old (45). Incident stroke is a common occurrence in CADASIL and through a two-year follow-up study 17 subjects reported a total 18 new stroke events over a two-year period. This corresponds to an incidence rate of 10.4 per 100 person years with 95% confidence interval (CI) 5.6 to 15.2 (46). More recently a larger cohort of 278 patients were followed up for 3 years and 55 (19.8%) developed incident stroke (47). Of this group preceding gait disturbance, active smoking and previous stroke were risks for incident stroke (47). The subcortical ischaemic events contribute to the development of vascular dementia and have a role in the development of lacunes and other abnormalities on MRI imaging seen in this population.

2.4.3 Mood disorder

Various mood disorder symptoms have been reported commonly in CADASIL and can be very significant in their impact of burden on quality of life. Apathy was present in 54 (41%) participants in an early CADASIL cohort study and was associated with depression as well as lower quality of life (48). Accepting the lack of structured criteria used to determine a diagnosis and resulting difficulty in differentiating between different mood disturbances, it is generally considered that psychiatric disorders might be present in near a quarter of patients studied (49). These include depression, occasional cases of bipolar disorder, behavioural and personality disorders as well as psychosis and panic attacks. The spectrum of mood disturbance is large and people with CADASIL are also prone to symptoms of irritability, and inappropriate laughing and crying emotions outwith associated depressive symptoms (50).

2.4.4 Cognitive impairment and dementia

A case-control study designed to determine the pattern of cognitive impairment in CADASIL described speed of processing as the primary domain of cognitive impairment in CADASIL, along with executive function and decreased attention problems to a lesser degree. These impairments developed early in the disease process, with mean age 47 years within the cohort (51). Global disability measured by modified Rankin scale score was associated with increasing age in a separate cohort which determined 60% of those above 60 years old to have dementia (52). This study also found impairment of executive function to be the most common symptom in the younger age group which progressed to a more severe global dementia affecting multiple domains at increased age above 60 years (52).

2.4.5 Seizures

Seizures have been reported as being associated with CADASIL and a recent study including 446 people with CADASIL found seizures reported in 8.6%, with just over a quarter having partial seizures (53). An earlier CADASIL study found fewer than 10% had a history of seizures and often the reported seizures in CADASIL co-existed with presence of stroke (54). Given the subcortical nature of the abnormalities typical of CADASIL, it is possible that the seizures occur as a result of cortical damage secondary to infarction and the CADASIL diagnosis is incidental (55).

2.4.6 Blood pressure in CADASIL

Hypertension is a common risk factor and likely causative in sporadic small vessel disease but in CADASIL a diagnosis of hypertension is less frequent, present in around 28% in one of the larger studies (53). One study of 127 patients with CADASIL found that hypertension, present in 20% of their cohort had no influence on clinical phenotype severity nor extent of MRI WMH abnormality (56), although a subsequent study of 147 patients demonstrated a correlation between hypertension and presence of cerebral microbleeds (CMBs) (57). More recently several studies have found an increased prevalence of hypertension in the more distal mutations EGFr 7 to 34 while also observing milder phenotype and less extensive MRI abnormalities (53, 58, 59). In a small case-

control study of 12 people with CADASIL were found to have lower daytime systolic, diastolic and mean arterial blood pressure and additionally found to have significant % reduction in mean arterial blood pressure over night time, than the control group (60). The recently published TREAT-SVD open-label randomised crossover trial investigated the effect of different anti-hypertensive medications on cerebral vasoreactivity measured using blood oxygen level-dependent (BOLD) imaging in response to hypercapnic challenge in CADASIL and sporadic small vessel disease (61). Unlike the sporadic small vessel disease cohort the medications, in particular amlodipine and losartan did have an effect on cerebral vasoreactivity in the 17 patients included with CADASIL (61). It remains feasible that blood pressure regulatory mechanisms or diurnal variation of blood pressure are contributory to the enlarged WMH volumes and to the symptomatology of CADASIL and further investigation of these mechanisms is required.

2.4.7 Other clinical features

Despite the presence of the aberrant NOTCH3 gene throughout the body the manifestation of CADASIL remains in the brain. There are very few reports of vascular disease elsewhere with one exception of observational high incidence of myocardial infarction in an early Dutch study (62). The patients involved in this study tended to have generalised coronary microvascular dysfunction with few showing typical focal stenosis. The lack of evidence of increased rates of cardiac disease in CADASIL comes even with imaging evidence of coronary artery dysfunction (63). Reasons for this lack of ischaemic heart disease and potential protective mechanisms merit further investigation.

2.4.8 Prognosis in CADASIL

CADASIL is associated with dementia and disability and since earlier studies of prognosis, there have been variable results in terms of life expectancy. An early study of prognosis in Germany found an average life expectancy for males to be reduced at 64.6 years but for females it was similar to the national average at 70.7 years (64). Risk factors for premature death included male sex. Common cause of death was pneumonia (38%) and more than one quarter of the deaths in this early study were sudden and unexplained (64). Fifty-seven (78%) of those who died in this study were completely dependent at time of death and the median age of being unable to leave bed in the cohort was 62.1 years for males and 66.5

years for females (64). A more recent study of including review of more than 900 people with CADASIL found male sex was associated with increased mortality in CADASIL and a median (IQR) age of death of 68 (60 to 73) (65). At the other end of the spectrum with further understanding of the relationship between genetic mutation and disease severity, cases are emerging of people affected by CADASIL in their 50s and beyond with completely normal imaging by MRI and without disability (66). As a result of the combination of early disability and relatively preserved life expectancy in CADASIL, the burden for patients, their carers' and for health service provision is significant.

2.4.9 Disability measures in CADASIL

Neuropsychological assessments covering different domains of cognition attention deficit, impairments of verbal and non-verbal memory, and visuospatial function have all been reported to varying degrees (52). These tests often assess either specific domains or more generally provide estimates of overall disability. There are advantages and disadvantages of both. Disability can be measured through the modified Rankin Scale, widely used as an outcome measure in stroke trials (67). This is a general scale with 6 points of disability on it. It is easy to use but the categories can be too broad to inform specific rehabilitation requirements and the categories are often dichotomised into good and bad outcomes. The Barthel index is used to assess ability to carry out activities of daily living and includes 10 broad categories, with a potential limitation the interpretation of independent and the lack of sensitivity to minor deficits likely often to be relevant to the lacunar strokes in CADASIL (68). The mini-mental state examination (MMSE) is used to screen for dementia by assessment of registration, recall, attention and ability to follow commands (69). This has been expanded upon in an attempt to cover more diverse cognitive domains in the Cambridge Cognitive Examination (CAMCOG) (70). Acutely the National Institutes of Health Stroke Scale (NIHSS) is used to assess severity of stroke and is reported as an outcome in CADASIL cohorts. Trails A and B are useful neuropsychological assessment tools in CADASIL as they combine to assess visual attention, and ability to switch tasks with Trail B particularly able to test executive function (71). The Mattis dementia rating scale (MDRS) is used to test cognitive function through domains of attention, initiation, construction and memory (72). These contain additional components that can have utility in assessing executive function. A structured interview for the diagnosis of Dementia of the Alzheimer type, multi-infarct dementia and dementias of other aetiology (SIDAM) aims to establish cognitive dysfunction and dementia (73). The Alzheimer's disease assessment scale (ADAS)-cog was designed to detect early changes of Alzheimer's disease (74).

Revisions of this scale to make more applicable to vascular dementia have been developed Vascular Dementia Assessment Scale cognitive subscale (VADAS)-cog (74). This was shown to be more sensitive to different extent of WMH in a large observational study of sporadic small vessel disease (22). Cognitive impairment domains in CADASIL are specific and often include processing speed disproportionately affected in comparison to executive function and attention (51). Care must be taken to ensure the correct cognitive assessment is applied to the appropriate clinical situation.

2.4.10 Correlation between genetics and prognosis

The pathognomonic mutations are located within exons 2 to 24 with clear clustering to exons 3 and 4 encoding for EGFr 2 to 5 (75). Most of the early cases of CADASIL had mutations located proximally within the gene. Over time many other mutations have become apparent in the CADASIL population and there are now a range of mutations including more distal mutations. There remains a clear preponderance for specific exon locations often in the proximal domains. Early studies did not delineate a relationship between mutation location and disease severity nor imaging markers, although this is likely related to the small number of cases involving distal mutations and the considerable heterogeneity of phenotype even within families with the same mutation (56). This has been superseded by a large study in Leiden that has resulted in a paradigm shift in thinking in CADASIL with respect to mutation location and its impact on disease phenotype. The initial study was a large genetic study interrogating 60,706 exomes in the ExAC database (76) for missense mutations in NOTCH3 (15). These variants were assessed for CADASIL pathogenicity missense mutations with gain or loss of cysteine residue in one of the 34 EGFr domains of NOTCH3. This noted a surprisingly common occurrence of NOTCH 3 abnormalities in the population as a whole at 3.4 in 1000 (15), far more than would be expected from CADASIL population disease estimates (8). Further analysis of the distribution of these genetic findings determined that proximal mutations as divided into EGFr domains 1 to 6 were commonly found in the symptomatic CADASIL population whereas mutations in EGFr domains 7 to 34 were predominantly found in an asymptomatic population with no prior diagnosis of CADASIL (15). This suggested that NOTCH3 abnormalities were more common than previously thought and there was a broader spectrum of disease expression between severely symptomatic CADASIL to others more mildly affected and possibly complete non-penetrance. Further validation of these findings in the Dutch population has resulted in a clear pattern that proximal mutations in CADASIL are associated with a more severe phenotype suffering stroke at a younger age

and with shorter life expectancy than their counterparts with distal mutations (EGFr 7 to 34) (58). This pattern has now been externally validated in other geographical populations in Asia as well as Europe (53, 59, 77, 78). These studies all found a similar, but counter-intuitive pattern of increased presence of hypertension in those with distal mutations. There have been subtle variations in both distribution of population among different exon locations as well as the severity of disease associated with specific sites. For example, in the Asian populations studied with exception of Japan, exon 11 is particularly well represented (79). There is also thought to be a relatively higher incidence of haemorrhagic stroke in Asia (80), when compared to European populations. Sub-analysis of one study that attempted to evaluate the impact of NOTCH3 mutation position on stroke and age of onset of encephalopathy found a signal that exon 10 through 17 had a lower risk of stroke than other locations (78). Further analysis and refinement of the initial genotype-phenotype correlation cohort has also found specific EGFr mutation locations that appear to have different risk level from the group average. As a result a three-tier risk genetic stratification method in CADASIL has been proposed (81). Domains 8, 11 and 26 were added to the high-risk group, formerly EGFr 1 to 6. The remaining EGFr domains were divided into 10 medium risk and 11 low-risk domains (figure 2.1). The number of individuals representing certain EFGFr repeats are small and further validation would be beneficial. The three-tiered risk stratification system demonstrated correlation with the vascular NOTCH3 aggregation load which in turn correlated with severity of clinical features (81). The value of this three-tiered risk category in conjunction with the presence or absence of family history of CADASIL can be used to assist careful discussions around prognostication.

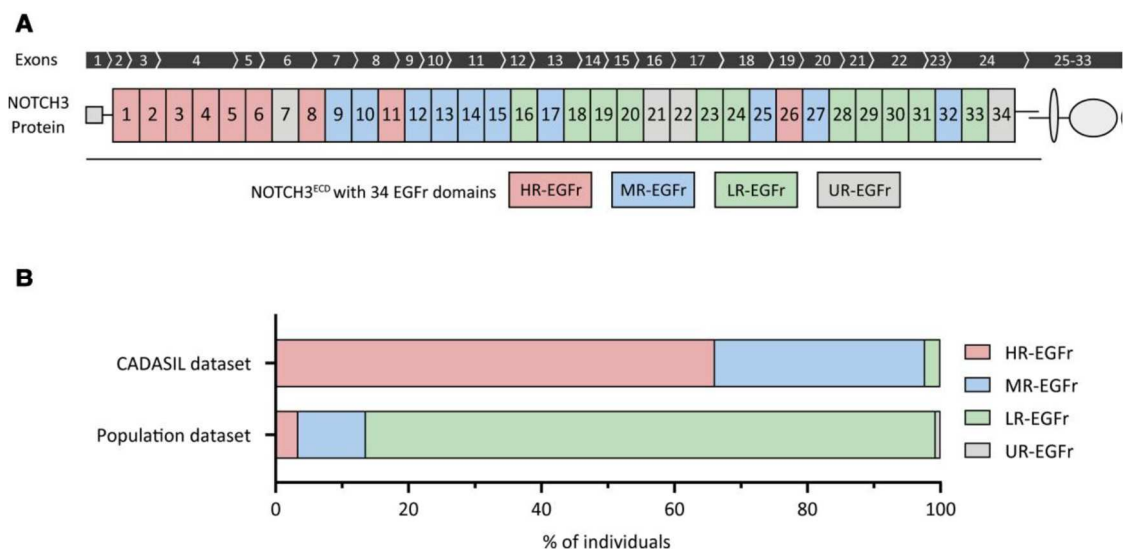


Figure 2.1 Representation of CADASIL EGFr domains divided into high, medium and low risk mutations along the NOTCH3 protein (81)

2.4.11 Magnetic resonance imaging in CADASIL

Cerebral small vessel disease is associated with a range of imaging features including white matter hyperintensities, lacunes, cerebral microbleeds, enlarged perivascular spaces and brain atrophy (82, 83) . These can provide prognostic information and have associations with the developments of dementia and death in general populations (84). These imaging features are also frequently present in CADASIL but the correlation between each imaging finding, and disease progression can be very different. Further longitudinal research would be necessary to completely understand the relevance and value of each individual marker with respect to disease progression. We will discuss in this chapter our understanding of what correlations exist between each of these imaging features and disease progression and disability in CADASIL. MRI changes are normally believed to precede the clinical manifestation of CADASIL by somewhere between 10 and 15 years (83).

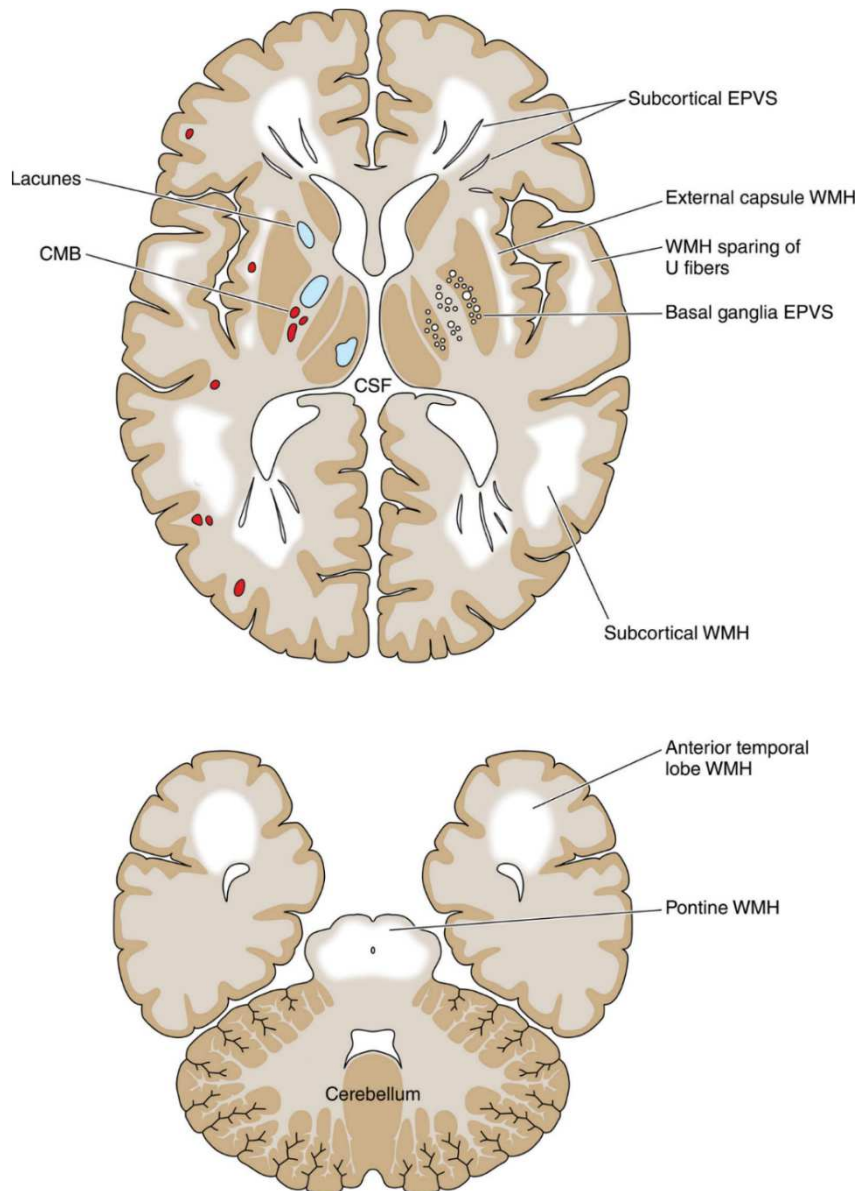


Figure 2.2 MRI imaging features of CADASIL (85)

2.4.11.1 White matter hyperintensity

White matter hyperintensity (WMH) is described as an abnormal signal hyperintensity in the white matter of variable size visible as hyperintensity on T2-FLAIR without cavitation (82). Lesions in subcortical grey matter or brainstem are not included, and where they are it should be described as subcortical hyperintensities (82). The presence of WMH has prognostic importance in the general population (84). In the CADASIL population WMH have been one of the hallmarks and are often very extensive (86). This does not always correlate with disease severity and longitudinal studies have not consistently shown it to be a useful determinant of poor prognosis (87). These WMH have a propensity to develop in the deep white matter and periventricular regions with presence in the anterior temporal

lobes reported as having high specificity for CADASIL (86%) in early CADASIL studies (86), and, with a lesser degree of specificity, involvement of the external capsule (88). The location of WMH abnormalities in the general population appears important prognostically (89) and in CADASIL the extent of WMH volume has been noted to be potentially less important than the presence of WMH in specific locations. Specifically, WMH in anterior temporal poles and superior frontal gyri were associated with better outcomes and whereas WMH in the pyramidal tracts were associated with poor prognosis (90). The Fazekas score is a simple visual score that allows assessment of extent of abnormality of WMH divided up into periventricular and deep white matter areas (91). This is rated with a score between 0 to 3 in each area. This score is well-validated and easy to use and correlates with WMH volume (92).

2.4.11.2 Lacunes

With reference to updated STRIVE imaging criteria a lacune is an ovoid or round cavity located in the subcortical brain regions, that is filled with fluid and has a maximum diameter of 15mm (93). Miller Fisher's early autopsy studies in the 1960s demonstrated various mechanical processes underlying vessel occlusion in lacunar infarcts. The arteries were occluded at a histological level through segmental disorganisation by enlargement, haemorrhage fibrinoid deposition or through thrombosis of microaneurysm (94). Lipohyalinosis became the accepted term for this combination of pathological processes underpinning cerebral small vessel disease. Lacunar stroke is defined based on clinical symptoms alone as a subcortical stroke that can include sensori-motor symptoms, pure sensory or pure motor symptoms, or an ataxic hemiparesis, with no loss of higher cortical functions (95). The radiological term lacune is likely the end product of the pathological processes involved following a small subcortical infarct in the territory of one perforating arteriole, haemorrhage of this arteriole or an incidental DWI positive lesion (93). There are now two distinct vascular pathologies thought responsible for cerebral small vessel disease. Thickening of an arteriole vessel wall through smooth muscle hypertrophy and fibrinoid tissue deposition are seen in conventional small vessel disease states of diabetes and hypertension but also in CADASIL (96). Arterial occlusion can also occur from intimal plaques of the upstream parent vessel occluding the arteriole at source also known as branch atheromatous disease (96).

Lacunes are of specific prognostic significance in CADASIL. One longitudinal study of 206 people with CADASIL followed up over 3 years found incident lacunes in 51 patients. Incident lacunes were associated with stroke and with measures of executive function and independence at follow-up (97). Other studies confirm the importance of lacunes over other radiological markers of disease progression and cognitive impairment (98). Incident lacunes also appear to favour development around the edge of WMH in CADASIL further suggesting an intimate connection between the development of both markers of small vessel disease (99). The precise anatomical variation of position of lacunes is variable but one early study of an Irish CADASIL population showed a propensity for lacunes to develop in the basal ganglia, thalamus, external capsule and brain stem (100) while another study found they most commonly appeared in the basal ganglia, thalamus, brainstem, frontal and parietal white matter regions (101). For reporting the anatomical distribution of lacunes we used an anatomical distribution based on an MRI case report form developed in Calgary for chronic ischaemic stroke and small vessel disease. This divided regions into subcortical white matter including frontal, parietal, temporal, occipital lobes, internal or external capsule. Subcortical gray matter split into putamen, globus pallidus, caudate, thalamus. Brainstem, divided into midbrain, pons and medulla, and cerebellum.

2.4.11.3 Cerebral Microbleeds

Cerebral microbleeds (CMBs) are a common imaging finding in CADASIL and can be extensive. They are small hypointense lesions ranging from 2 to 10 mm in diameter that can be visualised on T2*-weighted MR imaging sequences including gradient-echo (GE) and susceptibility-weighted imaging (SWI). SWI imaging have been found to be more sensitive than GE in detecting CMBs (102) and increased MRI field strength also increases sensitivity (103). The MRI lesions are thought to represent haemosiderin deposits in perivascular tissue because of vascular leakage of blood cells due to dysfunction of vascular smooth muscle cell walls (104). CMBs are associated with sporadic small vessel disease, cerebral amyloid angiopathy (CAA), Alzheimer's dementia and CADASIL (82). In CAA the distribution of CMBs is part of the Boston criteria (105) and CMBs are most likely to be found restricted to lobar, cortical or cortico-subcortical regions and peripherally in the cerebellum (106).

The understanding of CMBs importance for prognosis, risk of intracranial haemorrhage and in development of cognitive impairment is evolving. Cerebral microbleeds out-with CADASIL are associated with an increased risk of intracranial haemorrhage following

stroke in a population with atrial fibrillation (107) and in a meta-analysis of multiple studies this remained the case, however the absolute risk of ischaemic stroke was higher than that of intracranial haemorrhage (108). Cerebral microbleeds have been found to be present in at least 30% of early CADASIL cohorts (109), and have a strong relationship with increasing age (44, 109). A more detailed analysis of a European cohort of CADASIL demonstrated an association with deep and infratentorial microbleeds and presence of cognitive impairment and incontinence (44). CMBs in the lobar regions were associated with intracranial haemorrhage and dementia. A novel finding of an inverse relationship between presence of CMBs and migraine was found (44).

There are two prominent systems that have been developed to improve accuracy of microbleed measurement and to allow comparison studies and with the intention to improve intra-and inter-rater observer agreement. The Microbleed Anatomical rating scale (MARS) divides CMBs anatomical location into deep (basal ganglia, internal and external capsule, thalamus, corpus callosum, and deep and periventricular white matter), infratentorial (cerebellum and brainstem) and lobar locations including cortical and subcortical regions (110). The MARS scoring system differs from the Brain Observer Microbleed Scale (BOMBS) criteria as it includes specific lobar locations. The BOMBS criteria include consideration of whether there are 1 or 2 CMBs (111). They are then divided into cortex / grey-white junction, subcortical white matter, basal ganglia grey matter, Internal and external capsule, thalamus, brainstem and cerebellum. They are divided as less than 5mm or between 5 and 10mm (111). Both systems have been externally validated and appear to assist with consistency in microbleed imaging research (44, 112).

2.4.11.4 Enlarged perivascular spaces

The glymphatic pathway is responsible for fluid transport from cerebrospinal fluid (CSF) into the brain parenchyma. Initially devised from animal models it describes the flow of CSF from subarachnoid space into the brain substance through the perivascular spaces of small leptomeningeal arterioles (113). Perivascular spaces (previously known as Virchow-Robin spaces) are fluid filled spaces that surround small arteries and follow their course through grey or white matter (82). Pathology of the glymphatic system and its dysfunction has been associated with enlargement of perivascular spaces and cognitive impairment in animal models (114). Once enlarged they have a distinctive MRI appearance and appear

linear or parallel depending on vessel orientation, with diameter normally less than 3mm (82). Enlargement of the perivascular spaces is seen in association with other imaging features of small vessel disease including WMH and lacunar stroke (115). This study used 1.5-Tesla MRI to develop a method for measuring EPVS in both basal ganglia and centrum semiovale and has an associated detailed online guide to assist researchers elsewhere (115, 116). This uses the higher score if there is asymmetry. In CADASIL, enlarged Virchow-robin spaces have been reported as a common finding but except for age were not associated with clinical disease severity nor extent of WMH (117). A later study found a high burden of enlarged perivascular spaces within the white matter was associated with lower cognitive scores accounting for age and other MRI imaging biomarkers (118).

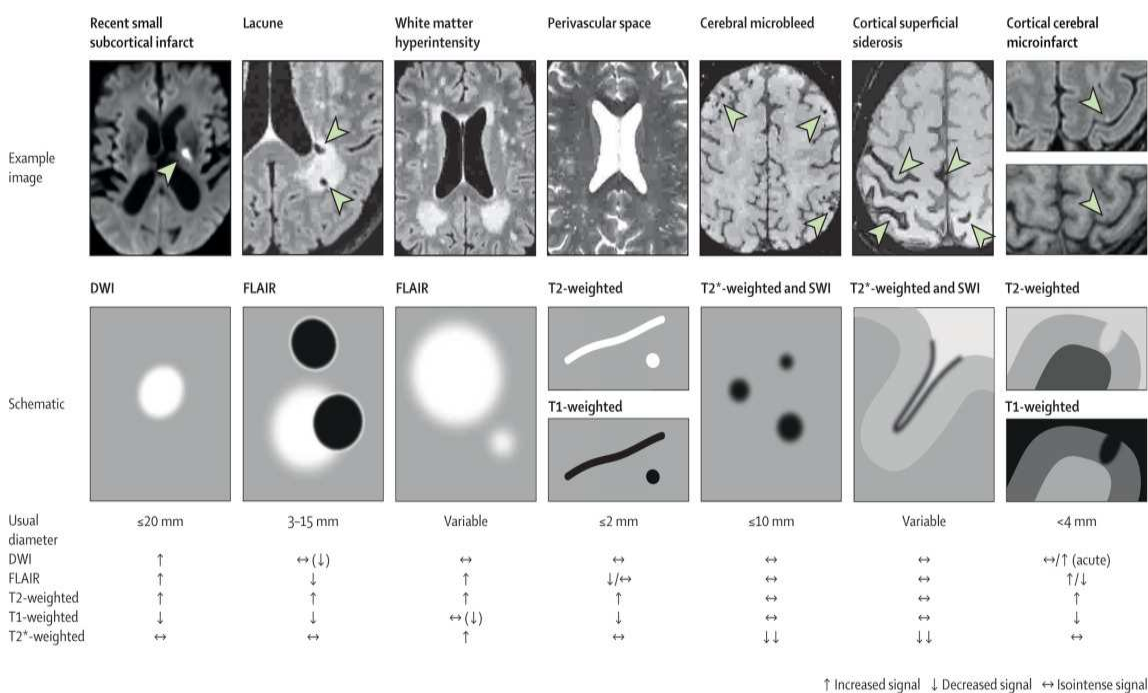


Figure 2.3 MRI imaging features of small vessel disease (82)

2.4.11.5 Brain atrophy

Measurement of brain atrophy can be challenging and is done using different methodologies (119). One of the commonly used measures in imaging studies is the brain parenchymal fraction (BPF). This is the ratio of brain volume to intracranial cavity volume (120). Brain volume and atrophy were identified as important measures with respect to cognitive function in CADASIL in different longitudinal studies (121, 122) but conversely an imaging study following up over 7 years did not corroborate the importance of brain atrophy measurements (87). A further study found that BPF was significantly associated

with cognitive and disability measures after adjusting for sex and age but was also associated with lacunar volume and age on multiple linear regression modelling (123).

2.4.12 High field strength MRI for structural imaging in small vessel disease

2.4.12.1 Benefits and pitfalls of 7 Tesla small vessel disease imaging

Limitations of spatial resolution through conventional MR imaging at 1.5Tesla have been reduced significantly by the introduction of high-field strength MRI. 7-Tesla MRI results in an improved signal-to-noise-ratio and increased spatial resolution(124). These features and improvements in image processing can be used to decrease acquisition time without compromising on image quality and reducing motion artefact (125). They have been associated with increased discomfort when compared to conventional scanners (126). Sources of discomfort included length of imaging, noise, narrow space, heat and general discomfort while sensations included vertigo metallic taste and nausea (126). Images can be affected by image degradation artefacts. 7-Tesla MRI has become a useful additional tool in imaging intracranial small vessel disease. At these high field strengths with the associated improvements in image quality and spatial resolution it is theoretically possible to directly visualise small lesions that were not previously appreciated at lower field strength. This could allow more accurate visualisation of more distal small vessels and sub-branches of arteries while also showing potential for analysing other aspects of disease processes. There are potential roles for vessel wall imaging, as well as visualisation and measurement of small vessel function as well as anatomy. Arterial spin-labelling and phase-contrast imaging also have potential to improve our understanding of underlying pathological processes when used at 7-Tesla. There are multiple challenges associated with the clinical use of 7-Tesla MRI. The signal to noise ratio can be limited through longer acquisition times and ability to reduce movement artefact. We will review in this chapter presenting the current status of 7-Tesla imaging and associated techniques in small vessel disease and CADASIL.

2.4.12.2 White matter hyperintensity

WMH on T2-FLAIR sequences are well visualised using conventional MRI at 1.5 and 3 Tesla. Studies have shown that there is little additional benefit from ultra-high field strength imaging for WMH detection (127, 128), although in future the better image

quality in conjunction with advanced imaging processing techniques may provide functional information. It is likely that different anatomical regions and WMH locations have different clinical importance (129).

2.4.12.3 Lacunes

Lacunes have relevance to small vessel disease and CADASIL and are associated with disease progression in CADASIL. In large observational study of vascular small vessel disease lacunes have been associated with specific features of WMH including larger volume and irregular confluent shape that have been, in turn, associated with more severe pathology and less good functional outcomes (130). 7-Tesla has been shown to demonstrate improved spatial resolution of lacunes or chronic infarcts and infarct morphology and associated findings including haemosiderin deposition (128). This group highlighted the longer scanning period required to achieve these outcomes and the importance of developing methods to reduce this inconvenience while maintaining optimal spatial resolution (128).

2.4.12.4 Cerebral microbleeds

There is evidence to show that 7-Tesla scans are more sensitive for the detection of cerebral microbleeds (131). 7-Tesla was superior to 1.5-Tesla in a majority of more than three quarters, with a near double count of CMBs and a larger median size of CMBs than for 1.5-Tesla imaging (127). This is in consistent with clinical studies comparing 3-Tesla and 7-Tesla for other neurological conditions (132).

2.4.12.5 Enlarged perivascular spaces

7-Tesla MRI has high sensitivity for visualisation of enlarged perivascular spaces using T2-TSE sequences and provides useful insight into morphological features and their associated perforator arteriole (133). One study of EPVS in people above 40 years found no relation between number of EPVS and age and cardiovascular risk factors but did find correlation between EPVS and other imaging markers namely, microbleeds in BG and CSO and WMH volume in basal ganglia (134).

2.4.12.6 Vascular imaging 7 Tesla MRA

An early advance with success at 7-Tesla was visualisation of intracranial vessels through non-contrast time-of flight magnetic resonance angiography (MRA) imaging, achieved in stroke patients in 2012 (135). The better image quality at 7T compared to 1.5T can be seen from figure 2.4 and the differences between healthy controls and people with stroke who demonstrated fewer visible lenticulostriate vessels (135).

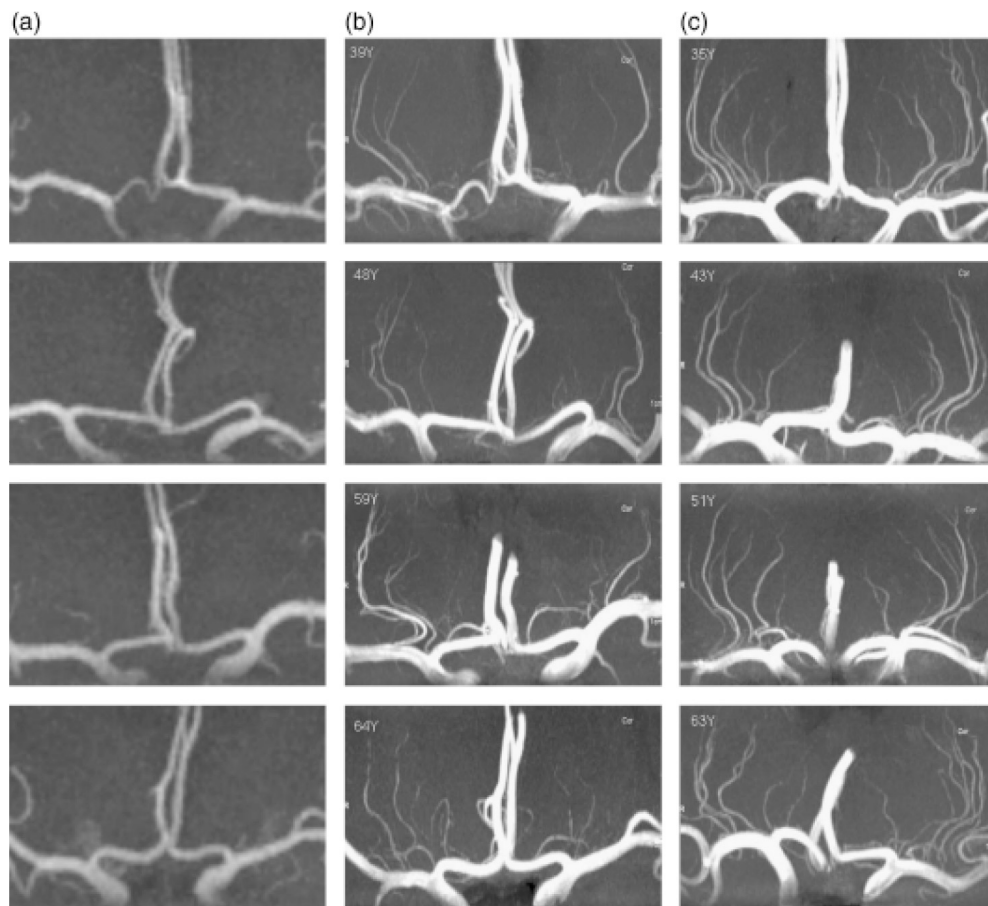


Figure 2.4 (a) 1.5T MRI (b) Stroke at 7T (c) control at 7T (136)

Properties of these small lenticulostriate arteries may be useful correlates of imaging or clinical endpoints in small vessel disease. There was a positive correlation between the number of branches from lenticulostriate vessels and disability measured through MMSE score in a CADASIL cohort (137). The CADASIL group were also found to have fewer lenticulostriate branches than their control cohort, this did not however result in significant

differences in basal ganglia lesions (137). A separate case-control imaging study of subcortical vascular dementia found fewer lenticulostriate vessels than controls, but those vessels were of greater diameter. This group also noted a correlation between number of branches and number of lacunes (138). As a result of the very small size of lenticulostriate vessels and limitations of signal to noise ratio, multiple attempts have been made to improve image quality, including through suppression of background, and improvements of movement artefact. Compressed-sensing TOF-MRA is a promising method used to try to reduce the time of imaging and improve image quality. One volunteer study has shown this to be an effective method that was able to show clearer definition and more distal branches of lenticulostriate vessels in a shorter scan time (139).

2.4.12.7 Vascular imaging of lenticulostriate in acute lacunar stroke and the susceptibility vessel sign

One component of intracranial small vessel disease is lacunar infarcts. These are responsible for around 25% of all strokes (95), and contribute to the development of dementia. The pathogenesis of lacunar stroke is not as well understood as that of cardio-embolic and large vessel stroke. It is thought the mechanisms of vessel occlusion are different and may include intrinsic disease of the arteriole resulting in endothelial dysfunction and vessel wall disruption (82). Additional insight can be derived from ultra-high field strength MRI as this should enable us to directly visualise the responsible occluded small vessels, with further information about vessel wall properties and clues to occlusive mechanisms. The susceptibility vessel sign (SVS) was initially reported from gradient echo T2*-weighted MR imaging (126). The SVS corresponds to MR signal loss as result of arterial thrombus correlating with vessel occlusion. Using SWI sequences on MRI, the presence of the SVS has been associated with larger, more disabling stroke of cardio-embolic aetiology (140, 141). There are technical challenges with visualising the SVS in acute lacunar infarct but retrospective studies have reported their presence which suggests an underlying thrombotic occlusion in a proportion of lacunar infarcts (142, 143). Ultra-high-field MRI has enabled us to directly visualise small vessels using both SWI and TOF-MRA (144) and should allow further validation of research suggesting presence of SVS in lacunar stroke.

2.4.12.8 Arterial Spin Labelling for cerebral blood flow at 3-Tesla in SVD and CADASIL

Maintenance of cerebral blood flow is essential to allow perfusion of the brain for normal function. This means that autoregulation mechanisms must maintain steady state even in situations of relative adversity including alterations in blood pressure and levels of oxygenation. The equation relevant to this is:

$$\text{cerebral blood flow (CBF)} = \text{cerebral perfusion pressure} / \text{cerebrovascular resistance}.$$

These qualities are altered by the cerebral small blood vessels adjusting to various internal and external stimuli. Cerebral blood flow can be measured quantitatively using the imaging technique called MRI arterial spin labelling (ASL) (145). For this technique, two images are required. A label image is acquired, oriented perpendicular to the relevant artery. Blood molecules crossing the labelling plane can be “tagged” by inversion of magnetisation through saturation pulses (146). There is a subsequent post-labelling delay to allow for blood perfusion caudally, in this case. A section of the image is recorded to image both the static brain space and the mobile “tagged” blood. The control image is recorded without labelling the blood. Subtraction of the labelled image from the control static image allows a representation of the signal carried by blood flow through arteries into tissues.

Mathematical models are utilised to allow calculation of cerebral blood flow. Development of ASL imaging has resulted in different methods of acquisition. There are three categories of ASL pulse sequence: continuous ASL, pulsed ASL and pseudo-continuous (PC)ASL. Pseudo-continuous ASL uses a series of very brief pulses to label the blood over a long duration of time. This has high labelling efficiency and better signal to noise resolution than continuous ASL (147). As such there is a natural contrast material, and no external contrast agent is required. This is a key benefit of ASL as it allows imaging in the paediatric population, with severe renal impairment where multiple follow-up images will be required (145).

A large study of people recruited from a memory clinic with and without cognitive impairment compared clinical characteristics, conventional MRI SVD markers, lacunes, CMBs, WMH, EPVS and degree of cognitive impairment (148). They found increasing age, hypertension, smoking, male sex, high cholesterol, number of lacunes and CMBs were associated with low cerebral blood flow (CBF). WMH volumes was associated just with spatial coefficient of variation, a measure of dispersion of a probability distribution, while

EPVS had no correlation with ASL parameters (148). High spatial coefficient of variation was associated with cognitive impairment, dementia and vascular dementia, emphasising possible merit of measuring hypoperfusion in assessment of vascular brain disease (148). ASL has been used specifically in CADASIL for a longitudinal study where over two years CBF was able to predict progression of WMH and low CBF at baseline correlated with decline in attention and memory (149). ASL has also been used in CADASIL to assess the function of the blood-brain barrier (150). This study found that the water exchange rate over the blood-brain barrier was decreased in CADASIL and that this was negatively correlated with WMH volumes, suggesting blood brain barrier dysfunction has some role in CADASIL progression (150). A separate study used dynamic contrast-enhanced MRI to measure blood -brain barrier permeability and voxel-based quantitative susceptibility mapping to measure iron deposition and found iron burden was associated with regional blood-brain barrier permeability and cognitive function (151). Up until this point ASL has been used at 3-Tesla, but further work is ongoing to try to enable the use of this technique at higher resolution but currently acquisition times and movement artefacts have prohibited use of ASL at 7-Tesla.

2.4.12.9 Phase-contrast imaging in 2 dimensions measuring pulsatility

Phase-contrast imaging uses MRI signal to quantify velocity. As particles move along a magnetic field they develop phase-shifts, proportional to spin velocities. Faster protons undergo a greater phase-shift. The maximum measurable velocity before aliasing occurs is called the encoding velocity (152). The encoding velocity is inversely proportional to the gradient so spatial resolution at low velocities is limited if the encoding velocity is set too high (152). The encoding velocity is selected by MRI operator to best match the target vessels.

There are already multiple clinical applications of phase contrast imaging including in cardiovascular medicine. In neurology blood flow for aneurysms and CSF flow in hydrocephalus make use of phase-contrast flow measurements. More recently it has been used to measure flow in small vessels intracranially. The better spatial resolution of 7-Tesla MRI allowed measurement of blood flow velocity and pulsatility in perforating arteries at the level of basal ganglia and centrum semiovale using quantitative flow analysis and a cardiac-gating method (153). This technique involves selecting two-dimensional slices or regions of interest at specific anatomical levels, manually identifying

small vessels then measuring mean, maximum and minimum velocities for these vessels to allow calculation of pulsatility index (153). This technique is susceptible to partial voluming effects and additionally the use of 2-dimensional planes means that it is likely that the majority of vessels will not have been perpendicular to the plane, and this would likely result in an underestimate of true velocity. This technique has been further developed and has been shown to result in higher pulsatility index in lacunar infarcts at the level of the basal ganglia and in deep intracranial haemorrhage (154). As part of the aging process and in response to cardiovascular factors, arterial stiffness increases over time (155). A consequence of this is an increased transmission of pulsatile energy to the small distal vessels that could result in small vessel damage (156). This arterial stiffness can be estimated through measurement of the damping factor, which is the ratio of flow velocity pulsatility in the lenticulostriate arteries to that in the middle cerebral arteries. A volunteer study analysed the damping factor and found it to decrease significantly with increasing age (157) and a recent study found the damping factor to correlate to the number of EPVS scene at 7-Tesla (158).

Imaging the lenticulostriate vessels even at 7-Tesla is challenging and it has been necessary to compare novel imaging techniques with current modalities. One study highlighting these challenges found that middle cerebral artery (MCA) velocity using phase-contrast was approximately 30% lower than that recorded using transcranial doppler (TCD) ultrasonography (159). Possible explanations include TCD method being averaged over multiple cardiac cycles, making it less likely to capture maximum velocity (159).

2.4.12.10 Phase contrast imaging in 3 dimensions using mean flow velocity in small vessel disease and CADASIL

In order to get the most representative values of flow velocity for individual vessels it is important to measure the flow velocity directly along the vessel course. Recognising the limitation of alignment of vessels in 2-dimensional cardiac gated imaging, groups have developed methodology for 3-dimensional phase contrast imaging in the brain. After initial success visualising lenticulostriate vessels using TOF-MRA at 7 Tesla (160), further developments in motion artefact suppression and reduction in acquisition times enabled the same group to perform 3-dimensional phase contrast imaging of lenticulostriate vessels (136). This method requires acquisition of and combination of TOF-MRA with phase contrast MRA. This research group performed a volunteer study selecting three different

encoding velocity (VENC) values to determine the optimal VENC for slow small vessels such as lenticulostriate arteries (LSA), in order to minimise aliasing and other causes of artefact (136). This technique has been used to assess differences between CADASIL and control cases in a Chinese population (161). This group used a VENC of 15 cm/s to focus on the lenticulostriate arteries. The pipeline of their analysis technique includes first performing an intensity bias correction, then extracting regions of interest focusing on the LSA from the maximum intensity projection (MIP) images. From here they perform noise-masking to minimise external interference that is threshold based, and then they reconstruct the velocity components using the collected MRI phase contrast data. This allowed a calculation of flow velocity in specific visualised lenticulostriate vessels (161). They found that the symptomatic CADASIL cohort LSA had a lower flow velocity than controls, but asymptomatic NOTCH3 cysteine-involving mutation carriers had mean flow velocity very similar to controls (161).

Attempts have been made to use 4-dimensional flow MRI to calculate average pulsatility over brain space. This included inputting and averaging arterial waveforms from large numbers of cross-sections over many small vessels (162). The results of this study showed comparable values for pulsatility and resistivity compared to prior 2-dimensional phase contrast reference values (162). For now, there are significant limitations of this technique as acquisition times are long and signal to noise ratio is very limited for individual small vessels as movement artefact over the cardiac cycle is very significant.

2.5 Aims and hypotheses of this thesis

Chapter 3 investigates the Neuroimaging correlates of disease progression and disability in CADASIL

Chapter 4 investigates the hypothesis that the CADASIL population in Scotland is increasing but the majority of symptomatic presentations remain within the proximal mutations EGFr 1 to 6.

Chapter 5 investigates the hypothesis that there is a specific pattern of distribution of lacunes, white matter hyperintensities and cerebral microbleeds in the Scottish CADASIL population.

Chapter 6 investigates a genotype-phenotype correlation in the Scottish CADASIL population and compares CADASIL with sporadic small vessel disease.

Chapter 7 investigates the rate of change of imaging biomarkers over time in CADASIL.

Chapter 8 investigates the possibility that we can use 7-Tesla MRI to detect a susceptibility-vessel-sign and associated small vessel occlusion in recent lacunar infarct.

Chapter 9 investigates the differences in sensitivity of 1.5, 3 and 7-Tesla MRI for detection of lacunes, cerebral microbleeds and enlarged perivascular spaces.

Chapter 10 investigates the hypothesis that we can use phase-contrast imaging at 7-Tesla in a healthy volunteer group to measure flow velocity through the lenticulostriate arteries.

3 Neuroimaging correlates of disease progression and disability in CADASIL: a systematic review

3.1 Abstract

Introduction

CADASIL commonly results in recurrent stroke, disability and dementia. Imaging abnormalities often precede clinical presentation. The imaging features in CADASIL and sporadic small vessel disease have some relationship with disease severity and disability, but the different component elements of imaging features do not have a consistent relationship with severity. The purpose of this systematic review is to establish the correlation of imaging findings with disability and to establish correlation between imaging changes over time with clinical deterioration.

Methods

We performed a systematic review searching MEDLINE, EMBASE, CENTRAL and Web of Science databases on 11th December 2018. This search was updated on 9th February 2024. We searched for papers reporting CADASIL, imaging findings and disability. We included observational studies including case-controlled studies, cohort studies in English language that had more than 10 patients from more than one family. We excluded studies that did not report primary data and conference abstracts.

Results

From the 5971 papers found using our documented search strategies, we excluded 5926. Thirty-six cross-sectional studies were included correlating imaging with disability and nine studies measured features longitudinally over between 21 and 84 months. Imaging features including white matter hyperintensities, lacunes, cerebral microbleeds, enlarged perivascular spaces, measurements of blood volume and flow with arterial spin-labelling, and diffusion-tensor imaging with disease progression. We found that brain parenchymal fraction and lacunar lesion load provide the most reliable correlates of disease burden as measured by Mattis Dementia rating scale, Trail making tests and Cambridge cognitive examination scores for cognition, and Barthel index and modified Rankin Score for disability. White matter hyperintensities generally shows poor correlation with disability.

The longitudinal studies include 3 patient cohorts of fewer than 300 people from specialist centres. We included 5 small studies of between 16 and 81 participants suggesting diffusion tensor imaging may provide sensitive detection of early disease states.

Discussion

Further use of novel MR imaging strategies using high field strength may help our understanding of CADASIL. We must continue to develop and validate imaging biomarkers that are sensitive to early changes in CADASIL.

3.2 Introduction

There is significant variation in onset of first symptoms and rate of disease progression within CADASIL. Better prediction of these variables would aid prognostication and communication of potential disease trajectories. There is currently no definite disease modifying treatment available and management usually consists of lifestyle modification including smoking cessation and control of co-existent risk factors for small vessel disease including hypertension and diabetes. The role of Aspirin is contentious as there is no evidence to support their use for primary prevention in CADASIL. Recent European guidelines have advised that there is no benefit in giving antiplatelets in the absence of prior stroke and also that there is no benefit from statins with normal cholesterol (163).

The clinical spectrum of CADASIL is evolving and recently there is increasing understanding that there is significant variability of disease progression depending on multiple factors including EGFr domain of genetic mutation (164). This milder phenotype may have more clinical overlap with spontaneous small vessel disease in older age. The earlier imaging studies in CADASIL predominantly consisted of those with proximal mutations which are now recognised as being associated with earlier clinical presentation and greater likelihood of clinical symptoms. MRI changes are normally believed to precede the clinical manifestation of CADASIL by somewhere between 10 and 15 years (165). The MRI features associated with CADASIL include white matter hyperintensities (WMH) seen on T2-weighted and T2 FLAIR (Fluid attenuation inversion recovery) sequences. Other potentially important MRI findings include lacunar lesions, cerebral microbleeds and enlarged perivascular spaces. In addition to conventional MRI, ongoing research is trying to establish the utility of novel and advanced imaging techniques to enable measurement of imaging correlates of clinical endpoints. The hope is to discover

more sensitive imaging biomarkers to monitor disease progression or measure response to therapeutic intervention. Additionally, with advances in imaging providing higher resolution with improved signal to noise ratio, direct visualisation of smaller vessels is now possible enabling measurement of vessel function. These techniques are expected to allow us to improve our mechanistic understanding of the underlying pathophysiological processes. Diffusion tensor imaging (DTI) detects movement of water molecules along white matter tracts and is used to assess white matter structural integrity in CADASIL patients (166). Phase-contrast imaging has emerged as a method for quantifying blood flow and is well established for studying large vessels extra-cranially including the aorta and heart. Phase contrast-imaging in the brain is also developing with ability now to measure flow velocity and pulsatility of small vessels which could give additional information regarding cerebral blood flow (136, 153, 161). Arterial spin labelling (ASL) can measure tissue perfusion or cerebral blood flow and blood oxygenation level dependent (BOLD) reflects localised variation in cerebral blood flow (167, 168). Increasingly it is anticipated that these imaging techniques may provide biomarkers that more sensitively and reliably correlate with disease state.

CADASIL progression is commonly associated with disability and cognitive deficits, and standardised measures to quantify these deficits may be used as markers of disease severity and disease progression. CADASIL has been shown to impact particularly on executive function, with reduced processing speed an early sign of disease (52). In neuropsychological assessments covering different domains of cognition attention deficit, impairments of verbal and non-verbal memory, and visuospatial function have all been reported to varying degrees (52). Disability can be measured through the modified Rankin Scale, widely used as an outcome measure in stroke trials (67, 169). The Barthel index is used to assess ability to carry out activities of daily living (68). The mini-mental state examination (MMSE) is used to screen for dementia by assessment of registration, recall, attention and ability to follow commands (69). This has been expanded upon in an attempt to cover more diverse cognitive domains in the Cambridge Cognitive Examination (CAMCOG) (70). Acutely the National Institutes of Health Stroke Scale (NIHSS) is used to assess severity of stroke and is reported as an outcome in CADASIL cohorts, although it has not been well validated in chronic stroke populations. Trails A and B are useful neuropsychological assessment tools in CADASIL as they combine to assess visual attention, and ability to switch tasks with Trail B particularly able to test executive function (71). The Mattis dementia rating scale (MDRS) is used to test cognitive function through domains of attention, initiation, construction and memory (72). These contain

additional components that can have utility in assessing executive function. A structured interview for the diagnosis of Dementia of the Alzheimer type, multi-infarct dementia and dementias of other aetiology (SIDAM) aims to establish cognitive dysfunction and dementia (73).

The characteristic MRI findings are frequently encountered in the diagnosis of CADASIL but there is still a paucity of literature clearly correlating initial MRI findings with disability. Furthermore, it is not clear yet how the progression of initial imaging abnormalities correlates with disease progression and disability. The purpose of this systematic review is to establish the current knowledge base for:

1. the correlation between imaging findings in CADASIL and disability at diagnosis and
2. to establish the correlation between progression of imaging findings and disease.

3.3 Methods

We conducted a systematic review of the literature to identify studies relevant to establishing correlation of imaging findings with disability and disease progression in CADASIL. We enrolled the assistance of specialist librarians to assist with devising a search strategy. Protocol published on PROSPERO can be viewed at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019119298.

We searched MEDLINE, EMBASE, CENTRAL and Web of Science databases on 11th December 2018. We searched for articles in the English language published between January 1993 and the date of search. This starting date was used as this was the year CADASIL was first reported as a distinct entity (170).

The complete search strategy is included in appendix 1. Example search strategy is shown below for MEDLINE using MESH terms to identify relevant articles.

1. CADASIL
2. Cerebral autosomal dominant arteriopathy with sub?cortical infarct? And
leu?oencephalopathy
3. Magnetic resonance imaging
4. MRI
5. MR imaging
6. Neuroimaging

- 7. Functional neuroimaging
- 8. Arterial spin labelling

- 1. 1 OR 2
- 2. 3 OR 4 OR 5 OR 6 OR 7 OR 8
- 1 AND 2

The search strategy was repeated using MEDLINE on 9th February 2024 to allow inclusion of more recently published studies. This search strategy included an additional search term: 'phase-contrast imaging' to include the most relevant advanced imaging techniques based on findings from the initial search.

INCLUSION

We included studies that reported both disability and imaging findings or a correlation between disease progression and imaging findings in human patients with CADASIL. We included observational studies, case-control studies, and cohort studies that had results for more than 10 people from more than one pedigree.

EXCLUSION

We excluded reviews that do not report primary data. We excluded articles not written in English and we excluded studies of adequate sample size that were taken from one family only. Conference abstracts were not included. We included reports using the same study cohort only where sufficiently novel imaging characteristics were presented. In situations where articles were excluded to avoid duplication, the largest cohort or that with the most comprehensive and relevant follow-up was included.

ANALYSIS

Identified titles and abstracts were reviewed by two researchers (SN and VB) and papers were excluded if not meeting the inclusion and exclusion criteria or clearly not relevant. Any disagreement was discussed and where necessary a further researcher (AM) had the final say on inclusion or exclusion. Since the majority of studies included were observational in nature it was felt that a descriptive narrative was more informative with quantitative meta-analysis not practicable. Complete articles were then reviewed and assessed using the Strobe criteria for observational studies (171).

3.4 Results

We identified 2313 studies after searching the online databases. 762 were removed as duplicates. The abstracts and titles of the remaining 1551 were reviewed by two researchers and after the abstracts were reviewed for inclusion and exclusion criteria, there were 67 papers remaining. These were then divided into cross-sectional and longitudinal studies. The complete papers were then reviewed and the STROBE statement criteria for reporting observational studies were used to assess the quality of the reported studies. For updated review on 9th February 2024. 3658 records through search strategy leaving 3353 after duplicates removed. After applying exclusion and inclusion criteria to abstract review 48 papers remain for full paper review. Following removal of studies that did not correlate imaging with disability, with fewer than 20 cases, and where multiple studies from same patient cohort were removed 7 further observational studies were included. Tables with all included studies summary in appendix.

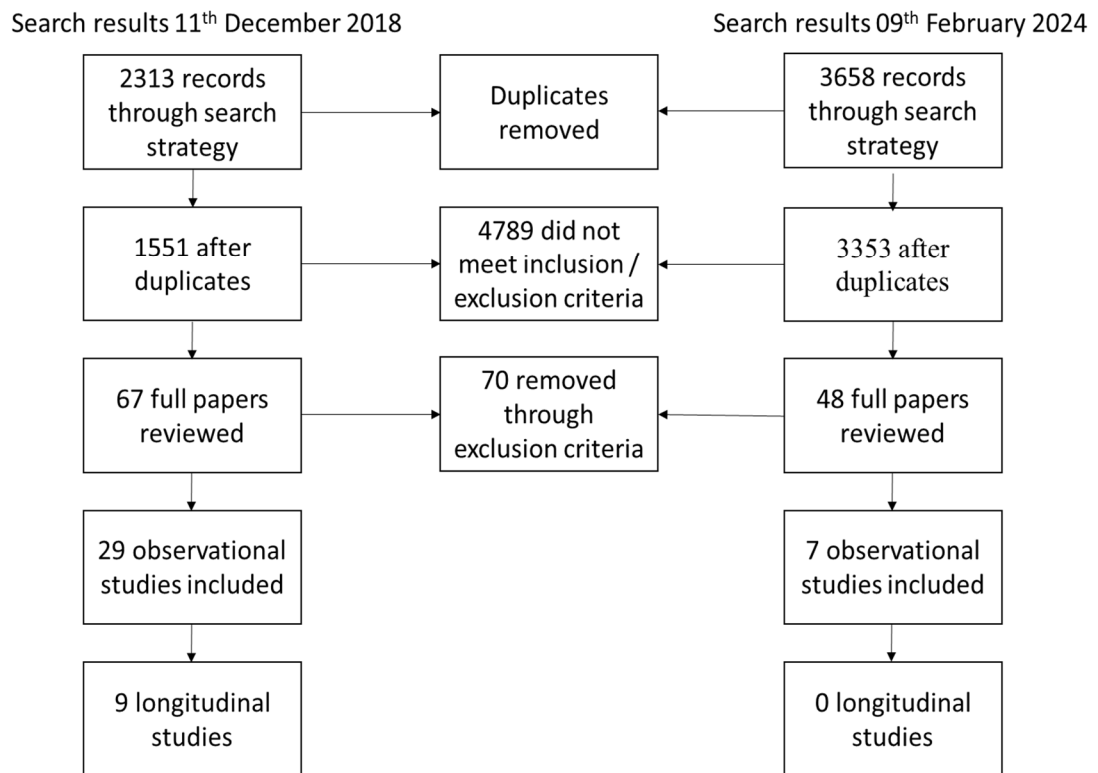


Figure 3.1 Search strategy and process of study selection

3.4.1 Imaging correlates of disability

White Matter Hyperintensities' impact on disability

WMH are one of the hallmarks of imaging findings in CADASIL. These white matter abnormalities may precede symptoms by many years and often have a characteristic anatomical distribution. Confluent subcortical white matter hyperintensities are found in the periventricular regions, the anterior temporal lobes, frontal lobes, basal ganglia, external capsule, and the insular region (172-174). While the presence of WMH in CADASIL is ubiquitous, the extent of any impact on disability is unclear. The early studies of CADASIL by Yousry and colleagues reported correlation between the volume of T1 white matter hypointensity and degree of disability and intelligence on multiple testing modalities (172, 175). There was an inverse correlation between lesion load and overall cognition, and lesion volume correlated significantly with age (175). Benisty compared patients with CADASIL that had only white matter abnormality on MRI to CADASIL with WMH and other MRI abnormalities. This study showed that even with extensive white matter abnormalities people with CADASIL can remain relatively asymptomatic, with only notable deficit found using (TMT) B time score a measure of processing speed (176). The group that also had lacunar lesions had a more significant impact on cognitive impairment (176) and the results of recent studies repeatedly questions the clinical importance of the extent of WMH abnormality.

Impact of WMH in specific anatomical locations on disability.

Chabriat 1998 noted a predilection for white matter abnormality in the periventricular and deep white matter areas, as well as noting their presence in the brainstem (173). Singhal's observational imaging study of 124 people found stroke and disability measured by rankin score correlated with Scheltens score as well as pontine and internal capsule involvement (174). Duering et al used mapping technique to compare specific locations of lacunes and WMH with reduced processing speed. Lesions of the anterior thalamic radiation and forceps minor impact on processing speed as determined by a composite score including components from of Trail Making Test (TMT)-A and (TMT)B. (177) . Another study similarly noted different brain areas of WMH have differing impact on cognition. The anterior temporal poles and superior frontal gyri appear to convey a more benign clinical picture while the pyramidal tracts and forceps minor were associated with increased disease severity measured by mRS and MDRS (178). Most recently a novel finding of WMH in the corpus callosum was shown to significantly correlate with MDRS and mRS in a cohort of 228 patients with CADASIL in France (179).

Lacunes and Brain Volume

Lacunes are small subcortical fluid filled cavities consistent with prior subcortical infarct or haemorrhage in the territory of a single perforating arteriole (82). The loss of space from these cavities has an interconnection with brain volume and tissue loss. These imaging features are therefore often reported together. Three of our included nine longitudinal studies have reported the effect of lacunar volume on cognitive impairment in CADASIL (180-182). Brain volume, brain atrophy and brain parenchymal fraction (BPF); the ratio of brain volume to intracranial cavity volume, are often reported in studies and have been associated with degree of cognitive impairment in CADASIL. Viswanathan has reported in two cross-sectional studies from the same cohort that BPF and volume of lacunes were the more consistently important MRI markers of disability and cognitive function (MDRS) when compared to WMH, cerebral microbleeds and mean apparent diffusion coefficient (ADC) (183, 184). A small prospective study has reported greater lacune volume and smaller brain volume as being associated with cognitive impairment. Furthermore, it reported a correlation between functional connectivity in CADASIL and performance speed and executive ability (185). At low levels of brain pathology, after correcting for age, level of education which was used as a measure of cognitive reserve, had an additional protective effect on processing speed in relation to lacunar burden and BPF (186). More recent studies remain consistent with lacunes count associated with Brief Memory and Executive Test (BMET) measure of vascular cognitive impairment (odds ratio 1.63) in 176 patients with CADASIL (98), normalised lacune volume significantly associated with mRS in 200 patients in Leiden (187) and high lacune count correlating with MOCA score in 44 patients Japan (188). Included studies from Korea have found correlation between number of lacunes and extent of white matter hyperintensities with disability and inverse relationship between MMSE and number of lacunes (189). Lee demonstrated that number of lacunes was the most significant indication of cognitive impairment in their patient cohort (190, 191). Different scoring systems have been used to assess the MRI abnormalities and the global cortical atrophy scale, devised to measure atrophy in multiple brain regions was associated with poor cognitive performance over a range of neuropsychological domains (192).

The interconnection between brain atrophy and lacune lesion burden has been noted through a larger study of 129 patients that demonstrated strong correlation between brain atrophy and multiple disability and cognition scales (193). BPF was significantly correlated

with volume of lacunes, apparent diffusion coefficient and age. This study also provided evidence that the correlation between impact of lacunar lesion volume and brain atrophy is not just through direct tissue loss, it appears to result in volume loss through remote or diffuse mechanisms (193). A final study reporting brain atrophy offers potential insight into the relationship between brain atrophy, lacunar volume and cognition through the concept of brain reserve (186).

Cerebral microbleeds

Cerebral microbleeds represent a common finding in aging and small vessel disease, present in 6.5% of healthy people between 45 and 50 years old increasing to 35.7% in those over 80 years of age (194). Cerebral microbleeds were measured in 18 included cross-sectional studies and 4 longitudinal. They have often been reported as having less significant impact on cognitive impairment in the CADASIL population (176, 183, 190). They have however been found to be more prevalent in those people with emotional disturbance, in a small and non-controlled study (195). With the recognised geographic differences in CADASIL, more recent studies from Asian populations have noted some significant correlation with CMBs and disability. Multiple studies of between 39 and 81 participants have found CMBs number to be associated with MOCA (188), MMSE (188, 196, 197) and susceptibility value in the thalamus for iron deposition correlated with mRS (198). Longitudinally the 7 year follow-up study of 62 patients found that CMBs were associated with CAMCOG total, Wechsler Memory Scale (WMS) and Stroop interference (87), and Chabriat larger study of 290 followed up for 3 years also found an association between CMBs and MDRS, Barthel index and mRS (182). Perivascular spaces are thought to have minimal impact on cognition and were not associated with the presence of microbleeds in a single-centre study (199).

Advanced Imaging Techniques

Advanced MR imaging techniques have shown promise in delineating greater detail of tissue damage in CADASIL patients. While it has proven difficult to accurately measure regional cerebral blood volume in white matter, it has been correlated with disability measures, namely MMSE and Rankin score (200). The regional cerebral blood volume was markedly lower in areas of white matter hyperintensity on T2 images, and both disability and cognitive impairment showed a negative correlation with cerebral blood flow in these areas of white matter hyperintensity (200). Around the same time a case-controlled study of magnetisation transfer imaging compared measures of this in proton-density visible lesions to normal appearing white and grey matter. The average magnetisation transfer

ratio in normal appearing white matter and proton-density visible lesions was significantly decreased with increasing age, Rankin score, and decreasing SIDAM score (201). Similarly, mean diffusivity maps have been shown to be elevated in abnormal white matter but more so the average mean diffusivity in normal appearing brain positively correlated significantly with disability in a small case-controlled study (202). Chabriat performed a small case-control study of symptomatic CADASIL patients with healthy asymptomatic controls using DTI to demonstrate a strong correlation between mean diffusivity and degree of diffusion anisotropy and both MMSE and Rankin score (166). Extracranial imaging has rarely been used as a biomarker for cognition in CADASIL. One study noted carotid intima-media thickness and age are inversely correlated to Mattis Dementia rating scale, although the effect was small (203). Diffusion tensor imaging analysis along the perivascular space (DTI-ALPS) is a measure of the dynamics of cerebral interstitial fluid associated with imaging markers of small vessel disease. Within this study DTI-ALPS has been found to be lower in CADASIL than in controls and also correlates with MMSE score in CADASIL patients (196).

3.4.2 Imaging correlates of disease progression longitudinally

We have seen that multiple MRI imaging abnormalities are present in CADASIL and the precise contribution of each abnormality to the clinical phenotype is complex. Clinical deterioration longitudinally in patients with CADASIL is common, and over a period of as little as 3 years a significant proportion of patients have been shown to develop incident stroke (19.8%), moderate or severe disability (9%) or dementia (20%) (182). CADASIL is an uncommon disease that consists of a prolonged pre-symptomatic phase that is potentially amenable to intervention. The small vessel disease structural MRI biomarkers associated with CADASIL are very variable in their clinical correlations and are therefore unreliable as clinical biomarkers. There is a need to develop more sensitive imaging biomarkers. These can be used to characterise baseline imbalances in important prognostic markers for a clinical study, and to adjust for these imbalances. They could be used as a surrogate endpoint for efficacy in a clinical trial to enable realistic sample size within the context of a rare disease. They can offer further mechanistic insight into CADASIL pathophysiology, as seen in spontaneous small vessel disease through developing an understanding of small vessel mechanics and function, such as through measuring cerebral blood flow, vasoreactivity, or vessel pulsatility. An important augmentation to the imaging studies assessing the association between MRI abnormalities and disability at diagnosis is the correlation between disease progression and longitudinal changes in MRI features.

There have been fewer studies investigating the longitudinal correlation of imaging biomarkers with disease progression and we will summarise the findings from the main studies investigating different modalities.

Brain atrophy has been shown to correlate longitudinally with disability and measures of global cognitive abilities. Peters et al demonstrated this and following 76 patients over 2 years found percentage brain volume change correlated significantly with Rankin, Barthel, NIHSS and SIDAM, but not MDRS (204). This result was not replicated in a 7-year cohort study of only 25 people with CADASIL (87). This study of longer duration reported lacunar lesions, ventricular volume and cerebral microbleeds to be associated with cognitive decline. WMH volume was not related to cognition (87). The larger cohort studies followed patients over 3 years. Most of these studies have been recruited from the co-operation between the CADASIL cohort studies in Munich and Paris. We have included multiple studies where they have reported different imaging modalities. Generalisability of these findings to other populations remains unproven without adequate external validation. Chabriat showed that CADASIL patients experience clinical deterioration frequently with almost half of those completing follow-up experiencing composite end point of incident stroke, dementia, at least moderate disability, or death over a 3-year period. Number of lacunes and BPF and to a lesser extent CMBs were reported as predictors of disease progression including clinical status, age, sex and degree of education (182). The same group utilised computer prediction modelling techniques combining clinical and imaging factors to predict deterioration over 3 years. Models based on lacunar volume and BPF performed best for MMSE, MDRS and mRS, although not for TMT-B (180). Expanding on earlier research showing the importance of lacunar volume, (Ling et al) demonstrated number of lacunes initially presented was an important determinant of the risk of developing further interval lacunes which in turn correlated with clinical deterioration (205). Incident lacunes impacted on cognitive and motor function, the effect most profound on tests of executive function and processing speed (205). The location of lacunes was reported to help further understand the potential reasons for specific impact on these cognitive functions. A later study by the same authors from the same cohort reported independent association between incident lacunes and stroke, while noting the importance of brain atrophy as a risk for developing stroke, dementia and disability (206). After appropriate adjustments, changes of MDRS, Barthel index and TMT-A were associated with brain atrophy, while TMT-B was associated with brain atrophy and incident lacunes. The mRS was associated with increase in WMH in this cohort (206).

In addition to the more conventional MRI abnormalities, strategies incorporating an assessment of brain morphology has been reported on a large cohort of 190 patients (181). This group measured cortical sulci length, mean depth, fold opening and cortical thickness. After adjustment for sex and education there was a significant association between reduction of cortical sulcal depth and TMT-A and TMT-B (181). Cortical thickness was associated with disability as measured by mRS and MDRS was associated with brain volume (181). It was felt that lacunar lesions in the deep cortical matter were primarily responsible for the cortical morphological changes.

DTI imaging has been used to study CADASIL longitudinally in an attempt to develop more sensitive biomarkers. Initially Molko et al followed 14 patients over 21 months reviewing 2 or sometimes 3 DTI trace histograms (207). There was an increase in mean value and decrease in peak height of trace histograms suggesting an increase in high diffusion voxels. This was in the absence of recordable decrease in mRS. For the 7 patients who received imaging on 3 separate occasions the later of the follow-up imaging showed similar results. At final follow-up the 5 patients who had deteriorated were noted to have a greater increase in diffusion than those who were clinically stable (207). This study is very small, and methodologically it is difficult to draw conclusions from this, but a further imaging study using diffusion MR histograms followed 62 patients over 2 years (208). T2 abnormality volume, average mean diffusivity and peak height of histogram were all significantly correlated with clinical scores. Change of average mean diffusivity over follow-up time was correlated significantly with Rankin, NIHSS and SIDAM scores and non-significantly with Barthel and MDRS (208).

3.5 Conclusions

During this systematic review we have reviewed the latest evidence on imaging correlates of disability in CADASIL. Due to the nature of CADASIL the available studies have been reported from a small number of research groups who have been monitoring patient cohorts for prolonged periods. The studies have consistently reported number and volume of lacunar lesions as an important determinant of disability. Brain volume and BPF have also been shown to be of clinical importance. Extent of WMH is important in making the diagnosis although appear to have relatively little impact on clinical deterioration. Novel imaging methods including diffusion tensor imaging have shown promise in detecting more subtle changes in white matter integrity that could be further validated to become

useful as imaging biomarkers in clinical trials. A significant limitation of the available studies is that they derive from small groups of affected individuals from specialist centres and the global generalisability of the imaging findings remains unclear. We must continue to research novel imaging techniques and validate those established in different CADASIL populations.

Appendix 1. Longitudinal studies

Table 0.1 Longitudinal studies summary

A u t h o r s (Y e a r)	C o u n t r y	Sa m p l e s i z e	A g e (% m a l e)	S e x	Ima g i n g (seq uenc es)	Segm entati on meth ods	Adj ust men t	Imagi ng para meter s	Disabilit y endpoi nts	Key result correlations (significant)	fol low -up (m ont hs)
L i e m 2 0 0 9 (8 7)	L e i d e n	62 (38 co mp lete d fol low - up) 25 mu tati on car rier s	40 · 3		1.5T T1 T2 FLA IR T2* GE	Semia utoma ted segme ntatio n Fuzzy cluste ring /regio n growi ng SNIP ER	Age diffe renc e betw een MC and non MC note d	Brain volum e, CSF ventri cular volum e, WMH volum e, Lacun e no. CMBs ·	MMSE CAMCO G WMS TMTA TMTB Stroop	Lacunes (CAMCOG Total and Language, Stroop interference) CMBs (CAMCOG total, WMS, Stroop interference) Brain atrophy (CAMCOG praxis) Ventricular volume (MMSE, CAMCOG total, WMS, Stroop interference)	84
C h a b r	P a r is / 	29 0 23 6	50 · 6	44 · 8 %	1.5T T1 T2	Manu al segme ntatio	Age and sex	Brain volum e,	MMSE MDRS mRS	Stroke (>3 lacunes, CM)	37 ±1. 8

A u t h o r s (Y e a r)	C o u n t r y	Sa m p l e s i z e	A g e	S e x (% m a l e)	Ima g i n g (seq u e n c e s)	Segm e n t a t i o n m e t h o d s	Adj u s t m e n t	Imagi n g p a r a m e t e r s	Disabilit y e n d p o i n t s	Key result c o r r e l a t i o n s (significant)	follo w - u p (m o n t h s)
ia t 2 0 1 6 (1 8 2)	M u n i c h	co m p l e t e d f o l l o w - u p	± 1 1 . 4		FLA IR T2* GE	n, expert neuror adiolo gist and conse nsus SIEN AX		WMH Lacun e no. and vol, CMBs . BPF	Barthel	Dementia (BPF<0.863, CM), Development of moderate or severe disability (BPF<0.863) Lacunes (MDRS) CMBs (MDRS, Barthel, mRS) BPF (MDRS, Barthel, mRS)	
H o l t m a n n s p o t t e r 2 0 0 5 (2 0 8)	M u n i c h	64	4 4 . 9 ± . 9	2 9(4 5. 3 %)	1.5T T1, T2, DWI DTI	Semi- autom ated thresh old proce dure follow ed by manu al refine ment SPM9 9		T2 lesion volum e whole brain, MD avera ge, MD Peak	mRS NIHSS Barthel MDRS SIDAM	Average MD longitudinally (mRS, NIHSS, SIDAM) T2 Volume, average MD, and peak height MD histogram significantly correlated with presentation clinical scores	26. 3± 1.2
J o u	F r a n	29 0	5 0 .	4 4.	1.5T T1 T2	Manu al segme	pena lized linea	Lacun e	MMSE MDRS	quantitative MRI markers (BPF and	36

A u t h o r s (Y e a r)	C o u n t r y	Sa m p l e s i z e	A g e	S e x (% m a l e)	Ima g i n g (s e q u e n c e s)	Segm e n t a t i o n m e t h o d s	Adj u s t m e n t	Ima g i n g p a r a m e t e r s	Disabilit y e n d p o i n t s	Key result c o r r e l a t i o n s (s i g n i f i c a n t)	fol l o w - u p (m o n t h s)
v e n t 2 0 1 6 (1 8 0)	c e/ G e r m a n y	(27 2 s y m p t o m a t i c) 23 6 f o l l o w - u p	6 ± 1 1 · 4	8 %	FLA IR T2* GE	ntatio n, e x p e r t n e u r o r a d i o l o g i s t a n d c o n s e n s u s	r e g r e s s i o n	volum e BPF CM WMH	mRS TMTB	lacune volume) stronger predictors of clinical course than baseline clinical status, age, sex, and level of education	
J o u r n a l o f N e u r o l o g y 2 0 1 2 (1 8 1)	P a r i s / M u n i c h	20 8 (19 0 a f t e r e x c l u s i o n)	5 4 · 5 ± 1 1 · 3	8 4 (4 4 %))	1.5T T1 T2* GE FLA IR	Manu a l s e g m e n t a t i o n, e x p e r t n e u r o r a d i o l o g i s t a n d c o n s e n s u s SIEN A Brain VisaF LIRT(FSL)	age, g e n d e r, b l o o d p r e s s u r e d i a b e t e s, c h o l e s t e r o l, a l c o h o l e d u c a t i o n, c e n t r e a n d b a s e v a l u e s o f m o r p h o l o g i c a l p a r a m e t e r s a n d c l i n i c a l	Mean depth, Fold open i n g, Cortic al thickn ess, WMH , lacune volum e, CM, BPF,	MDRS TMTA TMTB mRS	Increase TMTA and B associated with decrease of mean depth (not significant after adjustment for lacunes, WMH and CMBs). Decreased MDRS associated with decrease of brain volume. Increased mRS associated with cortical thickness	24. 4

A u t h o r s (Y e a r)	C o u n t r y	Sa m p l e s i z e	A g e	S e x (% m a l e)	Ima g i n g (s e q u e n c e s)	Segm e n t a t i o n m e t h o d s	Adj u s t m e n t	Imagi n g p a r a m e t e r s	Disabilit y e n d p o i n t s	Key result c o r r e l a t i o n s (significant)	follo w - u p (m o n t h s)
							score				
L i n g 2 0 1 7 (2 0 5)	P a r i s / M u n i c h	20 6	4 9 .5 ± 1 0 .6	9 3 (4 5.2 %)	1.5T T1 T2 GE FLA IR	Auto m a t e d p r o c e s s i n g f o l l o w e d b y m a n u a l c o r r e c t i o n	Age a n d s e x The n M R I p a r a m e t e r s a t b a s e l i n e	Lac u n e s n o.	MDRS MDRS- I/P TMTA TMTB mRS Barthel	Incident stroke independently associated with incident lacunes OR 2.6, but not significant after adjustment for MRI parameters. Decrease of the MDRS was 4 times as great with incident lacunes than without. Significant increase in TMTB, after adjustment	40. 8± 8.4
L i n g 2 0 1 8 (2 0 6)	P a r i s / M u n i c h	16 0	4 9 .6 ± 1 0 .7	7 6 (4 7.5 %)	T1 T2 GE FLA IR	Manu a l s e g m e n t a t i o n , e x p e r t n e u r o r a d i o l o g i s t a n d c o n s e n s u s	age, sex a n d c e n t e r e f f e c t	No. Lac u n e s nWM H CMBs BPF	MDRS MDRS- I/P TMTA TMTB mRS Barthel	Incident strokes associated with incident lacunes (OR 2.1) and brain atrophy (OR 0.7). Incident dementia associated with brain atrophy (OR 0.5) moderate or severe disability	37. 2± 0.2

A u t h o r s (Y e a r)	C o u n t r y	Sa m p l e s i z e	A g e	S e x (% m a l e)	I m a g i n g (s e q u e n c e s)	S e g m e n t a t i o n m e t h o d s	A d j u s t m e n t	I m a g i n g p a r a m e t e r s	D i s a b i l i t y e n d p o i n t s	K e y r e s u l t c o r r e l a t i o n s (s i g n i f i c a n t)	f o l l o w - u p (m o n t h s)
										associated (OR 0.5). After adjustment for age, sex and centre effect decrease of MDRS, MDRS-I/P & Barthel & increase of TMTA were associated with brain atrophy Increase of TMTB independently associated with brain atrophy and incident lacunes mRS associated with increase of WMH lesions	
P e t e r s 2 0 0 6 (2 0 4)	M u n i c h	80 (76 aft er ex c l u s i o n s)	4 5 · 1 9 · 7	3 4 (4 5 %)	1.5T T1 sp i n - e c h o T2	SIEN A, FSL Co- r e g i s t r a t i o n, e x p e r t r e v i e w e r c o r r e c t i o n	Age and sex	T2 l e s i o n v o l u m e N o r m a l i s e d b r a i n v o l u m e	mRS Barthel NIHSS MDRS SIDAM	Percent brain volume change correlated with Rankin score, Barthel, NIHSS, and SIDAM	26. 3 ±1. 1

Au- thors (Year)	Coun- try	Sam- ple size	Age (% male)	Ima- ging (sequen- ces)	Segm- entati- on meth- ods	Adj- ust- ment	Imagi- ng para- meter s	Disabilit- y end- point s	Key result correlations (significant)	fol- low- up (m- onths)
Mol- k- o- 2- 0- 0- 2- (20- 7)	Par- is	22(14 in lon- gitudi- nal)	57 ± 8	10(71%)	1.5T T1 T2 DWI DTI	Semi- autom- ated region growi- ng thresh- olds, cor- rec- ted by expert after visual inspec- tion	(DTI) Mean value, Peak value, Peak locati- on	MMSE mRS	MMSE positively correlated with peak height, negatively correlated with mean value and peak location of Trace (D)histograms. Rankin scores negatively correlated with peak height and positively correlated with mean value and peak location of Trace (D) histograms	21 ±6

Appendix 2. Cross-sectional studies

Table 0.1 Cross-sectional study summary

Authors (Year)	Country	Sample size	Age	Sex (% male)	Imaging (sequences)	Adjustment	Imaging parameters	Disability endpoints	Key result correlation
Chuin (1977)	Taiwan	49	60.4 ± 11.7	28 (57.1%)	3T GE	age, sex, education		MMSE	CMBs associated with reduced MMSE
Joly et al 2022 (98)	England and	176	50.95 ± 11.35	73 (41.5%)	T1, FLAIR SWI or GE	WM HC MBs normalized brain vol. age sex	Lacunes, WMH, CMBs, Brain volume	BMET, MOCA	Lacune count and BMET Odds ratio : 1.63, p = 0.014
Hack 2022 (187)	Holland	200	52.4 (11.8)	94 (47%)	T1 T2-FLAIR SWI DWI			mRS	normalized lacune vol., BPF, PSMD (Peak width of Skeletonized Mean Diffusivity) and nWMH associated with mRS
Shao-Lun Hsu 2024 (196)	China	81	57.2 ± 13.1	36 (44.4%)	DTI T2 FLAIR T1 SWI DTI		DTI-ALPS, (along the perivascular space); PSMD, peak width of skeletonized mean	mRS MMSE	multivariate regression analysis, only DTI-ALPS index and CMBs independently associated with MMSE, PSMD and CMBs independently associated with the mRS

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
							diffusi vity.		
Lin g, Ch en 20 20 (19 8)	C h i n a	3 9	44.1	18 (46 .2 %)	7.0- T T1 T2 FLA IR SWI	age	susce ptibili ty value as correl ate of iron deposi tion in deep grey matter	MMSE mRS	susceptibility of putamen associated with low MMSE susceptibility of thalamus associated with mRS
Ta nig uc hi 20 22 (18 8)	Ja p a n	4 4	58.1 ± 9.9	26 (59 .1 %)	NA		WMH Lacun es CMBs	MOCA MMSE	High CMBs associated with MOCA and MMSE High Lacunes associated with MOCA
Ou in2 02 3 (17 9)	F ra n c e	2 2 8	56.4	98 (43 %)	T1 FLA IR		WMH corpu s callos um	MDRS mRS	WMH Corpus callosum associated with MDRS and mRS
Be nis ty 20 12 (17 6)	P ar is / M u ni c h	6 6	42.3 ± 1.3	19 (28 .8 %)	1.5T T1 T2 GE FLA IR	age, gend er, educ ation al level , centr e	nWMH BPF CMBs	MMSE MDRS Grober and Buschke TMTA TMTB Wechsler Scale- VADAS- cog	nWMH significantly correlated with MDRS scores, TMT A, TMT B time, verbal fluency and delayed total recall. After adjustment for nWMH ≥1 CMBs associated with reduced performances for TMT B time and euroquol score

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
								FSCRT memory test IADL mRS Barthel	
Br ue nin g 20 00 (20 0)	M u n i c h	2 4	52.3 ± 11.7 year s	No t rep ort ed	1.5T T1 and T2 spin echo T2G E Dyn amic cont rast at Basa l gang lia	Nil	Regio nal CBV	mRS MMSE	rCBV significantly reduced in group with severe dementia than no or mild cognitive impairment Trend towards reduction in rCBV as mRS increases
Ch abr iat 19 98 (17 3)	P ar is	7 5 4 3 s y m pt o m at ic 2 5 as y m	Sym pto mati c 50.8 ±11 Asy mpt om. 38.7 ±10		1 to 1.5T T1 T2		T1 Hypoi ntensi ties T2 hyper ntensi ties (size and numb er)	mRS Dementia	Frequency and severity of lesions increases with age, suggestion of increasing lesions increasing disability but not statistically analysed.

Authors (Year)	Country	Sample size	Age	Sex (% male)	Imaging (sequences)	Adjustment	Imaging parameters	Disability endpoints	Key result correlation
		ptomatic							
Chabrias 1999 (166)	P	16 symptomatic 10 controls	59.3 ±67.6		1.5T T1 T2 DWI DTI		Mean diffusivity, Volume-ratio	MMSE Rankin	MD and volume ratio in T2 WMH strong correlation with MMSE and rankin score
Cullen 2016 (185)	Glasgow	22	49.8 ±11.2	11(50%)	3T T1 FLAIR BOLD		Lacunes number and volume, WMH, normalised for intracranial cavity volume, nBV,	SDMT (symbol digit modalities test) TMTA TMTB Wechsler Adult FAS letter fluency Stroop	Greater lacune volume and smaller brain volume associated with impaired processing speed and executive function.

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
Cu mu rei uc 20 06 (19 9)	P ar is	5 0	55.3 (35- 74)	26 (52 %)	1.5T FLA IR T2		Virch ow Robin Space (VRS) (<2m m) WMH	MMSE Rankin	Severity of dilated VRS did not correlate with mRS / MMSE nor degree of WMH
Di ch ga ns 19 99 (17 5)	M u ni c h	6 4	48.8 ±11. 8	29 (45 .3 %)	1 and 1.5T T1 T2 PD	Age sex	T1 hypo, T2 hyper , WMH	Rankin SIDAM MMSE	total lesion volume correlated with disability (Rankin Scale) both on T1- (SRCC = 0.51; p < 0.0001) and Proton Density (PD)- (SRCC = 0.63; p < 0.0001) weighted images. strong inverse correlation between total lesion volume and MMSE (T1: SRCC = -0.60, p < 0.0001; PD: SRCC = -0.69, p < 0.0001) and SIDAM (T1: SRCC = -0.59, p < 0.0001; PD: SRCC = -0.72, p = < 0.0001
Di ch ga ns 20 03 (20 2)	M u ni c h	1 3	51.5 ± 8.5	7 (53 .8 %)	1.5T MR Diff usio n	Age sex	Mean diffusi vity T2 hyperi ntensi ty, brain paren chym a ratio	mRS MMSE	Average mean diffusivity for normal appearing brain tissue was positively correlated with disability Δ Average mean diffusivity was strongly correlated with the Rankin disability score for whole brain

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
									parenchyma and normal appearing brain tissue SRCC = 0.915, SRCC =0.889
Du che sna y 20 18 (17 8)	P ar is / M u ni c h	3 0 1	50.6 ±11. 2	13 4 (44 .5 %)	1.5T T1 T2 GE FLA IR	Age sex educ ation	BPF Lacun e volum e WMH CMBs	mRS MMSE TMTB MDRS	Larger volumes of WMH in anterior temporal poles and superior frontal gyri are associated with milder forms of the disease, while larger volumes of WMH in pyramidal tracts or in the forceps minor are associated with severe forms, all else being equal
Du eri ng 20 11 (17 7)	P ar is / M u ni c h	2 1 5 (f ro m 3 2 0)	47.9 ±10. 7	(88) 40. 9%	1.5T T1 FLA IR		Lacun es no. and norma lised volum e nWM H BPF	TMTA TMTB NIHSS mRS Barthel MDRS	Anatomical study suggesting anterior thalamic radiation and forceps minor are functionally important areas for WMH and Lacunes
Du eri ng 20 18 (20 9)	M u ni c h	5 7	53.4 ± 10.7	19 (33 .3 %)	T1 T2 FLA IR GE Diff usio n		WMH vol, Lacun e vol. CMBs · brain volum e MD FA	TMTA TMTB Composite processing score mRS	Reduction in fractional anisotropy (FA) and increase in MD mainly driven by free water. Free Water showed strongest association with processing speed

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
Ian nu cci 20 01 (20 1)	M u n i c h	3 3	50± 10	21 (63 .6 %)	1.5T T1 SE 2D GE	Age	PD Magn etizati on transf er ration (MTR)	mRS SIDAM	multivariate analysis- average lesion MTR associated with both Rankin (P=0.03, corrected for age) and the SIDAM (P=0.02, corrected for age) scores
Jou ve nt 20 07 (19 3)	M u n i c h/ P ar is	1 2 9 fr o m 1 4 7	51.4 ±11. 49	56 (43 %)	1.5T T1 T2 FLA IR PD DWI	Age and sex	BPF, nLacu ne volum e, nWM H, CMBs ADC	MDRS mRS MMSE Barthel NIHSS	BPF correlated Spearman rank coefficient with: (after adjustment) MDRS _e 0.43 MMSE _e 0.28 Barthel _e 0.42 mRS _e -0.41 NIHSS _e - 0.41 BPF correlated with both mean ADC,Lacune volume, and number of CMBs, but not with vWMH
Jou ve nt 20 08 (21 0)	P ar is	5 4 F ro m 6 0	48.8 ±10. 8	25 (45 %)	1.5T T1 FLA IR T2 PD DWI		BPF Lacun es WMH CMBs ADC	MDRS mRS	Depth and surface area of cortical sulci obtained in 54 patients were strongly correlated with both cognitive score and disability scales

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
Jou ve nt 20 11 (21 1)	P ar is	1 1 9	51.3	57 (47 .9 %)	1.5T T1 T2 FLA IR PD DWI ADC	See resul t	Brain volum e, BPF, Cortic al depth and width and thickn ess, Lacun es CMBs WMH ADC histog ram	MDRS mRS Apathy (NPI score (neuropsych iatric inventory))	Apathy associated with WMH, Lacunes, ADC and BPF, not CM after adjustment for gender, age, level of education, depression, global disability scales, and cognitive scores and brain parenchymal fraction multiple features of cortical sulci correlated with apathy
Jou ve nt 20 16 (21 2)	P ar is / M u n i c h	1 6 6 fr om 1 9 9 0	52.2 ±9.7	84 (51 %)	1.5T T1 T2 GE FLA IR		BPF WMH Lacun es CMBs Sulcal morph ology	mRS	severity of disability is independently associated with the shape of the central sulcus, mostly driven by the vertical position and the size of the hand knob mRS was significantly associated with IRL (OR=1.23, 95% CI =1.09–1.41; P=0.002), the volume of lacunes measured within pyramidal tracts (OR=1.01, 95% CI

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
									=1.00–1.01; P=0.01), the number of microbleeds measured outside pyramidal tracts (OR=1.11, 95% CI =1.02–1.22; P=0.009), brain parenchymal fraction (OR=0.86, 95% CI =0.79–0.94; P<10–4), and level of education (OR=0.74, 95% CI =0.55–0.98; P=0.03).
Ki m 20 06 (18 9)	S e o ul	2 7	47.7	15 (55 .6 %)	1.5T T2 GE FLA IR AD C		WMH Lacun es CMBs	MMMSE Beck Depression mRS	mRS related to lacunes and WMH MMSE inversely related to lacunes
Le e 20 11 (19 0)	Je ju	4 0	58.1 ±9.9	25(62. 5%)	1.5T T1 T2 GE FLA IR	Age and educ ation	Lacun es CMBs nWM H	MMSE ADAS-cog TMTB Stroop IF	Significant correlation between lacunes and MMSE (β -.41, ADAS-cog β 0.58, TMTB β 0.28, Stroop IF β 0.55) in multivariate adjusting for age and years of education.
Ma we t 20 11 (20 3)	P ar is	1 4 4	52.6 8 11.9	72 (50 %)	1.5T T1 T2G E DWI FLA IR		BPF, Brain volum e, nWM H, nLacu ne	mRS MDRS	Multivariate using MDRS 127 as cut- off, higher cIMT and older age only factors independently associated with lower cognitive

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
					Cerv ical ultra soun d		volum e, CMBs ADC histog rams ICA and CCA stenos is (cIMT)		performances -AUC 0.833 [0.761; 0.905]
No h 20 14 (19 5)	S e o ul	2 3	55 ± 12.5	8 (34 .8 %)	3T T1 T2 GE FLA IR MR A		WMH (Faze kas), CMBs (≥10)	MMSE MRS Montgomer y-Asberg Depression Rating Internationa l Neuropsych iatric Interview Plus to assess depression, emotional incontinenc e, modified Spielberger Trait Anger Scale (Stroke sepecific QOL)	number of CMBs and anatomical location (thalamic or cortical) associated with depression
O' Sul liv an	L o n d	1 8	46.3 ± 11.1		1.5T		mean diffusi vity,	TMTA TMTB	Significant correlations between executive function score and MD or FA.

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
20 05 (21 3)	o n				T2 FLA IR DTI		FA	Digit Span Backwards, Digit Symbol, Wechsler Memory Composite measure of executive function	In the left cingulum bundle and adjacent to the left precuneus, significant correlations were found with both mean diffusivity and FA.
O' Sul liv an 20 04 (21 4)	L o n d o n	1 8	46.3 ± 11.1		1.5T T1 FLA IR DTI		FA, Mean diffusi vity, T2 lesion volum e	TMTA TMTB Wechsler Adult Intelligence Scale– Digit span backwards, digital symbol, National Adult Reading Test	no correlation between the executive function score and T2 lesion volume ($r=-0.29$, $p = 0.27$), Executive function correlated with diffusivity in both normal-appearing gray ($r = -0.73$, $p = 0.002$) and white ($r = -0.68$, $p = 0.004$) matter. The executive function score correlated with fractional anisotropy of normal appearing white matter ($r = 0.52$, $p = 0.038$),
Shi 20 18 (19 2)	B e i j i n g g	2 0	49.9 ± 10.0	14 (70 %)	3T T1 T2 FLA IR DWI SWI	Year s of educ ation	SVD score GCA scale	mRS, Deperssions cale, BMET, MOCA, MMSE, Stroop Color Word Test, Shape Trail Test	Multivariable linear regression: deep atrophy score and total GCA score were significantly associated with the cognitive impairment in global cognition and all domains of cognition after adjustment for

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
								SDMT. Auditory Verbal Learning Test- Huashan version. Rey- Osterrieth Complex Figure Copy, Boston Naming Test	the years of education <i>Unstandardised coefficient - B</i> BMET -0.216 MOCA -0.186 Executive function - 0.137 Info processing speed -0.258 Memory -0.068 Language -0.145 Visuospatial -0.273
Sin gh al 20 05 (17 4)	L o n d o n	1 1 2	47 (23- 82)		1.5T T1 T2 FLA IR PD	age	Schelt ens	Rankin MMSE Depressio Dementia	Dementia correlates with total Scheltens score and Subcortical White Matter scores. Rankin score was associated with age, and after co-varying for age with total Scheltens score (P =.023), no longer significant after co- varying for history of stroke
So ng 20 14 (19 1)	Je J u	5 8	60.7	30(51. 7%)	1.5T T1 T2 GE FLA IR	Age and educ ation al level	Lacun es nWM H	MMSE,AD AS-cog, VADAS- cog TMTA TMTB Stroop,digit cancellation task, SDMT,Seo ul-	Lacuness influence ADAS-Cog β 0.382 p<0.001 Lacuness influence Stroop β -0.237 p<0.05 nWMH not significant on multivariate model

Au tho rs (Y ear)	C o u n t r y	S a m p l e s i z e	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
								Instrumental Activities of Daily Living (S-IADL),	
Vis wa nat ha n 20 07 (18 4)	P ar is / M u ni c h	1 4 7	51.8 ±111 .2	63(42.9%)	1.5T T1 T2 GE FLA IR		nWM H no. and volum es lacune s, CMBs	MMSE MDRS mRS Barthel Dementia	Multivariate normalised lacunar Volume associated with low MDRS Normalised lacunar volume and CM associated with high mRS
Vis wa nat ha n 20 10 (18 3)	P ar is / M u ni c h	1 4 7	51.8 ± 11.2	63 (42.9%)	1.5T T1 T2 GE FLA IR DWI ADC	Age Sex	Brain volum e BPF nWM H nLV mean ADC	MMSE MDRS mRS Barthel	Multivariate linear regression: For Disability: BPF $\beta = -0.12$, $p < 0.0001$ LL $\beta = 6.19$, $p < 0.0001$ CMBs $\beta = 0.05$, $p < 0.002$ For MDRS: BPF $\beta = 1.37$, $p < 0.0001$ LL $\beta = -53.8$, $p = 0.0057$ Mean-ADC $\beta = -2.7$, $p < 0.012$
Yo usr y 19 99	M u ni c h	1 4	49.6 (32-63)	7 (50%)	1 or 1.5T T1 T2		Lesio n volum e	Expanded Disability Status Scale (EDSS), Wechsler Adult	EDDS correlated with lesion volume (PD) in parietal lobe, basal ganglia Neuropsychological impairment with T1

Au tho rs (Y ear)	C o u n tr y	S a m pl e si ze	Age	Se x (% ma le)	Ima ging (seq uenc es)	Adj ust men t	Imagi ng para meter s	Disability endpoints	Key result correlation
(17 5)					PD			Intelligence Scale	lesion voume: sig. in these domains: Intelligence, conceptual functions, attention memory, visuospatial functions
Zie ren 20 13 (18 6)	M u ni c h/ P ar is	2 4 7	49.6 (24– 75)	10 8(4 3.7 %)	1.5T T1 T2 FLA IR	Age and educ ation	BPF Brain volum e nLV nWM H	TMTA/B Wechsler Adult Intelligence Scale, Verbal fluency, Grober & Buschke Free and Cued Selective Reminding Task mRS	Linear regression Processing speed and verbal memory associated with normalised Lacune Volume

4 Population database description and epidemiology update CADASIL in Scotland and registry

4.1 Introduction

CADASIL is a rare genetic small vessel disease as defined by UK government definition of affecting less than 1 in 2000 people. Population estimates of early studies varied from 1.32 per 100,000 (9) to between 4.1 and 4.6 per 100,000 (8, 215) using various techniques estimation of population in different geographical locations. These methods have been considered to underestimate true prevalence as people may not have presented to health services due to minor or absence of symptoms or for other reasons of access, and historically genetic testing may have been limited. A more recent large genetic study (216) established a much higher prevalence of NOTCH3 mutations present in 3.4 per 1000 of a different European population. Despite more extensive genetic testing and increased awareness of the condition the specialist clinics managing CADASIL are not experiencing referrals or detecting cases of affected individuals at this level. This has led to the hypothesis that there are a large population of people with a NOTCH3 mutation who have mild or no significant symptoms. There is evidence that genetic mutations of the NOTCH3 gene from EGFr 7 to 34 are less severely affected and have less extensive imaging abnormalities (58).

The purpose of this study was to update the population data from our national Neurovascular specialist clinic that was initiated in 2002 and offers review for all CADASIL patients nationally across Scotland. We will update an estimate of mutation prevalence and describe current genotype variation in this study cohort.

4.2 Methods

A neurovascular genetics clinic has been running nationally for people with CADASIL in conjunction with specialist genetics services since 2002. People are accepted for review from anywhere in Scotland. We included adults with CADASIL confirmed through genetic testing. This requires a pathogenic mutation from analysis of the NOTCH 3 gene. Currently an extensive panel is investigated for abnormalities within exons 2 to 23. We used a method previously reported to estimate likely disease prevalence in untested relatives of confirmed CADASIL cases (8). Using family tree clinical records and genetics services we included 1st and 2nd degree relatives thought still to be alive and resident in Scotland who have not undergone genetic testing. We recorded the amino acid and

nucleotide change in each case. 1st degree relatives were assumed to have 50% risk of CADASIL and 2nd degree relatives assumed to have 25% risk. Care was taken to avoid duplication as multiple family members can be affected who share many of the same untested relatives.

We included all people in Scotland who attend the national clinic with confirmed CADASIL on genetic testing. The geographical areas were divided into Greater Glasgow and Clyde, Lanarkshire, Lothian, and the rest of Scotland. We used national baseline mid-year projections for population from 2020, the most recent available from the National records of Scotland created by the Scottish government (217). We input values to the formulae described to calculate confidence intervals. We believe that the total number of people reviewed in clinic still underrepresents the true CADASIL population in this country. Previously the difference between disease prevalence of 4.6 per 100,000 and mutation prevalence estimate of 10 per 100,000 (8) suggested approximately 50%. For multiple reasons people with CADASIL may not have been tested. At risk individuals may not wish to be tested, others will not have developed symptoms nor had reason to present to healthcare services. Others will be managed locally by other health professionals.

Statistically, we required a critical value of 1.96, for a confidence level of 95%. The confidence intervals were calculated using the following two formulae.

$$\text{Confidence interval} = p \pm Z_{\alpha/2} \sqrt{(1/n) \times p \times (1-p) \times \text{FPC}}$$

P= sample proportion, n= sample size, N= total population , FPC = finite population correction

$$\text{FPC} = (N-n)/(N-1)$$

4.3 Results

There are 151 people living with CADASIL under regular review in the CADASIL clinic in Glasgow. This group had 389 first degree relatives untested for CADASIL presumed living in Scotland who were deemed to have 50% risk of having CADASIL. There were 322 second degree living relatives, not known to be tested for CADASIL for whom the risk was considered 25%. There were two from within the cohort about whom we did not have genetic mutation details, leaving 149 with confirmed CADASIL pathogenic gene mutations. Seventy-three (49%) were residing in greater Glasgow and Clyde with 24 (16.1%) living in Lanarkshire. Seven (4.7%) lived in Edinburgh and Lothian, with the

remaining 45 (30.2%) living elsewhere in Scotland. All patients in the cohort had an exon mutation between exon 2 and 22. A majority 89 (59.7%) had a mutation on exon 4.

The population of Scotland on 30th June 2020 is estimated to be 5466000, with Greater Glasgow and Clyde 980430, Lanarkshire 661960, and Lothian 912490. The rest of Scotland's population was 291120 (217).

With a required confidence interval of 95% and a sample size of 151 in a total population of 5466000 we estimated the confidence intervals as recorded in table 4.1. The cysteine-altering pathogenic variants p.Arg141Cys c.421C>T, exon 4 was the most frequently present in 22 cases (14.6%).

Table 4.1 Estimated disease prevalence in Scottish regions and overall estimated mutation prevalence

	Disease prevalence (per 100,000)	Mutation prevalence
Glasgow	7.45 (5.8- 9.1)	
Lanarkshire	3.78 (2.7- 4.9)	
Lothian	0.77 (0.2- 1.3)	
Rest of Scotland	1.55 (1.1 – 2.0)	
Scotland overall	2.76 (2.3 - 3.2)	7.79 (7.05-8.53)

Table 4.2 Distribution of genetic mutations in Scottish CADASIL cohort.

Amino acid change	Nucleotide change	Exon	EGF repeat	Number of cases
p.Arg54Cys	c.160C>T	2	1	10
p.Arg110Cys	c.328C>T	3	2	6
	c.341-1G>C	4	3	1
p.Arg133Cys	c.397C>T	4	3	14
p.Arg141Cys	c.421 C>T	4	3	22
p.Cys144Phe43	c.431G>T	4	3	1
p.Arg153Cys	c.457C>T	4	3	6
p.Arg141Cys	c.499C>T	4	3	2
p.Arg169Cys	c.505C>G	4	4	3
p.Arg169Cys	c.505C>T	4	4	16
p.Arg182Cys	c.544T>C	4	4	1
p.Cys183Arg	c.547T>C	4	4	6
p.Arg182Cys	c.554C>T	4	4	1
p.Cys194Ser	c.580T>A	4	4	5
p.Arg169Cys	c.583C>T	4	4	2
p.Cys201Arg	c.601T>C	4	5	3
p.Arg207Cys	c.618C>T	4	5	1
p.Arg207Cys	c.619C>T	4	5	4
p.Cys 222 Arg	c.664C>T	4	5	1
p.Cys245Ser	c. 811T>A	5	6	1
p.Phe228Cys	c.683T>G	5	5	5
p.Cys245Ser	c.733T>A	5	6	1
p.Cys260Arg	c.778C>T	5	6	9
p.Cys260Tyr	c.779G>A	5	6	1

p.Cys245Ser	c.811T>A	5	6	1
p.Cys323Ser	c.967T>A	6	8	1
p.Arg332Cys	c.994C>T	6	8	2
p.Cys446Gly	c.1336T>G	8		1
p.Gly481Cys	c.1441G>T	9	12	1
p.Arg607Cys	c.1819C>T	11	15	1
p.Arg680Cys	c.2038C>G	13		1
p.Cys709Tyr	c.2126G>A	13	18	1
p.Arg717Cys	c.2149C>T	14	18	3
p.Gly723Cys	c.2167G>T	14		1
p.Gln741*	c.2221C>T	14	19	1
p.Phe984cys	c.2951T>G	18		1
p.Cys986Arg	c.2956T>C	18	25	3
p.Cys988Arg	c.2962T>C	18		1
p.Arg1006Cys	c.3016C>	19	26	1
p.Tyr1021Cys	c.3062A>G	19	26	1
p.Cys1099Tyr	c.3296G>A	20	28	1
p.Cys1222Gly	c.3664T>G	22	31	5
	Total		EGFR 1-6 EGFR 7-34	123 26

4.4 Discussion

CADASIL remains a rare genetic disease first described in the early 1990s. Despite the surprising genetic finding of frequent NOTCH3 abnormalities in the general population (15), there has not been a large increase in number of patients reporting CADASIL-related symptoms. Since our local population was last evaluated for potential disease and mutation prevalence, there has been some increase in the population attending clinic as a result of increasing awareness and testing. The current calculated disease prevalence is lower at 2.76 per 100,000, compared to the previous estimate of 4.6 per 100,000 (8). This calculated disease prevalence was estimated by averaging the CADASIL population in Scotland as if evenly distributed. It is very likely that the higher population density in Glasgow are more representative of the true population estimate. Potentially, even this higher prevalence of 7.45 per 100,00 remains an underestimate as a result of lower penetrant cases. The previous estimate was calculated from extrapolating geographical areas with highest prevalence, and this pattern remains present today. Twenty six (17.4%) of the current population have a distal mutation, thought to be associated with a milder disease phenotype and less extensive imaging abnormalities (58). Amino acid change p.Arg141Cys c.421 C>T abnormality is the single most common cysteine altering mutation located on Exon 4 present in 22 cases (14.6%). The dominance and high proportion of these cases may be evidence of the founder effect that has been noted as a possible influence on geographically isolated areas (79, 218). The study in Finland thought the common ancestor responsible for the founder mutation likely lived in the late 1600s (218). The current number of individuals attending the national clinic in Glasgow is 151, which is approaching 173, the number of predicted potential cases estimated in 2013 (8). CADASIL genetic testing is more routinely available, and awareness of the condition is increasing so it is possible that the prior estimate of 50% attendance at clinic is no longer valid and that a higher proportion of CADASIL cases are now in attendance. The vastly differing clinic attendance from Greater Glasgow and Lanarkshire compared to Lothian and the rest of Scotland would suggest that there are still many people with CADASIL being reviewed at local services, without alternative explanation for this geographical discrepancy. The non-specific nature of some symptoms of CADASIL will lead to delay in undergoing genetic testing and later referral to specialist clinic. As there is some overlap between sporadic small vessel disease and CADASIL in imaging features, some cases of CADASIL may be considered the result of conventional risk factors and genetic testing may not be undertaken. This may also be the case in older patients on the basis that CADASIL can be considered a cause of stroke in younger people. Despite its autosomal inheritance, an

absence of known family history is not a reliable guide to exclusion of CADASIL. It is also feasible that in the mildest disease states people do not require or seek medical input at all. There is ongoing requirement to establish the difference between CADASIL disease, NOTCH-3 genetic abnormality in a mild disease state and non-penetrance.

There would be considerable benefit in creating a national database that is updated and maintained to allow more accurate data and epidemiological information on the disease CADASIL in Scotland.

5 Anatomical descriptive study of small vessel disease biomarkers in CADASIL and sporadic small vessel disease

5.1 Introduction

A notable imaging feature in CADASIL is the extent of WMH. The distribution of this imaging abnormality appears to be different in CADASIL than with other causes of small vessel disease (219). Presence of WMH in the anterior temporal poles is frequently described as highly specific for CADASIL (220). This early study using automated image analysis also found significant predilection for the paramedian superior frontal area and temporopolar arcuate fibres in CADASIL (220). Another case-controlled study comparing imaging abnormalities in CADASIL with sporadic small vessel disease confirmed WMH in the temporal pole and external capsule as key findings in CADASIL and noted 3 of 20 (15%) had WMH abnormality in the corpus callosum (88). A more recent study of 228 people with CADASIL found WMH of the corpus callosum in 46% and found this to be associated with increasing clinical severity (179). Another older study of 112 people with CADASIL demonstrated the correlation between extent of WMH with increasing age and reported the external capsule, in addition to the temporal, frontal and parietal lobes to be frequently affected. Conversely low levels of abnormal WMH were seen in the corpus callosum, cerebellum and brainstem (221). Despite the high specificity often reported in CADASIL imaging studies, when reviewed retrospectively bilateral temporal pole hyperintensities are a non-specific MRI finding with many potential aetiologies including infective, neoplastic and metabolic causes (222). Different geographical regions have been found to have different clinical and imaging characteristics and the proportion with involvement of anterior temporal pole and external capsule has been found to be lower in Taiwan and Korea than in European populations (223, 224).

The purpose of this chapter is to provide a contemporary overview of the anatomical distributions of markers of small vessel disease in the Scottish CADASIL population and comparing the differences in anatomical distribution of WMH, lacunes and CMBs with a cohort of sporadic small vessel disease, presumed not to be genetic in nature.

5.2 Methods

We used the MRI imaging cohort of data obtained from the NHS safe haven (GSH/18/NE/007) of adult patients with CADASIL. All scans were analysed using Mango

imaging software version 4.1 <http://ric.uthscsa.edu/mango/index.html> (225). We included T1, T2-FLAIR, GE and SWI sequences and one rater reviewed for WMH, lacunes, CMBs as defined by the original STRIVE criteria (82). The anatomical locations of lacunes were recorded using an MRI case report form for chronic ischaemic stroke and small vessel disease devised in Calgary. The imaging for sporadic small vessel disease was obtained from the initial MR imaging as part of a clinical trial (226). Inclusion criteria were adults over 50 who had a stroke or transient ischaemic attack in the 4 weeks previous. The trial was testing effect of xanthine oxidase inhibition on white matter hyperintensity progression (226).

We used Chi-square test or Fisher's exact test to calculate differences in each anatomical location for WMH and lacunes and used binary logistic regression with presence of CADASIL as dependant variable for each anatomical location of WMH. A Mann-Whitney U test was used to evaluate difference in ordinal scales including Fazekas scale for WMH and number of lacunae and cerebral microbleeds between CADASIL and sporadic small vessel disease.

5.3 Results

5.3.1 White matter hyperintensities

Images of 106 people with CADASIL and a convenience sample of 94 randomly selected with sporadic small vessel were included for comparison of MRI features. The baseline demographics are presented in table 5.1. As well as having more extensive WMH abnormalities in CADASIL (Figure 5.1), there are notable differences in anatomical distribution of WMH. As in table 5.2 the only brain region that did not show statistically significant difference between CADASIL and sporadic small vessel disease was the cerebellum ($p=0.081$). Figure 5.5 demonstrates the differences in distribution of presence of WMH in CADASIL compared to sporadic small vessel disease.

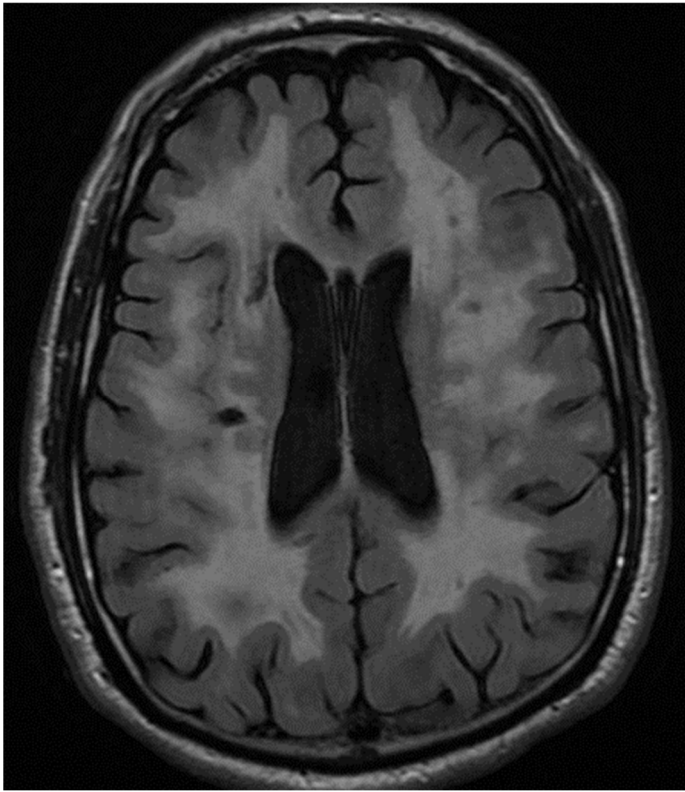


Figure 5.1 Example of extensive white matter hyperintensities and lacunes in CADASIL.

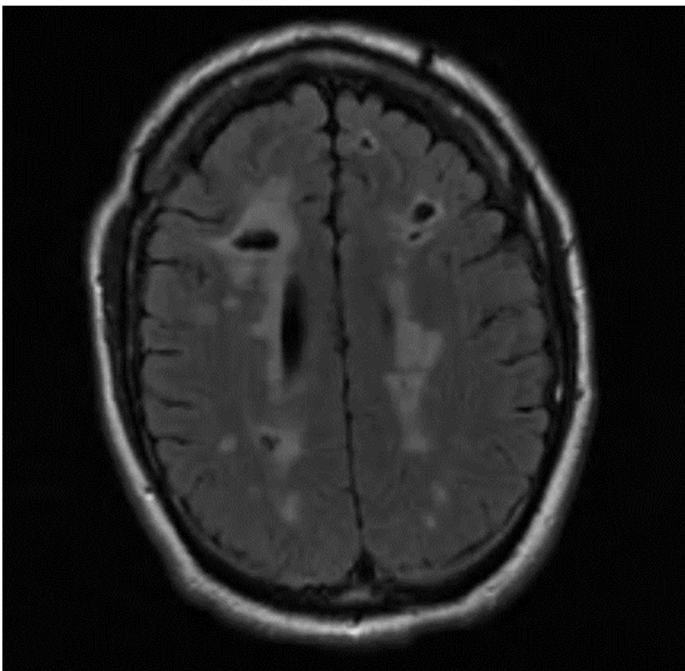


Figure 5.2 Example of multiple lacunes in CADASIL.

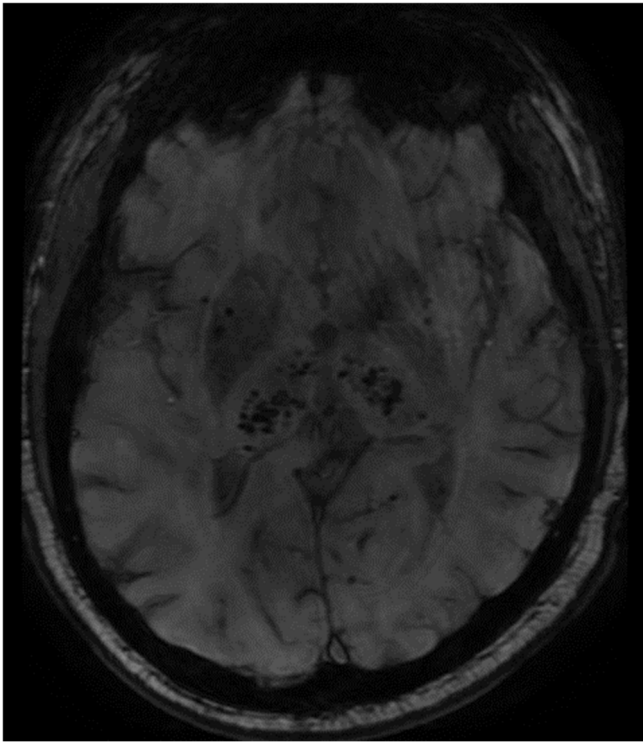


Figure 5.3 Cerebral microbleeds in the thalamus in CADASIL.

Table 5.1 Baseline characteristics for CADASIL and sporadic small vessel disease

	CADASIL	Sporadic SVD	p-value
n	106	94	
Age	46.4 ± 11.4 2 missing	65.8 ± 8.6	<0.001
Sex (male)	46 (44.2 %)	66 (70.2%)	<0.001
Hypertension	22 (22 %) 6 missing	42 (44.7%)	<0.001
Diabetes	9 (9.7 %) 7 missing	22 (23.4%)	0.017
Smoking	34 (41 %) 17 missing	47 (50%)	0.229

Table 5.2 Anatomical distribution of WMH and Fazekas score in CADASIL and sporadic small vessel disease.

Anatomical location WMH	CADASIL	Sporadic small vessel disease	p-value
Basal ganglia	76 (73.8%)	31 (33%)	<0.001
Thalamus	49 (47.6%)	25 (26.6%)	0.002
External capsule	67 (65%)	41 (43.6%)	0.002
Internal capsule	30 (29.1%)	14 (14.9%)	0.017
Corpus callosum	23 (22.3%)	9 (9.6%)	0.020
Brainstem	39 (37.9%)	21 (22.3%)	0.018
Cerebellum	20 (19.6%)	10 (10.6%)	0.081
Frontal	101 (98.1%)	77 (81.9%)	<0.001
Parietal	100 (97.1%)	58 (61.7%)	<0.001
Occipital	92 (89.3%)	42 (44.7%)	<0.001
Temporal	78 (75.7%)	23 (24.5%)	<0.001
Anterior temporal	83 (80.6%)	6 (6.4%)	<0.001
Missing data	3 (2.8%)		
Fazekas' PWM	3 [2 - 3]	1 [1 - 2]	<0.001
Fazekas' DWM	3 [2 - 3]	1 [1 - 2]	<0.001
Fazekas' total	5 [4 - 6]	3 [2 - 3]	<0.001

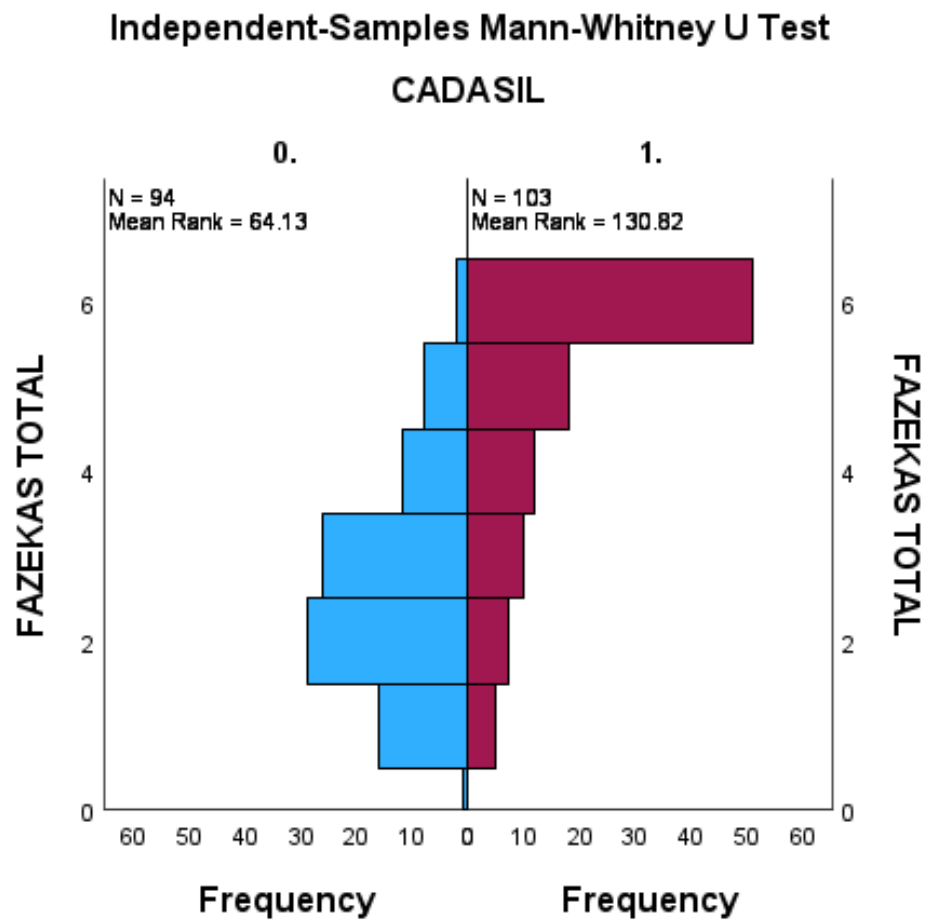


Figure 5.4 Differences in Fazekas score between CADASIL (1) and sporadic small vessel disease (0)

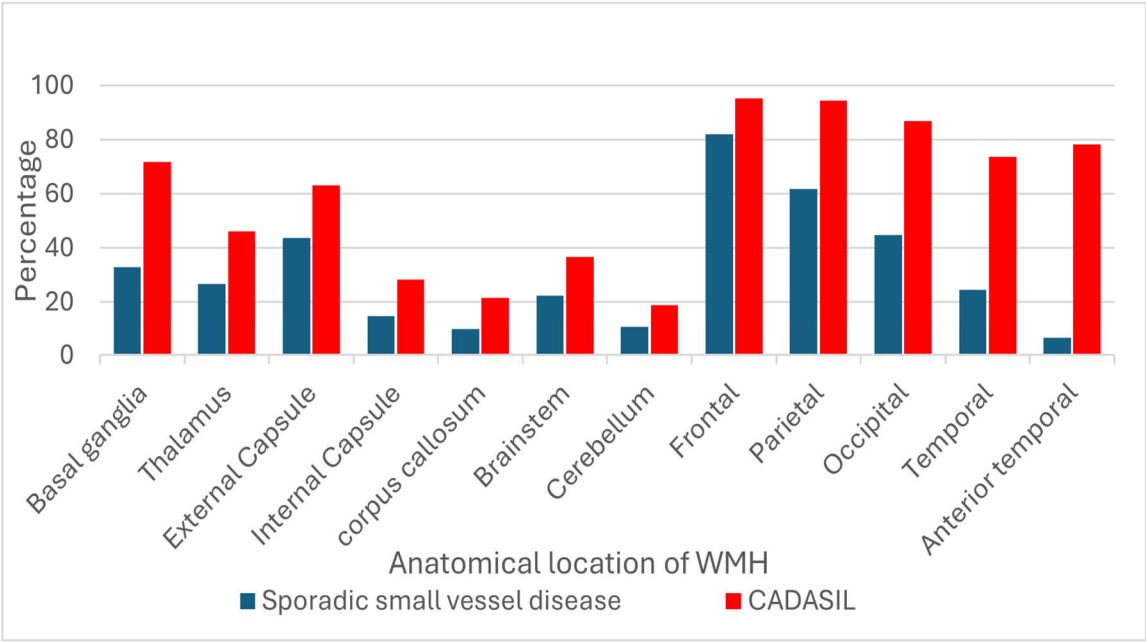


Figure 5.5 Comparison of WMH anatomical distribution CADASIL and sporadic small vessel disease

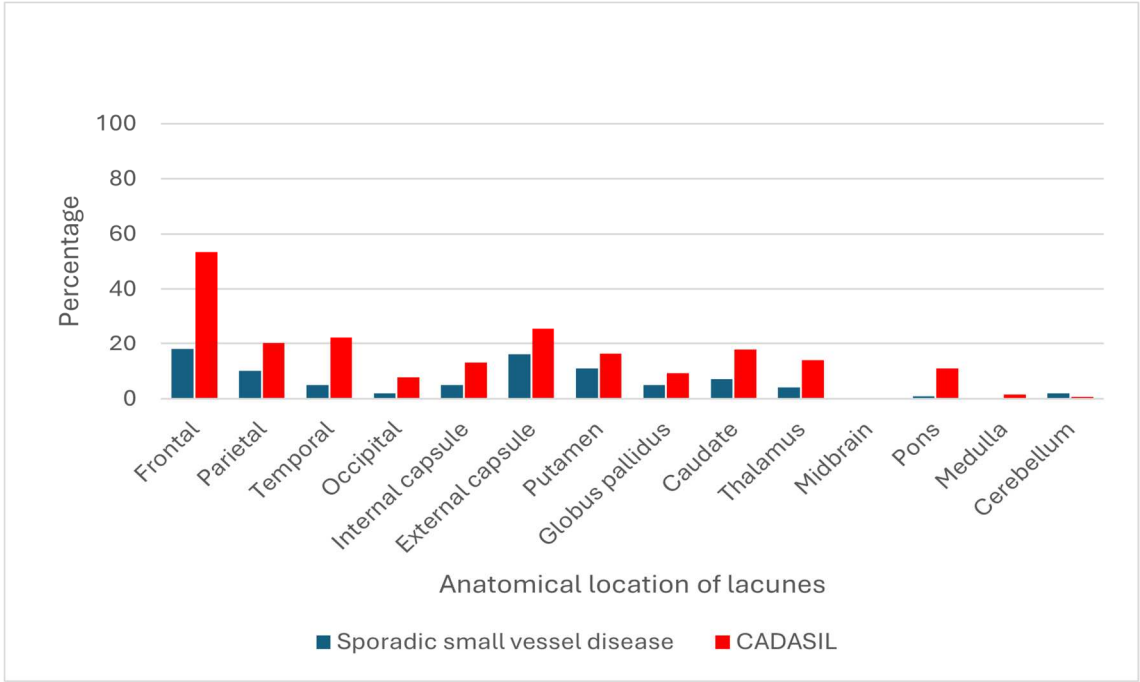


Figure 5.6 Comparison of lacunes anatomical distribution in CADASIL and sporadic small vessel disease

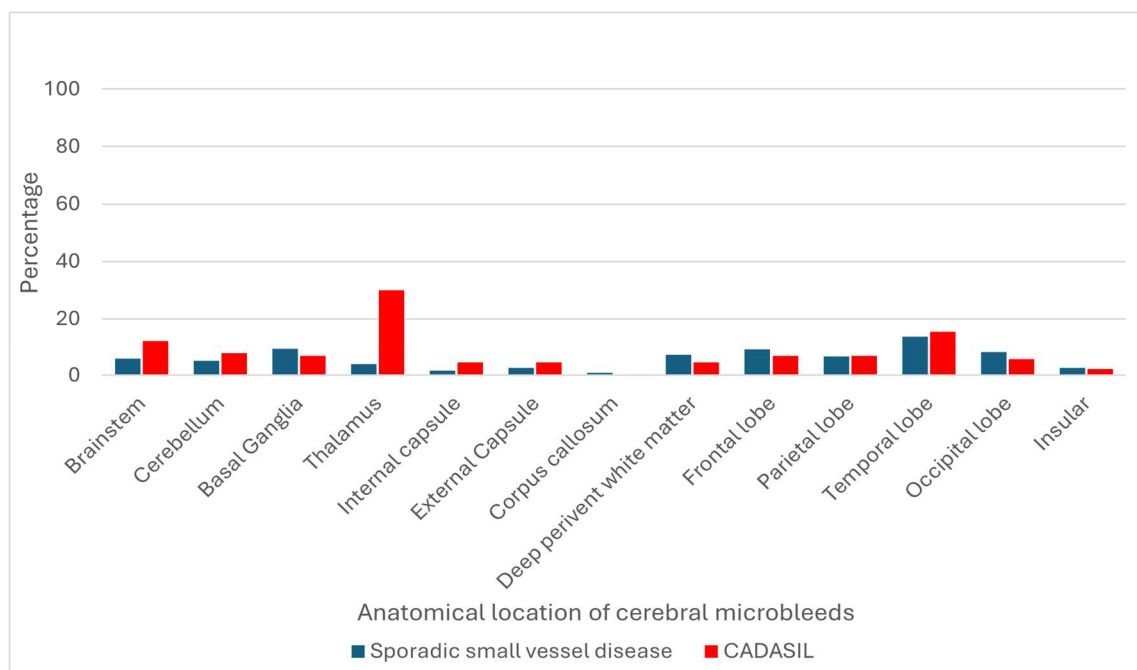


Figure 5.7 Comparison of cerebral microbleed anatomical distribution in CADASIL and sporadic small vessel disease

Table 5.3 Binary logistic regression model for location of WMH as independent variable and presence of CADASIL as dependent variable

Anatomical location WMH	Exp (B) Odd ratio presence in CADASIL	95% C.I. lower	95% C.I. upper	p-value
Basal ganglia	1.9	0.7	5.4	0.178
Thalamus	2.3	0.8	7.2	0.142
External capsule	2.4	0.8	7.7	0.130
Internal capsule	1.4	0.4	4.6	0.559
Corpus callosum	1.1	0.3	4.6	0.849
Brainstem	1.9	0.6	6.2	0.307
Cerebellum	1.0	0.2	4.2	0.983
Frontal	1.0	0.1	6.5	0.965
Parietal	6.5	1.3	32.2	0.023
Occipital	1.8	0.6	5.7	0.285
Temporal (excluding anterior temporal)	1.3	0.4	4.3	0.675
Anterior temporal	33.7	11.0	103.8	<0.001

5.3.2 Lacunes

Table 5.4 Differences in anatomical distribution of lacunes in CADASIL and sporadic small vessel disease

	Anatomical location	CADASIL	Sporadic SVD	p-value
Subcortical white matter	Frontal	69 (56.6%)	18 (18%)	<0.001
	Parietal	29 (23.8%)	10 (10%)	0.007
	Temporal	29 (23.8%)	5 (5%)	<0.001
	Occipital	12 (9.8%)	2 (2%)	0.024
	IC	17 (13.9 %)	5 (5%)	0.040
	EC	39 (32%)	16 (16%)	0.006
Subcortical gray matter	Putamen	21 (17.2%)	11 (11%)	0.190
	Globus pallidus	13 (10.7%)	5 (5%)	0.144
	Caudate	23 (18.9%)	7 (7%)	0.011
	Thalamus	20 (16.4%)	4 (4%)	0.004
Brainstem	Midbrain	0	0	-
	Pons	18 (14.8%)	1 (1%)	<0.001
	Medulla	2 (1.6%)	0	0.503
Cerebellar white matter	cerebellum	2 (1.6%)	2 (2%)	0.611
	CALGARY PROFORMA	2 [0 – 7.25]	0 [0 - 2]	

Table 5.5 Mann-Whitney U comparison of lacunes in CADASIL and sporadic small vessel disease

	Anatomical location	U test	p-value
Subcortical white matter	Frontal	8657	<0.001
	Parietal	6974	0.006
	Temporal	7241.5	<0.001
	Occipital	6581	0.016
	IC	6647.5	0.026
	EC	7090	0.006
Subcortical gray matter	Putamen	6512	0.156
	Globus pallidus	6447.5	0.123
	Caudate	6825.5	0.010
	Thalamus	6867.5	0.003
Brainstem	Midbrain	6100	1.00
	Pons	6940.5	<0.001
	Medulla	6200	0.199
Cerebellar white matter	cerebellum	6078	0.841
CALGARY PROFORMA TOTAL		8090	<0.001
mean $\pm$ SD	CADASIL 4.45 $\pm$ 5.831	sporadic SVD 1.29 $\pm$ 2.235	

Using the same cohort of patients, we compared the distribution of lacunes in CADASIL with sporadic small vessel disease (Figure 5.6). Using a binary logistic regression model with CADASIL presence as the dependent variable, presence of lacunes in the frontal lobe ($p<0.001$) and temporal lobe ($p=0.024$) were significantly associated with CADASIL. The distribution was compared using a Mann-whitney U test and results are shown in table 5.5.

Table 5.6 Binary logistic regression with presence of lacune as independent variable and CADASIL as dependent

	Anatomical location	Exp (B) Odd ratio presence in CADASIL	95% C.I. lower	95% C.I. upper	p-value
Subcortical white matter	Frontal	5.8	2.4	14.0	<0.001
	Parietal	2.1	0.7	6.5	0.206
	Temporal	3.6	1.2	11.0	0.024
	Occipital	1.4	0.2	8.1	0.7410
	IC	1.4	0.4	5.7	0.598
	EC	1.1	0.4	2.8	0.829
Subcortical gray matter	Putamen	2.5	0.8	7.8	0.110
	Globus pallidus	1.2	0.3	4.9	0.824
	Caudate	2.6	0.8	8.7	0.131
	Thalamus	1.4	0.3	5.4	0.669
Brainstem	Midbrain	-	-	-	-
	Pons	7.1	0.8	61.8	0.075
	Medulla	-	-	-	-
Cerebellar white matter	cerebellum	0.3	0.0	4.2	0.393

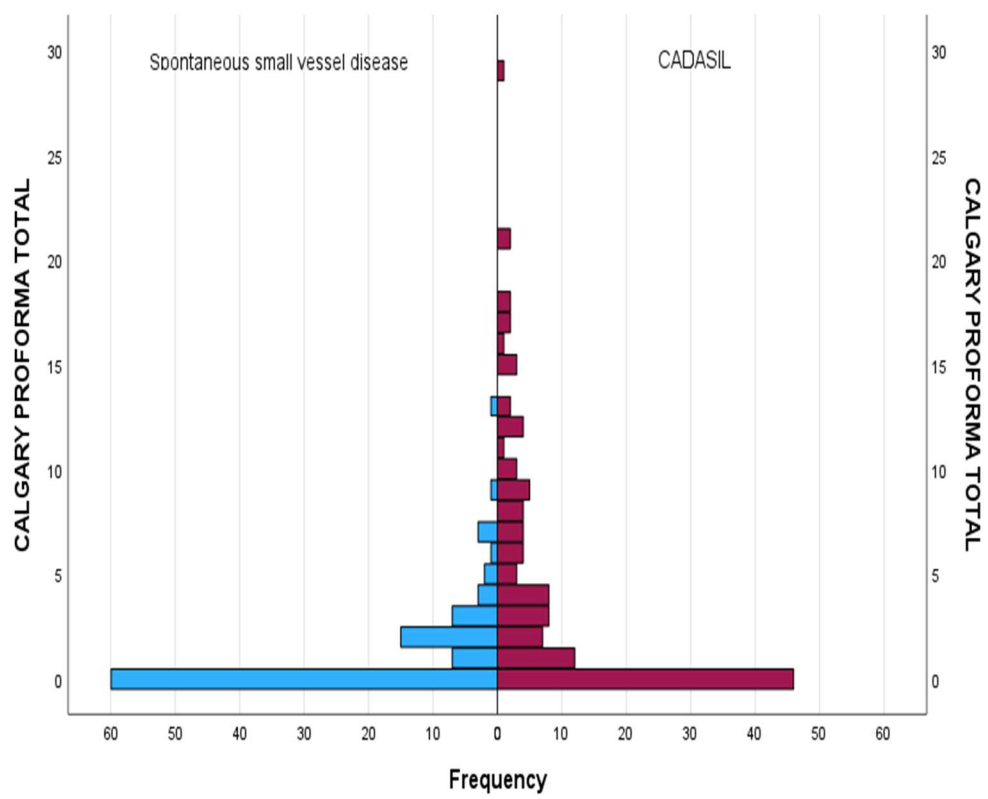


Figure 5.8 Distribution of lacune number in CADASIL and sporadic small vessel disease.

5.3.3 Cerebral Microbleeds

We reviewed the 90 CADASIL patients with available GE, SWI, or SWAN sequences, of which 31 (34.4%) had at least one microbleed present. The detailed anatomical distribution is displayed in table 5.8. There were 15 (16.7%) with CMBs in an infratentorial location, 18 (20%) with lobar CMBs and 28 (31.1%) with deep subcortical microbleeds. The proportion with 0, 1, 2, 3 to 5, 6 to 9, and >9 is shown in figure 5.9. Figure 5.7 demonstrates the difference in distribution of presence of CMBs in CADASIL and sporadic small vessel disease.

A Mann-Whitney U test was used to determine the differences in CMB count for CADASIL and sporadic small vessel disease in different anatomical locations. There were statistically differing distributions in the thalamus $p<0.001$ and brainstem $p=0.032$. The internal capsule was statistically approaching a significant difference ($p=0.079$), but no other regions differed significantly.

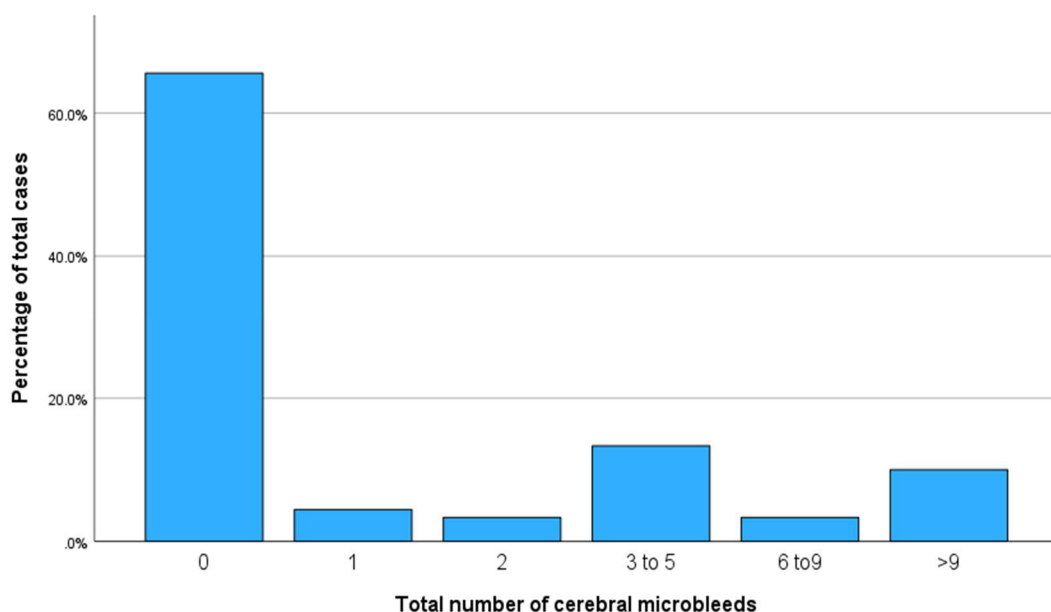


Figure 5.9 Proportion of CADASIL cases in each group based on number of cerebral microbleeds.

Table 5.7 Results of Mann-Whitney U test for differences in number of microbleeds between CADASIL and sporadic small vessel disease.

Anatomical location	CADASIL mean rank	Sporadic small vessel disease mean rank	Z score, p-value
Brainstem	254.1	238.6	-2.14, p=0.032
Cerebellum	246.9	240.3	-1.02, p=0.308
Basal ganglia	236.5	242.6	-0.77, p=0.441
Thalamus	293.9	229.5	-8.09, p<0.001
Internal capsule	247.2	240.2	-1.757, p=0.079
External capsule	245.3	240.6	-0.98, p=0.327
Corpus callosum	240.0	241.8	-0.83, p=0.406
Deep periventricular white matter	236.3	242.7	-0.918, p=0.359
Frontal lobe	240.0	242.5	-0.70, p=0.482
Parietal lobe	241.8	241.4	-0.05, p=0.959
Temporal lobe	246.5	240.4	-0.62, p= 0.534
Occipital lobe	236.9	242.6	-0.75, p=0.455
Insula	240.9	241.7	-0.18, p=0.857
Total	249.7	239.6	-0.722, p=0.471

Table 5.8 Comparison between proportion of patients in each group affected by microbleeds.

Anatomical location	CADASIL Presence of CMBs yes /no %	Spontaneous SVD Presence of CMBs yes /no %	p-value
Brainstem	11/79 (12.2%)	23/369 (5.9%)	0.034
Cerebellum	7/83 (7.8%)	20/372 (5.1%)	0.319
Basal ganglia	6 / 84 (6.7%)	36 / 356 (9.2%)	0.445
Thalamus	27 /63 (30%)	15 / 377 (3.8%)	<0.001
Internal Capsule	4 / 86 (4.4%)	6 / 386 (1.5%)	0.096
External capsule	4 / 86 (4.4%)	10/382 (2.6%)	0.255
Corpus callosum	0 / 90 (0%)	3 / 389 (0.8%)	0.537
Deep periventricular white matter	4 / 86 (4.4%)	28 / 364 (7.1%)	0.252
Frontal lobe	6 / 84 (6.7%)	35 / 357 (8.9%)	0.488
Parietal lobe	6 / 84 (6.7%)	26 /366 (6.6%)	0.991
Temporal lobe	14 / 76 (15.6%)	54 / 338 (13.8%)	0.662
Occipital lobe	5 / 85 (5.6%)	32 / 360 (8.2%)	0.277
Insula	2 / 88 (2.2%)	10 / 382 (2.6%)	0.605
Total	31 / 59 (34.4%)	143 / 249 (36.5%)	0.717
median [IQR]	0 [0 - 3]	0 [0 - 1]	

We divided the CADASIL cohort into those with higher numbers of lacunes (>6) and cerebral microbleeds (>6) and those with low numbers of both and performed a binary logistic regression analysis to assess the effect of migraines, stroke, hypertension, diabetes, smoking, or proximal /distal mutation on high levels of CMBs and lacunes. Only history of stroke: odds ratio 4.7 (95% CI 1.59 -14.3), $p=0.005$ and smoking: odds ratio 3.2 (95% CI 1.14 to 8.78) $p=0.027$ were significantly associated with the group who had higher numbers of CMBs and lacunes.

5.4 Discussion

The main finding for this study is an updated anatomical description of the distribution of WMH, lacunes and CMBs in a CADASIL population. There are differences between the location of abnormalities in comparison to sporadic small vessel disease and as the clinic spectrum of CADASIL evolves over time it is important to be aware of and update associated changes in imaging findings.

WMH in the CADASIL cohort was much more extensive than in sporadic small vessel disease with total Fazekas score differing between median 5 and 3 (Table 5.2 FAZEKAS distribution). This might increase the likelihood of presence of WMH in each anatomical region in CADASIL. The most striking finding is confirmation of the previously reported association of CADASIL with WMH in the anterior temporal lobe. In this cohort this was present in over 80% of people with CADASIL compared to less than 10% without corresponding to an odds ratio of 33 (95% C.I. 11-104). The other anatomical location strongly associated with CADASIL was the parietal lobe with an odds ratio of 6.5 (95% C.I. 1.3 – 32.2). From the binary logistic regression model there were two areas in which lacunes appeared significantly associated with CADASIL: the frontal lobe odds ratio 5.8 (95% C.I. 2.4 – 14) and the temporal lobe with odds ratio 3.6 (95% C.I. 1.2 - 11).

There is thought to be a close relation between the development of lacunes and WMH and a larger study found incident lacunes had a predilection to develop around the perimeter of patches of WMH (99). CMBs are a key imaging finding in small vessel disease and CADASIL with a significant increase with increasing age. The specific correlation between extent of CMBs and disease severity has been difficult to quantify, but there appears to be an association between CMBs and history of stroke (227). Early CADASIL studies showed high numbers of CMBs with a preponderance for cortico-subcortical regions, the thalamus and brainstem (104). Different scoring systems have been developed

to provide information on anatomical distribution of CMBs that can vary significantly (110, 111). This should aid our understanding of the relative importance of CMBs in specific locations. Our analysis of the distribution of cerebral microbleeds in CADASIL showed very similar distribution to a recent comprehensive assessment of cerebral microbleeds in a CADASIL cohort (44). This group studied a large cohort of 125 people with CADASIL and found that there was a correlation between CMBs located in deep and infratentorial regions with dementia and incontinence, while lobar CMBs were associated with intracranial haemorrhage and dementia. Interestingly migraine was less frequently present in those with lower numbers of CMBs (44). This study found CMBs present in 34 % of the population, similar to our cohort of 36%, divided into 22% lobar, 18% infratentorial and 29% deep subcortical. There were individual cases that a high number of CMBs located in the thalamus. Thalamic microbleeds clustering has been recognised before in CADASIL in Asian populations in association with the R75P NOTCH3 mutation (228), but in this population there was no obvious genetic predisposition. The thalamus has long been recognised as an area of the brain more likely to be affected by CMBs in CADASIL (104, 109) and this remains in cohort affected by frequent CMBs and occasional clusters.

6 Genotype-phenotype correlations in CADASIL and comparison with sporadic small vessel disease

6.1 Comparison of proximal versus distal mutations and low medium and high-risk mutations

6.2 Abstract

Introduction

CADASIL is a small vessel disease caused by a mutation affecting one of the cysteine-encoding regions of the NOTCH3 gene. It has recently emerged that the location of this pathogenic mutation is a key determinant of disease severity and correlates with extent of imaging abnormalities. We reviewed a Scottish CADASIL cohort to externally validate the hypothesis that mutations in epidermal growth factor-like repeat (EGFr) domain 1 to 6 result in a more severe phenotype than those in EGFr 7 to 34. We also compared the CADASIL population with a cohort of older people with sporadic small vessel disease.

Methods

We compared age at first stroke, white matter hyperintensity volume, lacunes and other clinical and imaging biomarkers between 129 people with CADASIL and 460 controls with sporadic small vessel disease. We divided the CADASIL cohort into those with proximal (EGFr 1-6) mutations and distal (EGFr 7-34) mutations, then reviewed after dividing into low, medium and high-risk mutations.

Results

There were 108 with proximal mutations, mean age 46.8 ± 11.5 , 47 (46.5%) with history of stroke. There were 21 with distal mutations, mean age 53.5 ± 11.4 , 9 (50%) with history of stroke. The proximal cohort had 15 (14.3%) with hypertension compared to 8 (38.1%) from the cohort with distal mutations. There were non-significant trends towards more extensive white matter hyperintensities and first stroke at a younger age compared to those with distal mutations. CADASIL patients had more extensive white matter hyperintensity, more lacunes and microbleeds than sporadic small vessel disease and the CADASIL patients with distal mutations differ from the matched small vessel disease cohort with more extensive WMH and more lacunes.

Conclusion

Proximal CADASIL mutations have more extensive MRI imaging abnormalities and present at younger age than those with distal mutations. Among those with distal mutations, higher prevalence of hypertension suggests a possible interaction that may be relevant to symptomatic presentation.

6.3 Introduction

Recent population genetic analysis has identified much higher prevalence of NOTCH3 genetic mutations within the general population (216) than previously thought likely from analysis of prevalence of CADASIL disease (7, 8). This unexpected finding may be relevant for observations of CADASIL being diagnosed later in life and with more minor symptoms and disability compared to the earliest studies reporting CADASIL (64, 229). Further studies investigating the potential influence of cardiovascular and genetic factors support the location of the mutation on the NOTCH3 gene as a key determinant of disease severity. Divided into proximal mutations (affecting EGFr domains 1-6) and distal mutations (affecting EGFr domains 7-34), proximal mutations are associated with a more severe phenotype in line with traditional cases of CADASIL and distal mutations are associated with a milder phenotype and were found more commonly than expected in the general population (16, 58). Further analyses have resulted in a proposed subclassification of CADASIL cohorts into low, medium and high risk mutations based on both clinical manifestations and imaging biomarkers (81). The extent of imaging abnormalities similarly appear to depend on mutation location with proximal mutations more severely affected with more extensive WMH (58). The correlation between genotype and phenotype has been validated externally in other populations with proximal mutations associated with younger age of stroke and also a suggestion that hypertension may be more common in distal mutations (59, 78).

The purpose of this study was to review a Scottish CADASIL cohort and externally validate correlations between NOTCH3 pathogenic variant location with clinical and imaging features. We additionally undertook comparisons with a population with presumed sporadic small vessel disease to quantify the imaging and clinical differences between CADASIL and sporadic small vessel disease. We also assessed intra-rater and inter-rater reliability for measurement of WMH and CMBs.

6.4 Methods

6.4.1 Study Cohort

Participants were all those who have attended a neurovascular clinic at the Queen Elizabeth University Hospital, Glasgow, United Kingdom over a 20- year period. Subjects were adults with a CADASIL diagnosis confirmed by presence of pathogenic NOTCH3

mutation on genetic testing. They must have completed one MRI scan during the course of routine clinical assessment or as part of previous clinical research studies (43). Exclusion criteria were contraindications to MRI. The control data set was derived from a clinical trial recruiting patients with traditional risk factors for stroke not known to have a CADASIL diagnosis (226). This included adults over 50 years with recent stroke or TIA in previous 4 weeks (226).

6.4.2 Clinical data

The following data were collected for all participants: demographics, sex, age at time of MRI, presence of hypertension, diabetes, cholesterol, migraine, stroke, antiplatelet prescription, statin prescription, smoking status, and alcohol history. Genetic mutation, age of onset of first stroke and previous encephalopathy were recorded specifically for the group with CADASIL.

6.4.3 Brain imaging

The imaging for CADASIL cohort was performed throughout Scotland but predominantly at the Neurological institute in Glasgow. For inclusion in the present analysis, the minimum requirement for imaging was Fluid attenuation inversion recovery (FLAIR) for white matter hyperintensity measurement and lacune review. T2 for assessment of enlarged perivascular spaces (EPVS), T2*- susceptibility-weighted imaging (SWI), Gradient-echo (GE) or susceptibility-weighted angiography (SWAN) for cerebral microbleed analysis. Where multiple T2*-weighted sequences were performed preference was given first to SWI then SWAN then GE. Most scans were performed on clinical 1.5- Tesla scanners, with some completed on the 3 Tesla GE Signa Excite scanner (GE Medical systems, Milwaukee, USA). Most often they had three-dimensional (3D) T1-weighted and T2-weighted images. For the comparison sporadic small vessel disease cohort the imaging was performed on 1.5 or 3-Tesla MRI. Sequences included T1-weighted, T2-weighted and FLAIR, DWI and SWI imaging (226).

6.4.4 Image processing

Images were transferred from clinical Picture Archiving Communications System (PACS) to a Safe Haven imaging data bank by trained research radiographers.

White matter hyperintensities (WMH), lacunes, cerebral microbleeds and enlarged perivascular spaces were defined as reported in the STRIVE criteria for imaging standards (82). Number of lacunes were recorded and cerebral microbleeds were counted using the Microbleed Anatomical rating scale (MARS) (110). EPVS were assessed using the comprehensive instructions on Enlarged perivascular spaces (EPVS): a visual rating scale user guide (230).

Mango imaging software <http://ric.uthscsa.edu/mango/index.html> was used to complete measurements and for visual review of images (231). We used the Brain extraction tool (BET) plugin based on the FMRIB Software Library program to remove non-brain tissue from the initial image of the whole head (232). This allowed for measurement of brain volume. Using the FLAIR sequence areas of hyperintensity in the brainstem and white matter were included to create a region of interest (ROI) using a combination of creating thresholds based on visual inspection (Figure 6.1). This was further visually inspected and modified to ensure the inclusion only of subcortical hyperintensities. These values were normalised by dividing them by the brain volume. This was done on two separate occasions by one reader (SN) blinded to genetic abnormality, more than one month apart to establish and an intra-class correlation measure. The Fazekas score was recorded for all participants to allow comparison between our measured values and a previously validated WMH grading system.

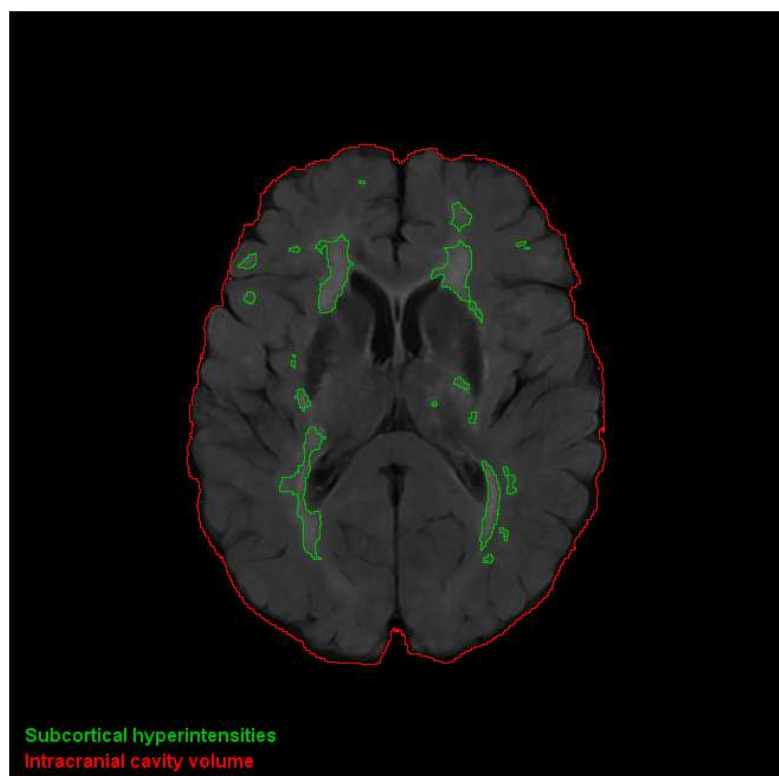


Figure 6.1 Example image with white matter hyperintensities and intracranial cavity volume.

6.4.5 Statistical analysis

Statistical analysis was performed using IBM SPSS version 29 (IBM, Armonk, New York, USA).

Data were inspected for normality using normality curve and statistically using Shapiro-Wilks test. Differences in baseline clinical characteristics were compared between proximal EGFr 1-6 and distal EGFr 7-34 mutations in CADASIL and also compared between CADASIL cohort and sporadic small vessel disease cohort. The differences between these characteristics including age and vascular risk factors were calculated using independent sample t-test for normally distributed continuous variables and Chi-squared or Fisher's exact test for categorical variables. A Mann-Whitney U test was used to calculate difference between ordinal variables. If continuous variables were not normally distributed a transformation was undertaken.

As there was a large difference in age between the CADASIL and control cohort, we derived a sub-cohort from the larger control cases using a matching method including age, hypertension and smoking history as contributing factors with tolerance of 5 years for age. Cox-regression analysis was used to create a Kaplan-Meier survival analysis curve for age at first stroke in groups of proximal and distal CADASIL mutations.

We used Kruskal-Wallis tests to compare lacune count, cerebral microbleeds and EPVS score, and median nWMH volume between each of high, medium, and low risk CADASIL mutations and the matched sporadic small vessel disease cohort.

A scatter plot was drawn for Fazekas scale against WMH volume (Figure 6.2). To establish intra-rater measurements a scatter plot and Bland-Altman chart were created for separate measurements of WMH volume (Figures 6.3 and 6.4). To assess inter-rater reliability, we performed a one sample t-test then intraclass correlation coefficient. A small number of cases were reviewed for cerebral microbleeds by two reviewers, the author, and a consultant neurologist with an interest in small vessel disease imaging to assess inter-rater reliability for cerebral microbleeds by ICC. The results of WMH volumes for the complete dataset were compared with the results from a Stroke consultant reported elsewhere (226) and a scatter plot drawn and an ICC calculation for WMH inter-rater reliability (Figure 6.5).

6.5 Results

6.5.1 Intra- and inter-rater reliability for WMH measures

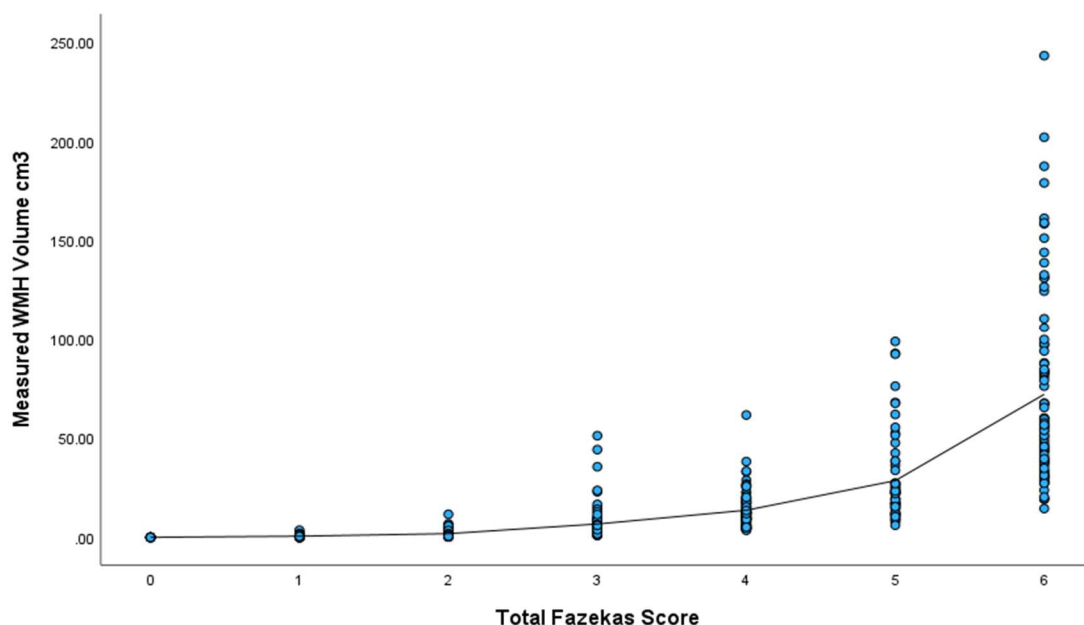


Figure 6.2 Correlation between calculated WMH volume and measured Fazekas score

One rater (SN) measured WMH volume for all images included in both available datasets as well as calculating Fazekas score. The two results are plotted against each other above (Figure 6.2) and we performed a spearman's rank correlation for correlation. Spearman's $\rho = 0.931$, $N = 558$, $p < 0.001$.

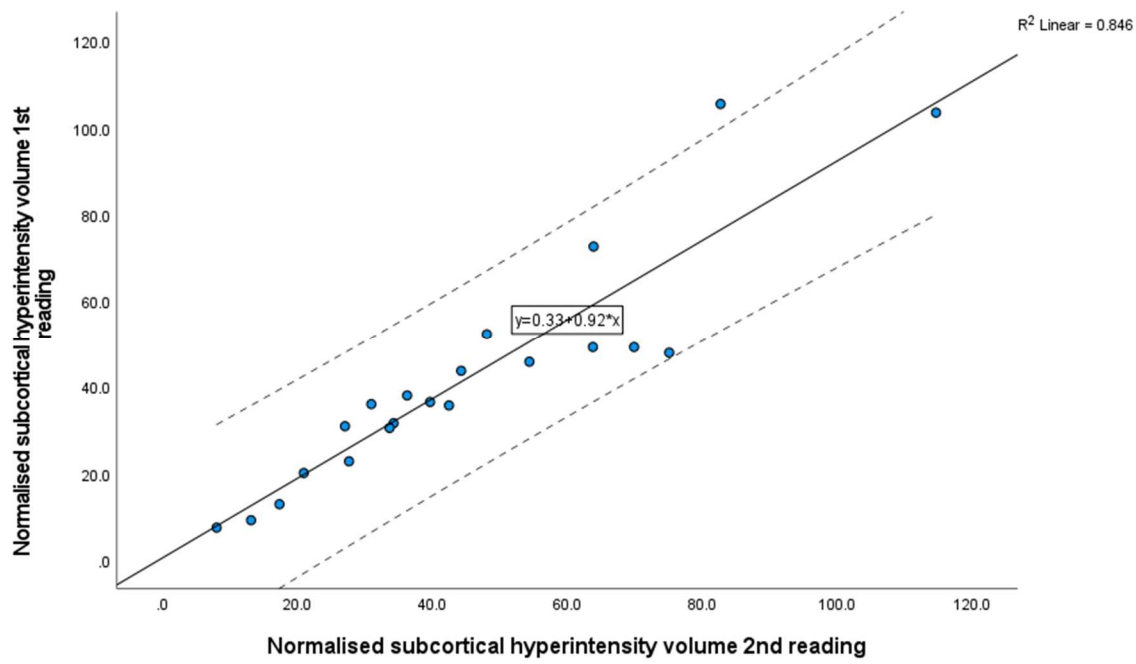


Figure 6.3 Repeat measure of normalised WMH volume in CADASIL

Bland-Altman plot was done to assess bias on intra-rater repeats.

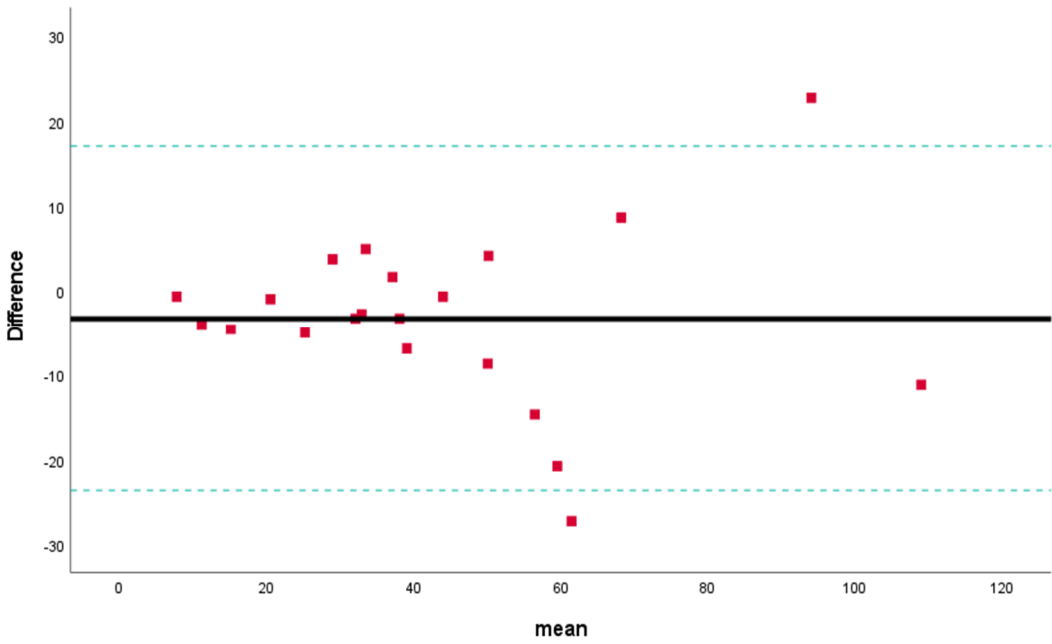


Figure 6.4 Bland-Altman plot for repeat measures of WMH volume in CADASIL

Linear regression of difference on mean was performed and the unstandardised beta coefficient for the mean was $= -0.001$ $p = 0.994$. Intraclass correlation for these values was 0.920 single measure (confidence interval 0.814 to 0.967) $p < 0.001$ indicating very good agreement.

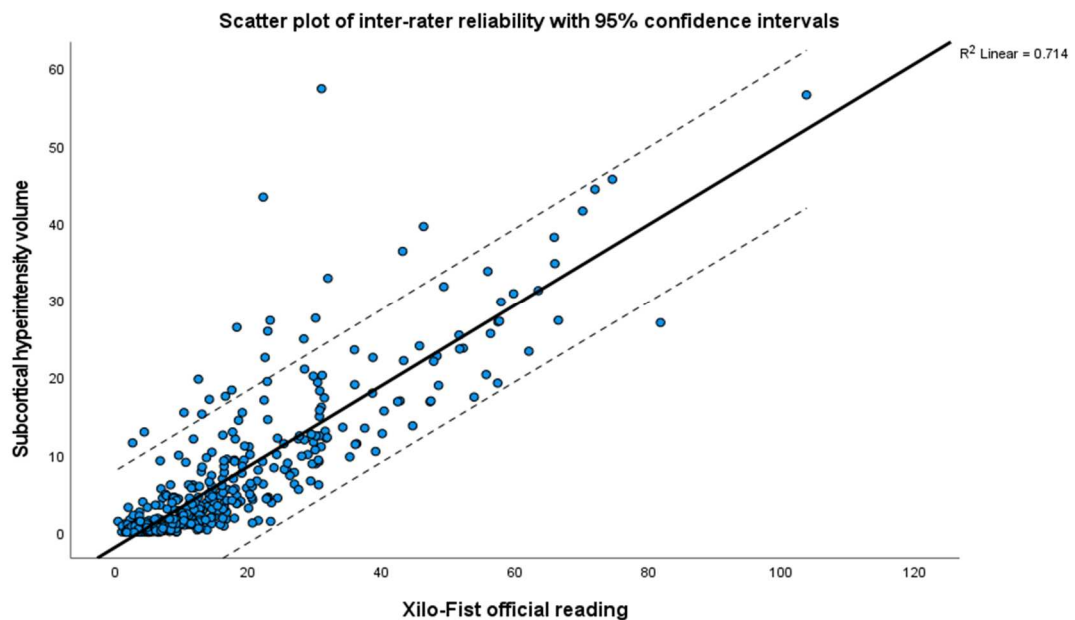


Figure 6.5 Comparison of measured WMH volume readings in this study with official study data

Using results analysed elsewhere comparison was made between white matter hyperintensity volume as plotted above (226). (Figure 6.5) The degree of correlation was measured by calculating an intraclass correlation coefficient. This was 0.755 single measures (confidence interval 0.711 to 0.793) $p < 0.001$.

The inter-rater reliability analysis for cerebral microbleeds using the MARS scoring system (SN and AS), the average measures showed excellent inter-rater reliability with intraclass correlation coefficient 0.909 (95% confidence interval 0.779 to 0.962) $p < 0.001$.

6.5.2 Comparison of EGFr domains 1 to 6 with EGFr domains 7 to 34

A total of 129 patients with CADASIL were included, of whom 108 (83.7%) had proximal mutations. Of these, 74 individuals (57.4% of whole group) had mutations affecting exons 3 or 4 (Figure 6.9). The 21 (16.3%) individuals with distal mutations were distributed across 11 different EGFr domains between 8 and 31. The demographic profiles of both

proximal and distal groups are shown in table 6.1 with details of individual mutations listed in appendix 1.

The group with proximal mutations were younger 46.8 versus 53.5 years ($p=0.05$) at time of imaging and had a significantly lower proportion with a diagnosis of hypertension (14.3% versus 38.1%, $p=0.010$). Although non-significant there were lower percentages of smoking history and diabetes in the proximal group.

We found the WMH burden was higher in the proximal mutations as well as median lacune counts although neither was statistically significant (table 6.2). These differences in WMH volume have been demonstrated with reference to sporadic small vessel disease in figures 6.7 and 6.8 including propensity matched cohort.

6.5.3 Comparison of CADASIL with sporadic small vessel disease

In comparison to the CADASIL cohort the sample of sporadic small vessel disease was older 65.7 ± 8.7 versus 47.5 ± 11.7 years, with higher proportion male sex 317 (68.9%) versus 57 (44.2%), more smoking 46 (41.8%) versus 245 (53.3%), hypertension 23 (18.3%) versus 220 (47.8%), and diabetes 9 (7.4%) versus 99 (21.5%), see table 6.3 for clinical differences between cohorts.

The CADASIL cohort had significantly larger volumes nWMH 5.9 versus 1.7 ($p<0.001$), Fazekas score 5 [4 to 6] versus 3 [2-4], ($p<0.001$) and lacune count 2 [0-8] versus 1 [0-2] ($p<0.001$), as demonstrated in table 6.4. These findings were consistent when comparing the sub-cohort matched on age, hypertension, and smoking history (Tables 6.5 and 6.6). The differences in imaging distributions between the CADASIL cohort and sporadic SVD are shown in figures 6.12, 6.13, 6.14 and 6.15. Although non-significant the distribution between CADASIL and sporadic SVD for CM is shown in figure 6.16 and for EPVS in figure 6.17.

6.5.4 Stroke free survival analysis comparison EGFr domains 1 to 6 and EGFr domains 7 to 34

Age at first stroke comparison between proximal and distal CADASIL mutations showed median 49 [43 - 55] for proximal and 54 [39.5 - 63] for distal, that was non-significant $p=0.312$. The Kaplan-Meier survival curve using Cox-regression analysis that showed as

age increases there is a suggestion of divergence with proximal mutations having first stroke at younger age, not statistically significant $p=0.109$ (Figure 6.6).

6.5.5 Imaging analysis of high, medium and low risk CADASIL with matched cohort of spontaneous small vessel disease

Although the numbers for medium and low risk categories were very small, we performed a Kruskal-Wallis analysis comparing high, medium and low risk CADASIL cases (based on the proposed classification of Hack et al (81)) against the matched cohort of sporadic small vessel disease. This showed significant differences between categories in WMH, lacunes and EPVS (Table 6.7). No difference was detected in cerebral microbleeds. The distribution of the differences in WMH volume between low medium and high risk mutations and sporadic small vessel disease including propensity matched cohort is shown in Figures 6.10 and 6.11.

Table 6.1 Summary characteristics of Scottish CADASIL population

	EGFR 1-6	EGFR 7-34	P-value
n	108	21	
Age at MRI	46.8 ± 11.5	53.5 ± 11.4	0.05
Male / female (% male)	46 / 62 42.6%	11 / 10 52.4%	0.409
Smoking, yes /no (%)	36 / 56 (39.1%) 16 unknown	10 / 8 (59.6%) 3 unknown	0.196
Hypertension, yes /no (%)	15 / 90 (14.3%) 3 unknown	8 / 13 (38.1%)	0.010
Cholesterol	4.5 ± 1.0	4.5 ± 1.2	0.130
Diabetes, yes /no (%)	7 /95 (6.9%) 6 unknown	2 / 17 (10.5%) 2 unknown	0.197
Stroke, yes / no (%)	47 /54 (46.5%) 7 unknown	9 /9 (50%) 3 unknown	0.786
Age of first stroke	49 [43 - 55]	54 [39.5 - 63]	0.312
Previous encephalopathy (%)	8 (7.4%)	1 (4.8%)	0.588
Age at first encephalopathy	47 [42 - 52]	49	0.750

Table 6.2 Imaging features of proximal and distal mutations in Scottish CADASIL cohort

	EGFR 1-6	EGFR 7-34	P-value
n	108	21	
Brain volume	1489.1 (188.6)	1517.8 (184.4)	0.262
nWMH sqrt transform mean $\pm$ SD	6.1 $\pm$ 2.7	5.1 $\pm$ 2.5	0.069
Fazekas' periventricular median [IQR]	3 [2-3]	2 [2-3]	0.459
Fazekas' deep white matter median [IQR]	3 [2-3]	3 [1.5 -3]	0.426
Fazekas's total median [IQR]	5.5 [4-6]	5 [3.5-6]	0.539
Lacunes median [IQR]	3 [0-8.5]	1 [0 -5.5]	0.202
CM median [IQR]	0 [0-2]	0 [0-1]	0.951
EPVS	3 [3-4]	4 [3-5]	0.286

Table 6.3 Comparison clinical characteristics CADASIL and sporadic small vessel disease cohort

	CADASIL	Sporadic small vessel disease	P-value
n	129	460	
Age at MRI	47.5 ± 11.7	65.7 ± 8.7	<0.001
Male / female (% male)	57 / 72 (44.2%)	317 / 143 (68.9%)	<0.001
Current or ex-smoker, yes /no (%)	46 / 64 (41.8%) 19 unknown	245 / 215 (53.3%)	0.031
Hypertension, yes /no (%)	23 /103 (18.3%) 3 unknown	220 / 240 (47.8%)	<0.001
Cholesterol	4.5 ± 1.0	3.9 ± 1.0	<0.001
Diabetes, yes /no (%)	9 / 112 (7.4%) 8 unknown	99 / 361 (21.5%)	<0.001

Table 6.4 Imaging features of Scottish CADASIL cohort and sporadic small vessel disease

	CADASIL	Sporadic small vessel disease	P-value
n	129	460	
Brain volume	1493.9 ± 187.5	1477.4 ± 157.1	0.162
nWMH sqrt transform mean ± SD	5.9 ± 2.7	1.7 ± 1.3	<0.001
Fazekas' periventricular median [IQR]	3 [2- 3]	1 [1-2]	<0.001
Fazekas' deep white matter median [IQR]	3 [2-3]	1 [1-2]	<0.001
Fazekas's total median [IQR]	5 [4 to 6]	3 [2-4]	<0.001
Lacunes median [IQR]	2 [0-8]	1 [0-2]	<0.001
CM median [IQR]	0 [0-2]	0 [0-1]	0.097
EPVS median [IQR]	4 [3-4.5]	4 [3-5]	0.420

Table 6.5 Comparison characteristics CADASIL and sporadic small vessel disease cohort propensity score matching cohort

	CADASIL	Sporadic small vessel disease	P-value
n	129	61	
Age at MRI	47.5 ± 11.7	58.5 ± 5.6	<0.001
Male / female (% male)	57 / 72 (44.2%)	41 / 20 (67.2%)	0.03
Current or ex-smoker, yes /no (%)	46 / 64 (41.8%) 19 unknown	29 /32 (47.5%)	0.470
Hypertension, yes /no (%)	23 /103 (18.3%) 3 unknown	14 / 47 (23%)	0.450
Cholesterol	4.5 ± 1.0	4.0 ± 1.1	0.02
Diabetes, yes /no (%)	9 / 112 (7.4%) 8 unknown	16 / 45 (26.2%)	<0.001

Table 6.6 Imaging features of Scottish CADASIL cohort and sporadic small vessel disease propensity score matching cohort

	CADASIL	Sporadic small vessel disease	P-value
n	129	61	
Brain volume	1493.9 ± 187.5	1494.8 ± 152.7	0.488
nWMH sqrt transform mean ± SD	5.9 ± 2.7	1.4 ± 1.2	<0.001
Fazekas' periventricular median [IQR]	3 [2- 3]	1 [1-2]	<0.001
Fazekas' deep white matter median [IQR]	3 [2-3]	1 [1-1]	<0.001
Fazekas's total median [IQR]	5 [4 to 6]	2 [1-3]	<0.001
Lacunes median [IQR]	2 [0-8]	1 [0-2]	<0.001
CMBs median [IQR]	0 [0-2]	0 [0-1]	0.382
EPVS median [IQR]	4 [3-4.5]	4 [3-5]	0.126

Table 6.7 Results of Kruskal-Wallis analysis of high, medium and low risk CADASIL against matched cohort of sporadic small vessel disease

Median [IQR]	High risk	Medium risk	Low risk	Sporadic small vessel disease	P-value (Kruskal- Wallis)
n	113	6	10	61	
nWMH	36.2 [17 .1 – 56.9]	33.4 [23.2 - 65.8]	21.1 [5.3 – 54.9]	1.1 [0.3– 3.8]	<0.001
Lacunes	3 [0 -8.25]	0.5 [0-2]	0.5 [0-9]	1 [0-2]	<0.001
Cerebral microbleeds	0 [0-2]	0 [0-1]	0 [0-3.5]	0 [0 - 1]	0.842
EPVS	3.5 [3-4]	5 [4.5 – 5.5]	4[3 – 5.25]	4 [3 -5]	0.035

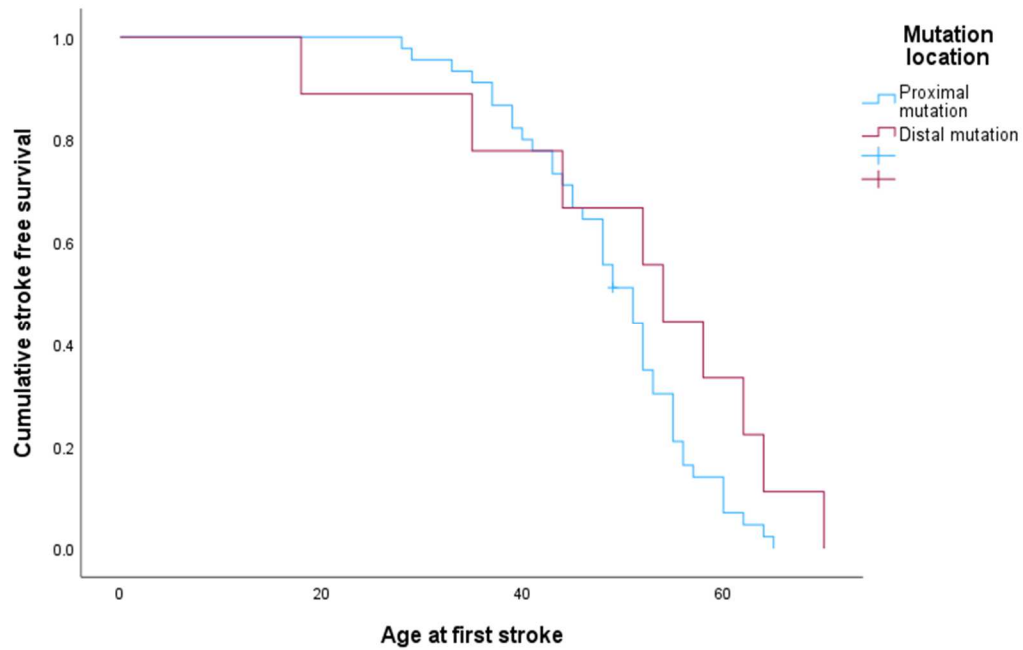


Figure 6.6 Kaplan-Meier survival curve for age of first stroke in CADASIL

Log-Rank (Mantel-Cox) Chi-square 2.569, $p=0.109$.

We performed a cox-regression with the categorical variable hypertension as a covariate, selected as significantly different between proximal and distal mutations. This made no difference and was not included in final model. The stroke-free survival curves are shown above (Figure 6.6).

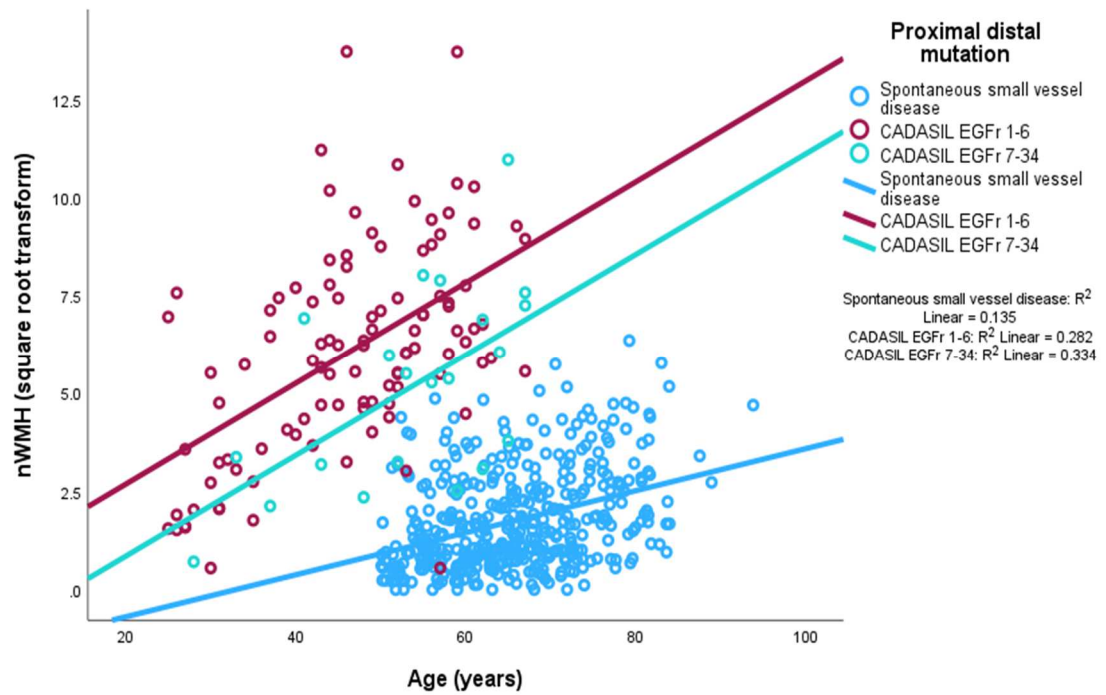


Figure 6.7 Comparison of WMH against age for CADASIL proximal and distal mutations and sporadic small vessel disease

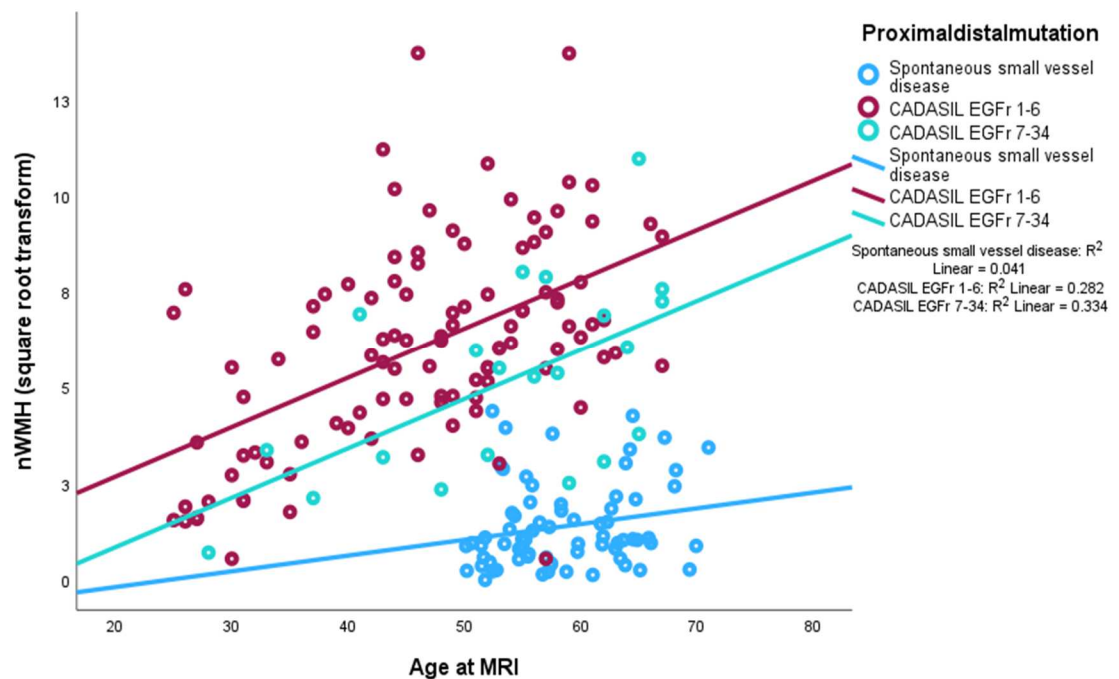


Figure 6.8 Comparison of WMH against age for CADASIL proximal and distal mutations and propensity matched sporadic small vessel disease

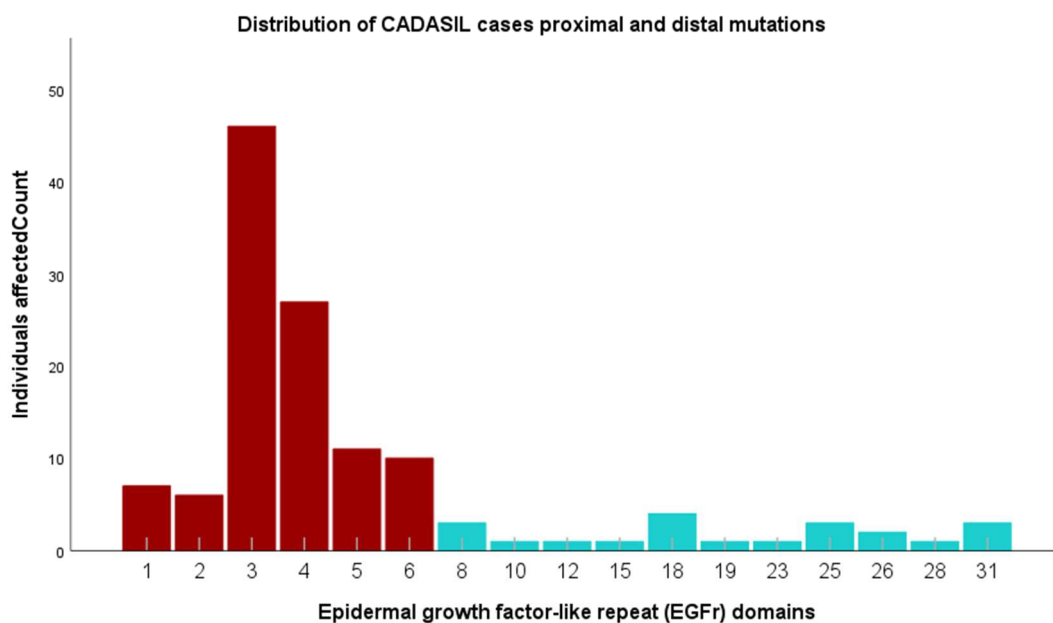


Figure 6.9 Distribution of CADASIL cases across different EGFr domains

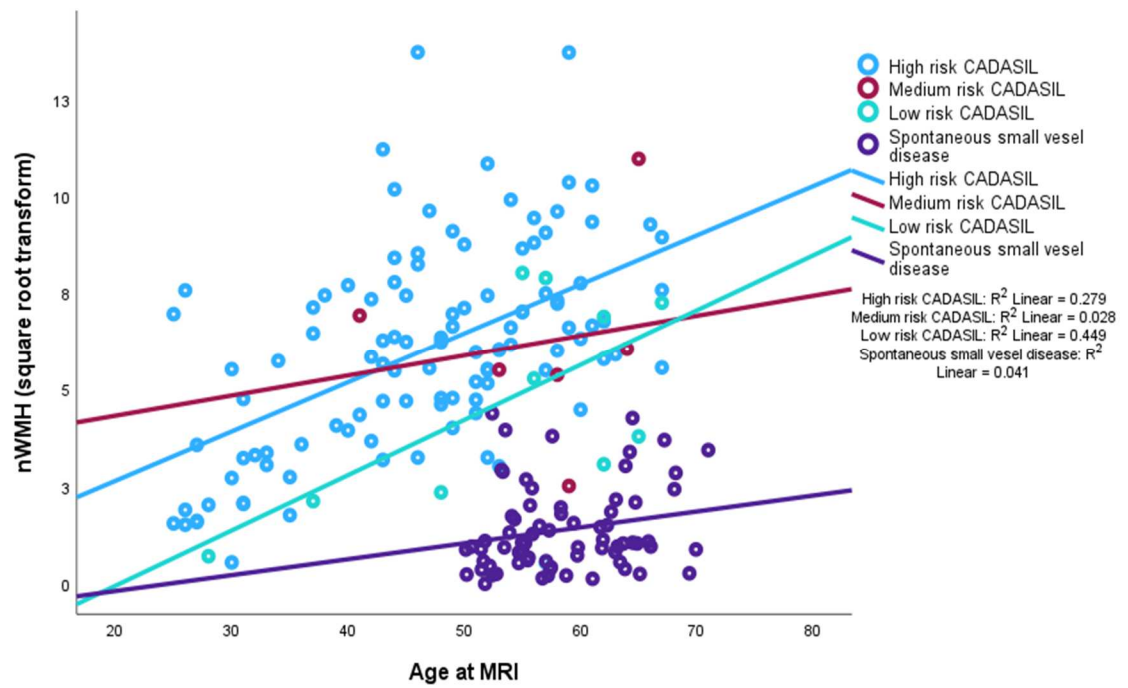


Figure 6.10 Comparison of WMH against age for CADASIL low, medium and high risk mutations and a propensity score matched sporadic small vessel disease

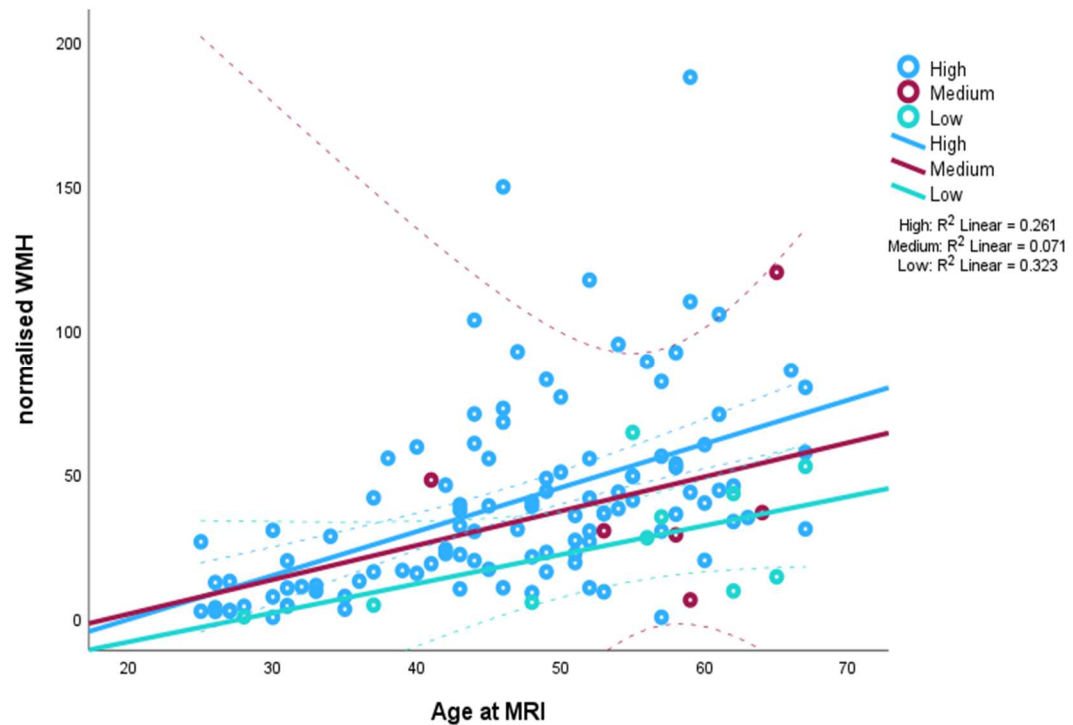


Figure 6.11 Age against normalised WMH divided into low medium and high-risk CADASIL genetic mutations.

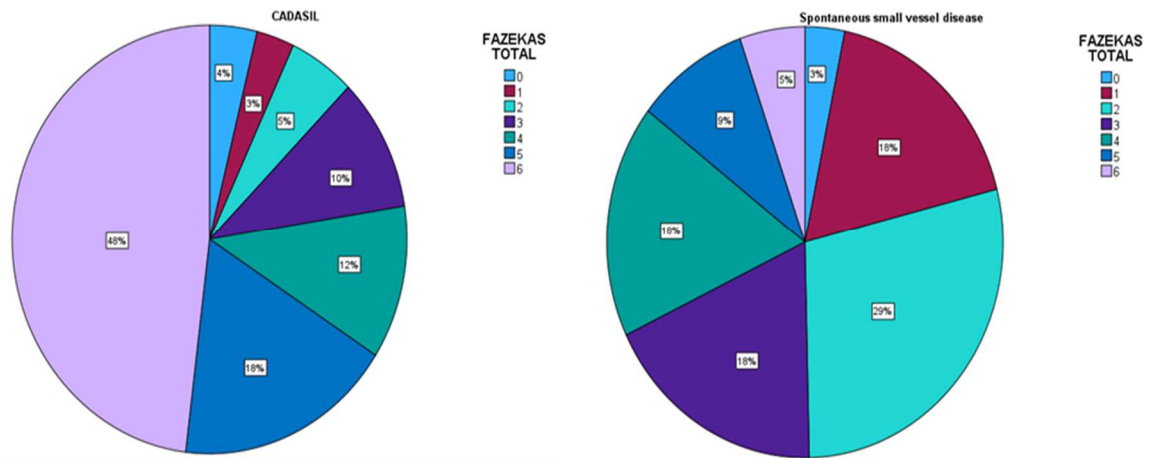


Figure 6.12 Comparison of Fazekas score distribution in CADASIL and sporadic small vessel disease.

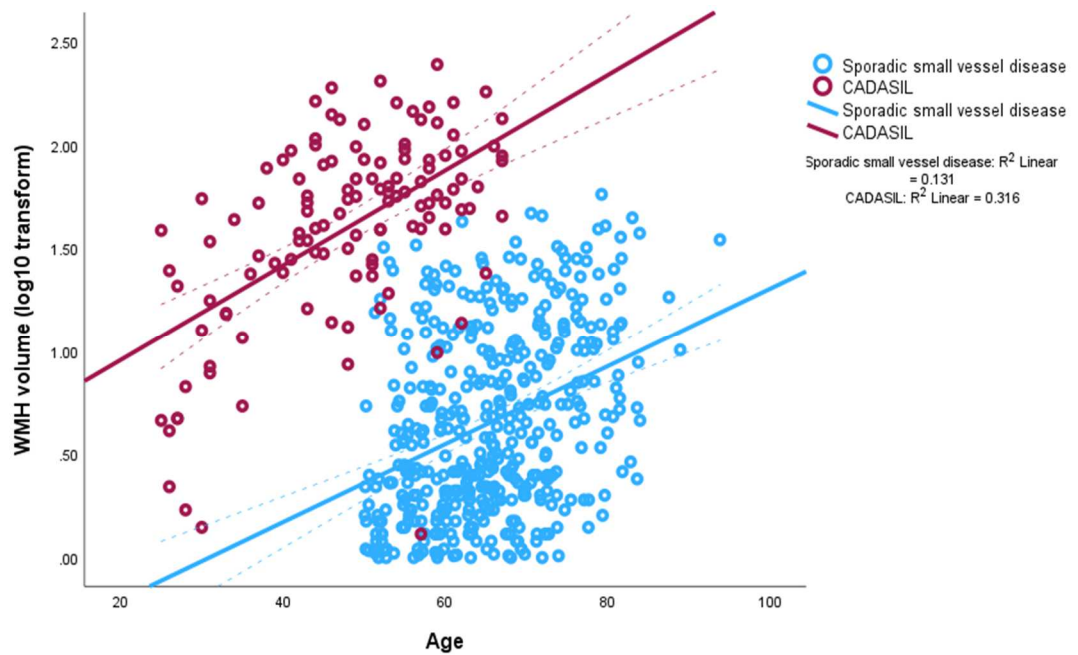


Figure 6.13 Comparison of age versus WMH volume in CADASIL and sporadic small vessel disease

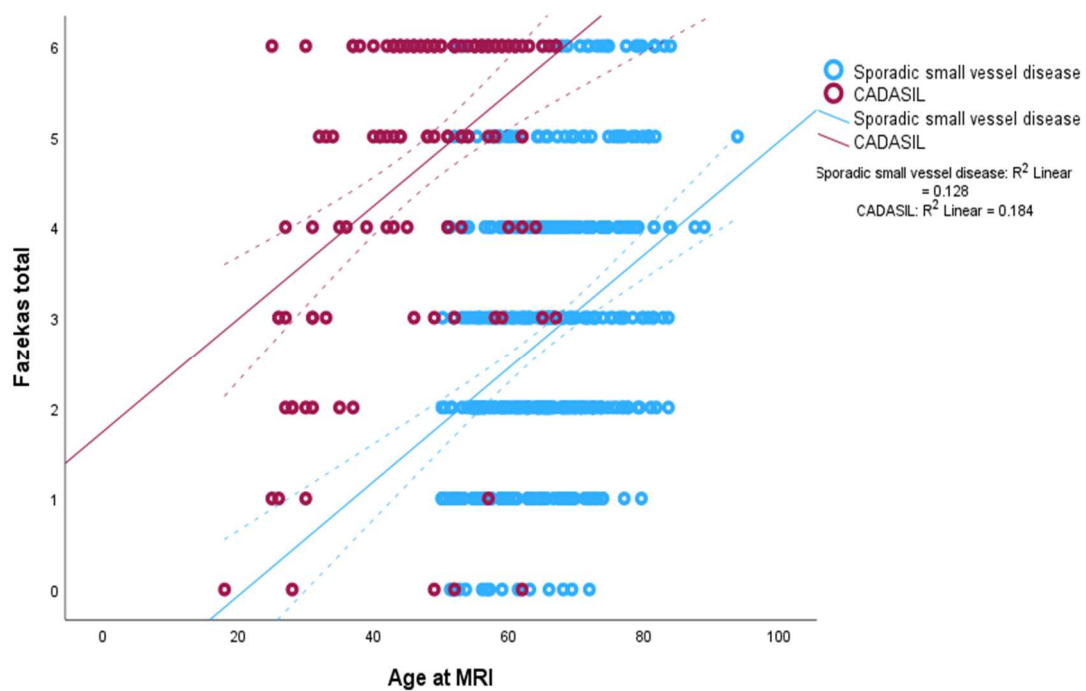


Figure 6.14 Age versus Fazekas score in CADASIL and sporadic small vessel disease

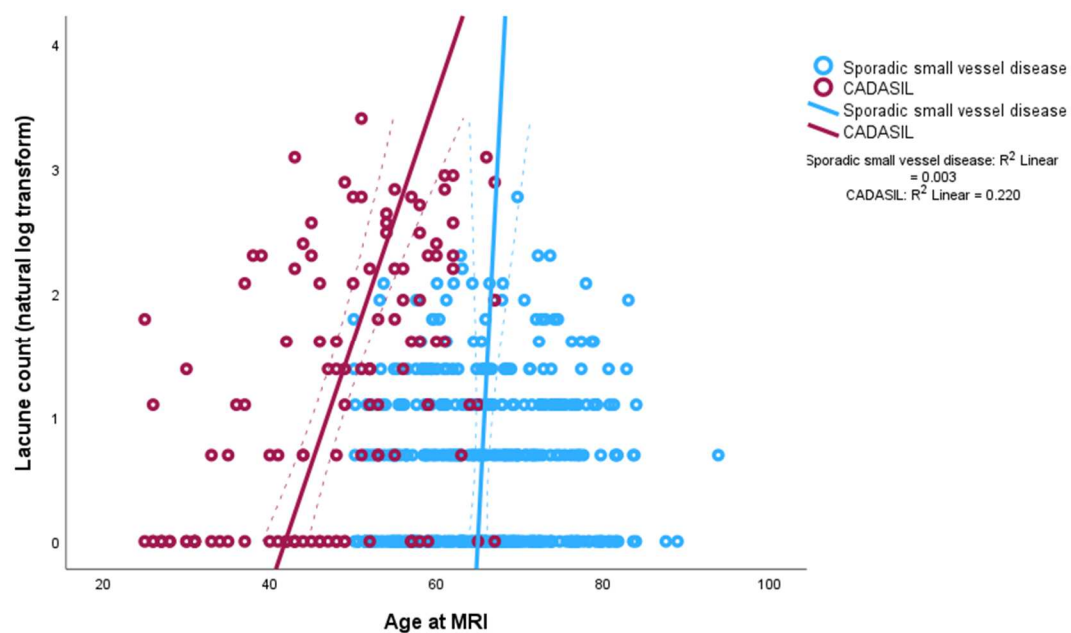


Figure 6.15 Age versus lacune count in CADASIL and sporadic small vessel disease

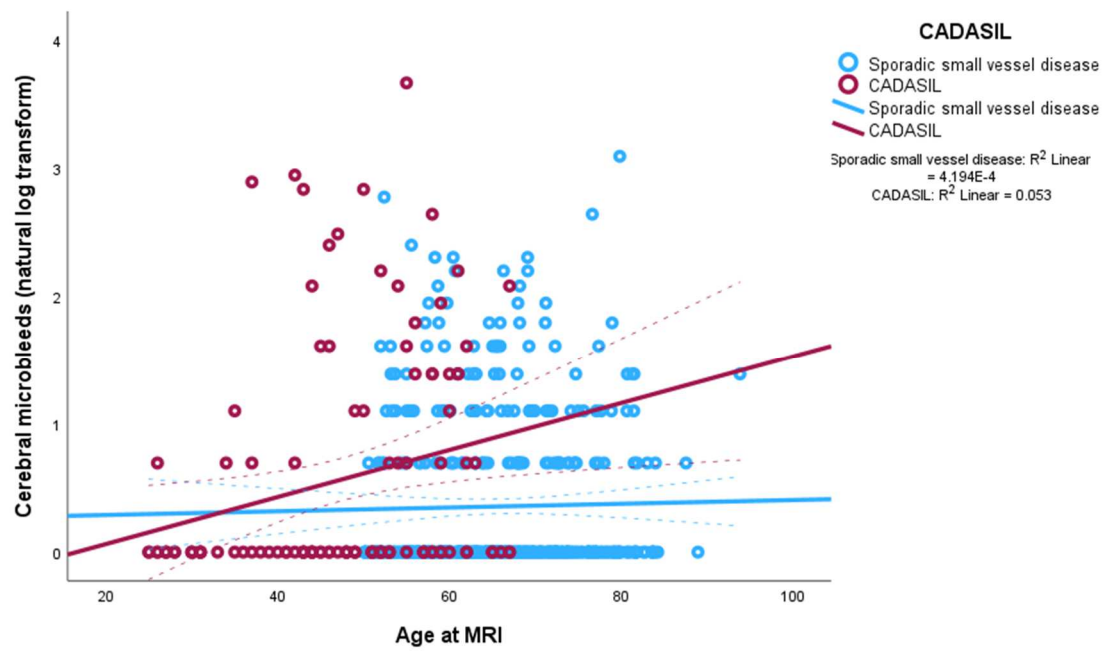


Figure 6.16 Age versus cerebral microbleed count in CADASIL and sporadic small vessel disease

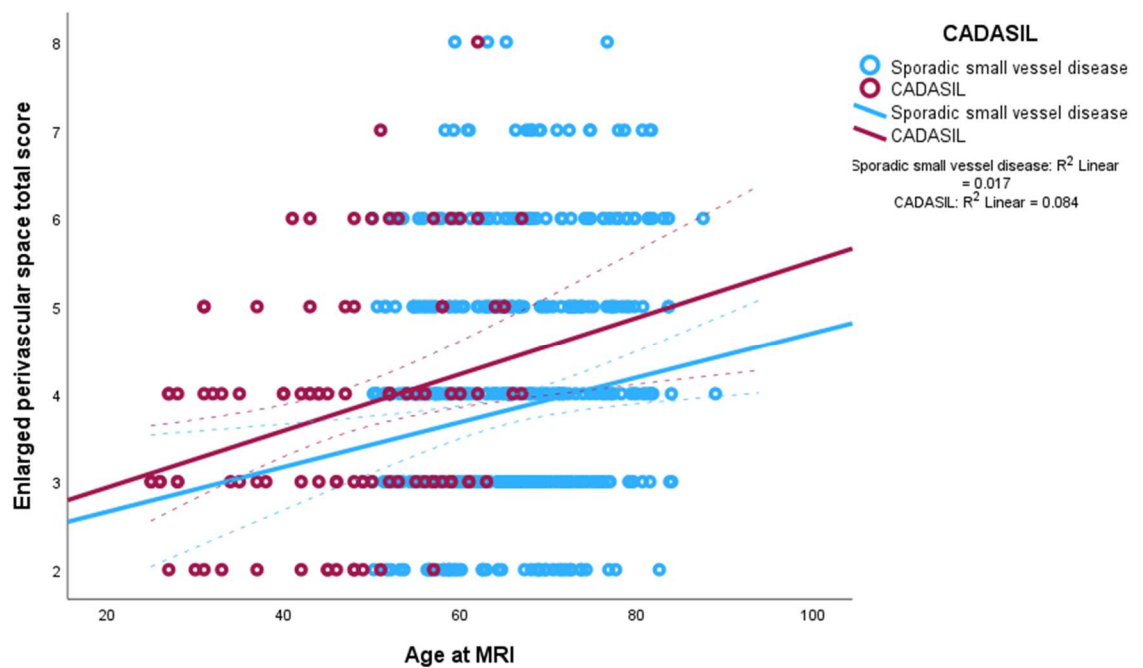


Figure 6.17 Age versus enlarged perivascular spaces in CADASIL and sporadic small vessel disease

6.6 Discussion

This imaging study on the Scottish CADASIL population was undertaken with the purpose of validating previous correlations between location of pathogenic variant and disease severity. Our findings corroborate the observation that proximal mutations are associated with more extensive imaging abnormalities, specifically WMH volume and lacune count. We also found that those with proximal mutations were younger and were less likely to be hypertensive. When further dividing the CADASIL cohort into high, medium, and low risk mutations according to the proposed risk stratification of Hack and colleagues (81) and comparing with a cohort derived from a study of sporadic small vessel disease with more conventional risk factors, we found similar correlations between WMH volume and lacune count with increasing age. There has been a recent paradigm shift in the risk stratification of CADASIL arising from the unexpected finding of a higher than expected frequency of NOTCH3 pathogenic variant in the general population at around 1 in 300 individuals (15). This number contrasted significantly from previous population estimates of clinically relevant CADASIL cases (8, 9). These findings have resulted in the proposal that there is a larger proportion of people with NOTCH3 genetic abnormality that have a very mild form of the disease or may have no relevant symptoms with prognosis very similar to a general population. Additional investigation of EGFr location as a risk for disease severity has resulted in division of CADASIL populations into proximal EGFr 1 to 6 and distal EGFr 7 to 34 mutations, with proximal mutations showing a more aggressive disease trajectory (164). The imaging findings of our study confirm that WMH volumes increase with age at similar rates in proximal and distal mutations and the burden appears higher with proximal mutations. The volume and rate of increase in both appears higher than is the case for sporadic small vessel disease. The extent of WMH burden is of clinical importance in the general population since abnormalities have been found in meta-analysis to be significantly associated with the development of stroke, dementia and death (233). The influence of WMH on disease progression in CADASIL appears less clearly established, with Chabriot and Liem findings no significant association over 3 and 7 years (182, 234), where Ling noted progression in WMH volume over time and an association between this and decrease in modified Rankin score (235). Our population is consistent with previous results showing that proximal mutations were imaged at younger age and that a higher proportion of people in the cohort with distal mutations had been diagnosed with hypertension. This has been noted before in several studies (53, 59, 164) with no definitive explanation confirmed. It is thought that potentially additional risk factors may be required in distal mutations to result

in clinical developments resulting in clinical presentations. Cardiovascular profile has been shown to be important in disease manifestation and may be deleterious in modulating clinical expression of CADASIL (187, 236). Whilst this dichotomised system using EGFr domain to risk stratify has altered CADASIL prognostication, additional attempts have been made to refine this. There are cases of distal mutations who suffer disproportionately clinically and there are known examples of distal mutations with very extensive WMH, lacunes and cerebral microbleed burden. The clinical and imaging findings do not uniformly correlate. Hack et al have developed a system of low, medium and high-risk CADASIL mutations which included EGFr domains 8,11 and 26 in the high risk category. Our analysis, although limited by small numbers in the more distal categories, does suggest that the WMH burden and lacune count follow similar patterns (81). A different study of a separate European population found that EGFR 10 to 17 was associated with a significantly lower risk of stroke (78). Our study population is similar to other European cohorts, with a heavy predominance of the population with abnormality in domains EGFr 3 and 4 (81). This contrasts to certain studies in Asian populations where abnormalities in EGFr domain 11 can be most prevalent (237, 238). While it is well known that CADASIL is associated with characteristic imaging findings including extensive WMH we have quantified this in a comparison with a control cohort that do not have known CADASIL. Since numbers of patients with low and medium risk mutations were small, further research is needed to establish the boundaries between people with CADASIL and a distal, low risk mutation or an unaffected individual with an incidental NOTCH3 abnormality without symptoms.

This study has the strength of being a unique CADASIL population based on a national clinical service with clinical genetics. The imaging data were largely gathered from clinical radiology scans. This provides insight that differs from prospective research imaging studies: age at MRI is a relevant clinical result as imaging is requested at time of diagnosis and then only if significant clinical change necessitates update and is likely indicative of disease severity. The quality of the scans was variable as they were taken on different machines nationally and at differing field strengths. The small number of distal mutations included limits generalisability but is a common finding with CADASIL genotype-phenotype correlation studies.

We have summarised our unique Scottish CADASIL population and have validated previous results confirming the severity of clinical manifestations and extent of imaging abnormalities in proximal mutations differ from distal. We note significant outliers within

this group and that further work is required to delineate EGFr domains associated with worse prognosis as well as to understand reason for the high proportion of hypertension in distal mutations.

7 Rate of progression of imaging markers in CADASIL: a retrospective imaging analysis

7.1 Introduction

CADASIL is associated with characteristic MRI findings including extensive WMH, lacunes and cerebral microbleeds. These markers have variously been associated with dementia and death, but the correlations between extent of WMH, lacunes and CMBs in CADASIL appear different from sporadic small vessel disease. A 7 year follow-up study found that lacune number and CMBs were associated with cognitive decline but WMH volume made no difference (87). A more recent larger study of 161 patients followed over 3 years found lacunes and change of brain volume to be the most sensitive markers for cognitive decline (235). For investigation of therapeutic interventions to have clinically relevant imaging biomarkers is vital and the rate of progression of each of the image markers and their correlation with disability endpoints is a vital part of this process. Technically there are challenges with measurement of WMH longitudinally using human raters and with assistance of machine learning (239). The boundary of between normal and abnormal WMH is not standardised and variability of this can lead to discrepancies between measurement, this can be particularly troublesome with larger volume WMH (240). The aim of this chapter is to retrospectively investigate and calculate the rate of change in volume of WMH, number of lacunes and microbleeds between MRI scans and to investigate differences between proximal and distal mutations.

7.2 Methods

We used a subsection of the MRI imaging data obtained through the NHS safe haven (GSH/18/NE/007) of adult patients with CADASIL. To estimate the rate of change of MRI imaging markers over time we included only those who had two or more MRI scans done. To allow time for new WMH, lacunes and CMBs to appear we excluded those whose scans were fewer than 12 months apart. We used MANGO imaging software version 4.1 as previously described to measure the volume of white matter hyperintensity and divided this by the brain volume. We also recorded the lacunes from the T2-FLAIR sequences and counted CMBs from the SWI or GE sequences. We took the two most far apart scans chronologically and calculated the difference in imaging markers between them, and

divided this by the time between each scan to generate an annual rate of volume increase for WMH and an annual rate of increase in number of lacunes and CMBs.

To calculate the difference between clinical features of proximal and distal mutations we used a chi-squared test or Fisher's exact test as indicated.

7.3 Results

Of the 108 patients involved in the study, we had to exclude 57 as only had single analysable MRI and another 13 as the interval between imaging was less than 12 months. This left a cohort of 38 with two MRI images more than 12 months apart. Baseline clinical data is shown in (Table 7.1). 28 (73.7%) had a proximal CADASIL mutation and 10 (26.3%) had a distal mutation. The distal mutations had a higher proportion with hypertension and had higher mean age of 54.3 versus 44.8 at time of first scan.

There were no statistically significant differences between rate of increase in WMH, lacunes nor CMBs in proximal nor distal mutations, there was however a trend towards more rapid increase in volume of WMH and lacunes in proximal mutations (Figure and table 7.2)

Table 7.1 Baseline clinical characteristics proximal and distal mutations

Clinical feature	EGFr 1 to 6	EGFr 7 to 34	p-value
n	28	10	
Age at MRI (mean $\pm$ s.d.)	44.8 $\pm$ 11.7	54.3 $\pm$ 10.7	
Smoker, yes/no (%)	7/21 (25%)	5/5 (50%)	0.144
Hypertension, yes/no (%)	4/24 (14.3%)	4/6 (40%)	0.010
Diabetes, yes/no (%)	3/25 (10.7%)	1/9 (10%)	0.721
Migraine, yes/no (%)	16/12 (57.1%)	6/4 (60%)	0.589
Stroke, yes/no (%)	12/14 (46.2%)	4/6 (40%)	0.583

Table 7.2 Differences in rate of progression each year of imaging markers in proximal and distal mutations

MRI	EGFr 1 to 6	EGFr 7 to 34	p-value
median (IQR)			
Rate of change of normalised WMH	1.7 (0.5 – 7.6)	0.6 (0.2 – 4.7)	0.353
Rate of incident lacunes per year	0.15 (0 – 1.0)	0 (0 – 0.5)	0.479
Rate of incident CMBs per year	0 (0 – 0.7)	0 (0 – 1.0)	0.559

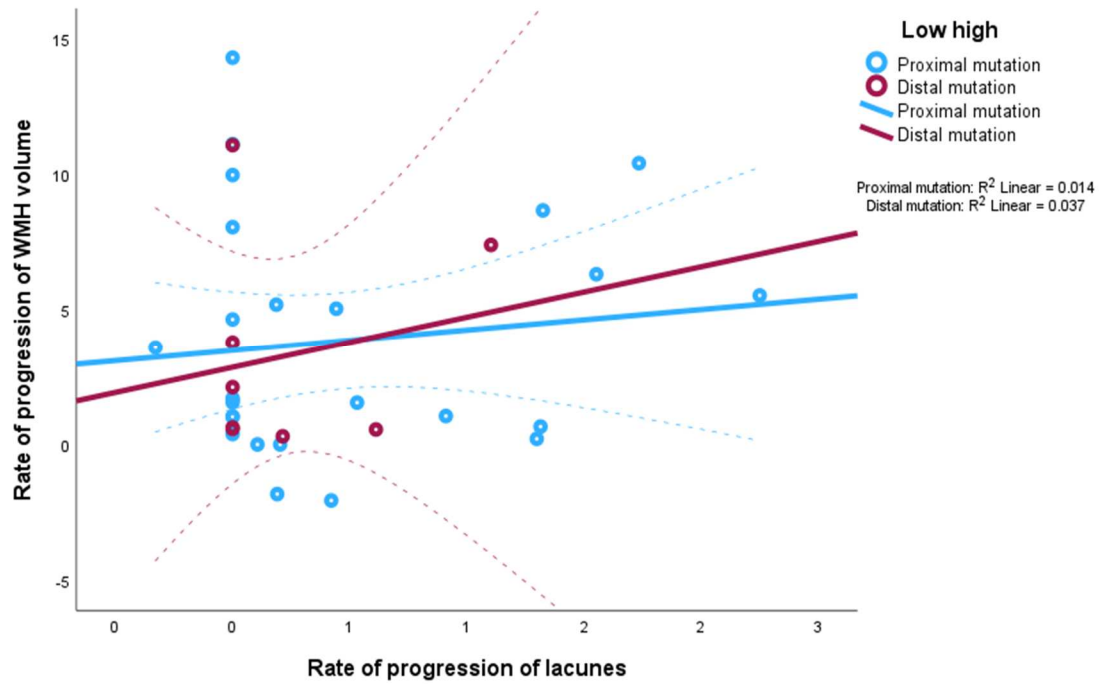


Figure 7.1 Correlation between rate of progression of WMH and lacunes in proximal and distal mutations

Table 7.3 Differences in clinical features between proximal and distal mutations

Clinical feature	EGFr 1 to 6	EGFr 7 to 34	p-value
n	28	10	
Age at MRI (mean $\pm$ s.d.)	44.8 $\pm$ 11.7	54.3 $\pm$ 10.7	
Smoker, yes/no (%)	7/21 (25%)	5/5 (50%)	0.144
Hypertension, yes/no (%)	4/24 (14.3%)	4/6 (40%)	0.010
Diabetes, yes/no (%)	3/25 (10.7%)	1/9 (10%)	0.721
Migraine, yes/no (%)	16/12 (57.1%)	6/4 (60%)	0.589
Stroke, yes/no (%)	12/14 (46.2%)	4/6 (40%)	0.583

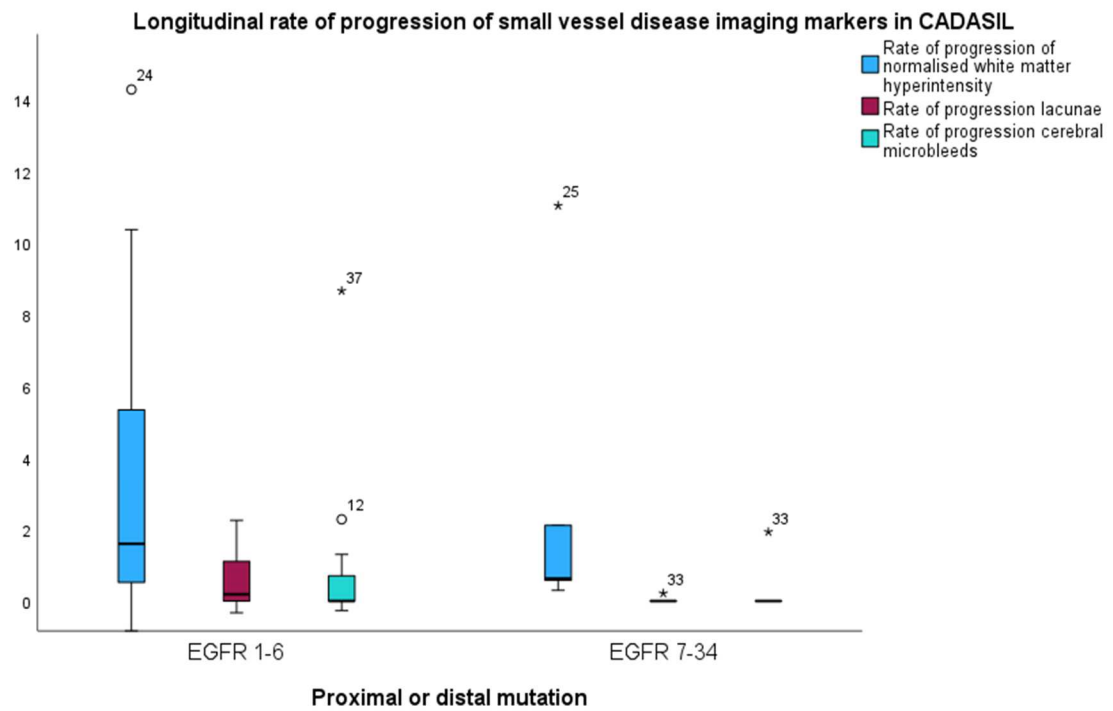


Figure 7.2 Boxplot with differences in rate of progression of WMH, lacunes and CMBs in proximal and distal mutations

7.4 Discussion

We have used the routinely acquired imaging data obtained through the NHS safe haven to measure the rate of progression of three commonly used imaging markers in CADASIL. Despite the small numbers involved and the younger age and lower level of hypertension, those with a more proximal mutation may have a numerically more rapid progression of WMH volume and development of lacunes than those with a distal mutation. This would be consistent with the current belief that distal mutations constitute a milder form of the disease with later onset stroke, lesser disability, and less extensive MRI imaging abnormalities (58, 78). Prospective studies in CADASIL have variable rate of progression of lacunes ranging from median 0.5 to 1 new lacune per year, with the same studies reporting between 0 and 1 new microbleed annually (149, 235), showing more rapid progression than in our retrospective cohort. Calculation of change in proportion of intracranial cavity annually would allow comparison with these studies.

Increasing research is being undertaken into the importance of WMH, lacunes and CMBs and their relative role in the development of cognitive impairment, gait disturbance and

their impact on prognosis. The challenges and importance of measuring their progression longitudinally are well recognised in sporadic small vessel disease.

This study is based on a small sub-section of a national CADASIL imaging study. The imaging itself had been undertaken as part of routine clinical care that would be sought either as part of clinic practice or in response to a clinical change. Geographically the scans were performed at multiple sites around Scotland, on different scanners, with different sequences and field strength, and consequently different imaging qualities. The images were rated by one rater using visual correction for boundary of WMH. Larger studies in CADASIL and small vessel disease have used specific software packages and semi-automated imaging analysis and segmentation techniques and there would be potential benefit for validity and external reproducibility if we used the same analysis software. These factors may introduce limitations to the generalisability of these measurement values.

8 LACUNAr infarcts, Microvascular Arteriopathy: a 7-Tesla Analysis of Thrombosed Arteries (LACUNAR MATATA)

8.1 Introduction

Lacunar stroke is a component of cerebral small vessel disease, which accounts for nearly 25% of all strokes (95), and is the second commonest cause of dementia. The pathogenesis of lacunar infarct is less well understood than for large vessel and cardio-embolic stroke. The proposed methods of vessel occlusion in small vessel disease include intrinsic disease of the arterioles resulting in endothelial dysfunction and vessel wall disruption (241). Recent clinical trials have shown benefit from agents with endothelial stabilising properties (242) which are thought unlikely to deliver benefit if mechanism of occlusion was purely thrombotic, while thrombolytic therapy appears to have comparable efficacy in lacunar stroke (243). Some radiological lacunar infarcts may have aetiology from branch atheromatous perforator obstruction (96) while others arise from cardio-embolic propagation of small thrombi (244). Mechanism of vessel occlusion is less well established in small vessel disease due, in part, to the small size of these perforating vessels limiting direct visualisation using conventional imaging techniques. 7-Tesla MRI has signalled the first opportunity to visualise directly the lenticulostriate arteries using non-invasive TOF-MRA (160).

The susceptibility vessel sign (SVS) is an MR imaging finding of signal loss seen on gradient-echo (GE) or susceptibility weight imaging (SWI) corresponding to arterial thrombus causing vessel occlusion. SVS has been associated with cardio-embolic stroke, larger infarct, and more disabling stroke (140, 141, 245). SVS in lacunar infarct is thought less common but has been reported in retrospective studies (142, 143), implying an underlying thrombotic occlusion in a proportion of lacunar infarcts. The development of ultra-high-field MRI allows direct visualisation of small vessels using both SWI and TOF-MRA (144).

We aimed to use 7-Tesla MRI in acute lacunar infarct to establish whether obstruction of small vessels could be visualised on TOF-MRA and whether associated SVS could be detected.

8.2 Methods

Participants

We recruited adults above 18 years old, who had capacity to consent, presenting acutely to the Queen Elizabeth University Hospital in Glasgow, with symptoms of lacunar stroke, small subcortical infarction less than 20mm in axial diameter (STRIVE criteria) (82), within the basal ganglia or centrum semiovale confirmed on 1.5T or 3T MRI within the previous 7 days. We defined stroke using the Bamford classification. We excluded participants if MRI confirmed brainstem infarct, any other stroke lesion, or if any established 7T MRI contra-indications including non-fixed metallic implants, tattoos and cardiac pacemaker. We recorded age, sex, co-morbidities, blood pressure, glucose, cholesterol, medications, and National Institutes of Health Stroke Scale (NIHSS). Short form stroke impact scale was recorded at day 30. The study received approval from the local institutional ethical committee (20/WS/0012). All patients provided written informed consent prior to inclusion. Study recruitment continued from 1st August 2021 until 3rd September 2022.

MRI protocol

We performed 7-Tesla MRI on the Siemens Magnetom Terra (Siemens Medical Solutions, Erlangen, Germany) including a 3-dimensional T1 sequences, T2 weighted FLAIR (fluid-attenuated inversion recovery), SWI, Diffusion weighted imaging (DWI) and TOF-MRA centring on lenticulostriate vessels.

MRI analysis

Two consultant neuroradiologists and a consultant stroke neurologist independently reviewed SWI and TOF-MRA blinded to radiological and clinical data. An SVS was deemed present if there was an area of hypo-intensity on SWI sequence in the territory of an ipsilateral small vessel, immediately proximal to DWI infarct. TOF-MRA signal dropout confirmed presence of small vessel occlusion. Agreement was needed on how likely hypointensity was to be SVS. Either 'likely SVS', or where cerebral microbleed or haemorrhage into infarct could not be excluded, it was considered 'possible SVS'. A consensus review with all three raters was performed for all cases where there was not complete agreement. Mango imaging software (<http://ric.uthscsa.edu/mango/index.html>) was used to map the lacunar infarct locations (231).

8.3 Results

Twenty participants underwent 7-Tesla MRI (table 8.1) a median of 5.5 days post onset, with a median age of 67.5 (IQR 64-81), a median NIHSS of 3 (range 0 to 8), four (20%) had AF, and 6 (30%) had carotid stenosis.

Four (20%) were thought highly likely to have SVS on axial SWI imaging (Figure 8.1), with 3 additional (15%) considered to have possible SVS. However, only one (5%) had occluded vessel on TOF-MRA, and there was no associated SVS on SWI. Three of the four SWI images thought most likely SVS positive were retrospectively visible on prior 1.5-Tesla or 3-Tesla MRI.

Table 8.1 Clinical data for study participants.

Variable	N (% or IQR)
Age median (IQR)	67.5 (64 - 81)
NIHSS	3 (2 - 4.75)
Time between onset and 7T MRI	5.5 (2.25 - 8)
Hemisphere left	10 (50)
IHD	2 (10)
Stroke/TIA	5 (25)
Diabetes	6 (30)
PVD	3 (15)
HTN	15 (75)
AF	2 (10)
Smoker	6 (30)
<i>Stroke aetiology after work-up</i>	
Atrial fibrillation	4 (20)
Carotid stenosis	6 (30)
<i>Clinical outcome</i>	
Short form stroke impact scale	23 (17.5 to 31)
<i>Imaging outcomes</i>	
Likely SVS	4 (20)
Possible SVS	3 (15)
Occluded vessel TOF-MRA	1 (5)

Of the 4 subjects with likely SVS on SWI 2 (10%) had atrial fibrillation, 2 (10%) had ipsilateral carotid stenosis but none had an occluded vessel on TOF-MRA.

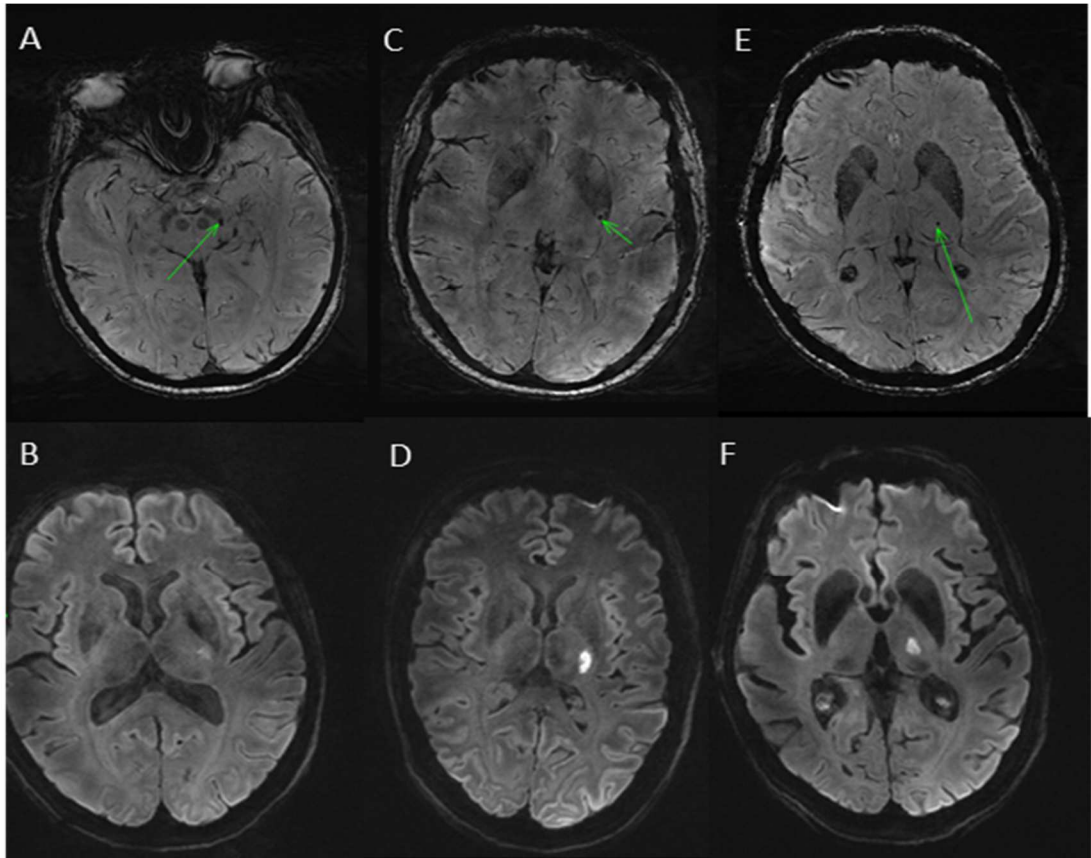


Figure 8.1 (A-F). SWI with DWI sequences showing likely SVS and infarct locations

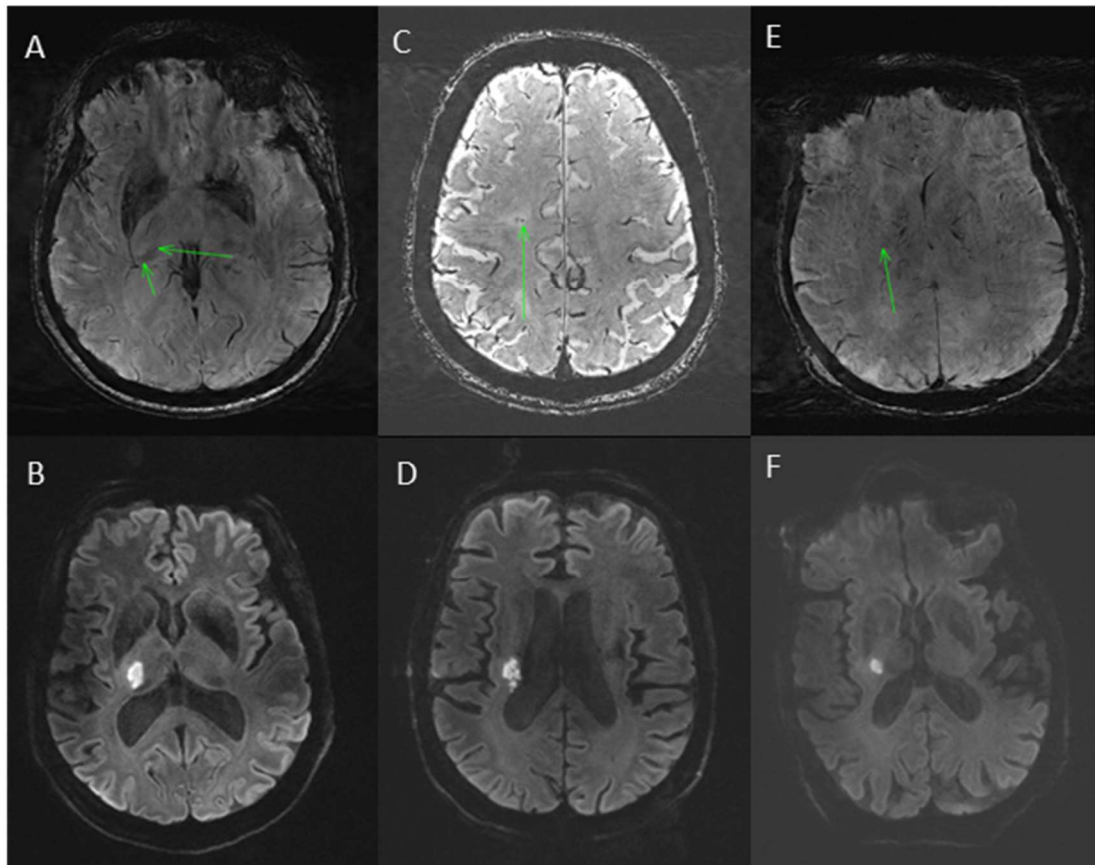


Figure 8.2 (A-F). SWI, with DWI sequences showing possible SVS and infarct locations.

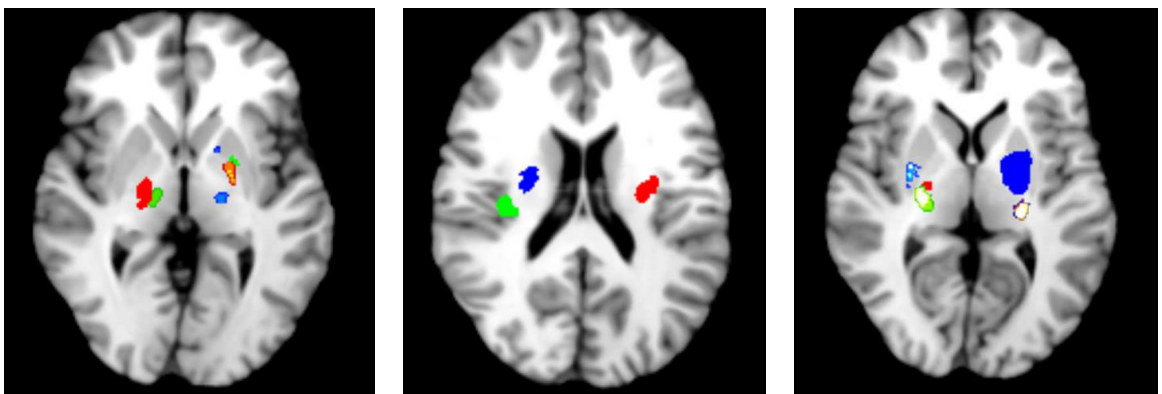


Figure 8.3 Axial MRI sample distribution of lacunar infarct location in our study population.

8.4 Discussion

Statement of findings.

Whilst we detected possible SVS in up to 35% of patients presenting with lacunar stroke, none of these patients had corresponding occlusion of the perforating artery confirmed on TOF-MRA. This could indicate that the pathology of the lacunar stroke is due to intrinsic pathology of the vessel wall, rather than emboli or thrombotic occlusion.

Strengths and weaknesses.

This study used ultra-high field MRI to image the small vessels of patients with acute lacunar stroke. The 7T MRI was performed median 5.5 days after stroke in which the 7T MRI was performed means that the absence of SVS may be the result of complete recanalization of the involved small vessels. An early imaging equivalent of SVS in lacunar stroke had evidence of vessel abnormality weeks after initial event (246). A more recent study demonstrated more rapid SVS disappearance alongside, rather than before recanalization in acute embolic infarction (247). We found 4 subjects with likely SVS and 3 with possible SVS which is a higher proportion than previous studies reviewing lacunar infarct using T2* weighted imaging at lower field intensity (142, 143). The additional review of TOF-MRA at 7T represents a strength of the study. However, the absence of associated vessel occlusion on TOF-MRA in each of the suspected SVS suggests this may represent intra-arteriole haemosiderin deposits rather than thrombotic occlusion of these vessels. Alternatively, the clot composition in these infarcts may not be red cell dense and may never have been visible. Susceptibility-weighted imaging (SWI) is thought to be more sensitive than TOF-MRA for SVS in large vessel occlusion (248) and 3D TOF-MRA vascular imaging confirmation is necessary to confirm SVS by thrombotic occlusion.

Further study and identification of SVS in acute lacunar infarct including those with co-existing atrial fibrillation or carotid disease may help delineate true small vessel aetiology from cardioembolic or large vessel and guide therapeutic interventions based on optimal understanding of underlying associated mechanisms.

9 Comparison of small vessel disease imaging markers at 7-Tesla with lower field strengths

9.1 Introduction

The quality of images using 7-Tesla MRI means that imaging markers of small vessel disease including lacunes, cerebral microbleeds and enlarged perivascular spaces may be more clearly visualised using 7-Tesla than 1.5 and 3 Tesla MRI. Early studies show that higher field strength improves the detection of CMBs (103, 131), thought to be under-detected by conventional MRI in comparison to post-mortem pathological studies (249). Field strength is referenced as important in the definition of cerebral microbleeds in the original STRIVE criteria (82). The additional imaging detail at higher field strength may assist with decreasing false positive detection of CMBs caused by other processes including microcalcifications and microaneurysms (249). There may be additional structural detail gained from imaging of lacunes at ultra-high field strengths, with recent updates to STRIVE criteria confirming existence of lacunes less than 3mm in diameter (93). 7-Tesla MRI may allow clearer differentiation between small lacunes and larger EPVS. EPVS are usually smaller cerebrospinal fluid filled structures surrounding small vessels in the brain (82). The importance of EPVS as pathological markers in small vessel disease is less well understood although theories including dysfunction of glymphatic drainage have been suggested (113). Most studies report EPVS using visual assessment techniques (250, 251), although increasing efforts have been made to develop automated tools to detect EPVS. Some of these show higher numbers of EPVS detected at 7-Tesla than with conventional imaging (252).

Scoring systems developed to improve accuracy and external validity of measurement of small vessel disease imaging markers include the MARS microbleed rating scale was developed using 1.5-Tesla MRI (110). Enlarged perivascular spaces (EPVS) can be measured using the Edinburgh EPVS rating scale, which provides an online easy to follow guide for detection of EPVS (230). The images used to develop this guide are likely to have been taken from lower field-strength MRI although this was not specified in the study (251). The increased sensitivity and detection of higher numbers of these MRI markers of small vessel disease at 7-Tesla may be impacting on the validity of these scoring systems using higher field strength imaging. Further validation of these scoring systems at 7-Tesla is required to ensure they remain applicable.

We used data from a prospective observational imaging study in acute stroke to compare detection of lacunes, WMH, CMBs and EPVS at 7-Tesla MRI with 1.5-Tesla and 3-Tesla MRI.

9.2 Methods

Participants

Images were used from the observational study '7T MRI in lacunar stroke' approved by the local institutional ethical committee West of Scotland REC number (20/WS/0012). Inclusion criteria included confirmed lacunar infarct on MRI <20mm diameter in the basal ganglia or centrum semiovale on 1.5 or 3-Tesla MRI.

MRI protocol

We performed 7-Tesla MRI on the Siemens Magnetom Terra (Siemens Medical Solutions, Erlangen, Germany) including a 3-dimensional T1 sequences, T2 weighted FLAIR (fluid-attenuated inversion recovery), SWI, Diffusion weighted imaging (DWI) and TOF-MRA centring on lenticulostriate vessels. A 1.5-Tesla clinical MRI or 3-Tesla MRI had already confirmed lacunar infarct.

MRI analysis

One rater reviewed T1-MPRAGE, T2-FLAIR, T2-TSE and SWI sequences and recorded Fazekas score, cerebral microbleed (CMBs) anatomical rating scale (MARS), number of lacunes and enlarged perivascular space (EPVS) scale at routine (1.5 or 3-Tesla) and 7-Tesla. This was repeated 4 weeks later. At the time of second review, the same rater reviewed side by side linked images of the 1.5/3-Tesla MRI and the 7-Tesla images and marked individual CMBs and lacunes in an attempt to isolate the differences in numbers from field strength alone. The rater was not blinded to the field strength.

Statistical analysis

Intraclass correlation coefficients (ICC) were used to assess differences between continuous variables (lacunes and microbleeds) and Cohen's Kappa (κ) coefficient was calculated to assess differences between ordinal variables (Fazekas score and EPVS scale rating 0 to 5).

9.3 Results

Twenty participants underwent 7-Tesla MRI a median of 5.5 days after stroke onset, with a median age of 67.5 (IQR 64-81), a median NIHSS of 3. Four (20%) had AF, and 6 (30%) had carotid stenosis. Five scans (25%) were performed on the 3-Tesla GE Signa Excite HD scanner and the remaining 15 (75%) were done on 1.5-Tesla clinical MRI. Table 9.1 reports the results from the review of both field strength images simultaneously (with exception of Fazekas).

Table 9.1 Comparison of 1.5 / 3-Tesla MRI with 7-Tesla

SVD imaging feature	1.5 or 3-Tesla median (IQR)	7-Tesla median (IQR)	p - value
WMH	3 [2- 4.75]	2.5 [2 - 4.75]	p<0.001
Total Fazekas			
Lacunes	1 [0-2]	1 [0-2]	p<0.001
Cerebral Microbleeds	1 [0-2]	1 [0-2.75]	p<0.001
EPVS scale (0-5)	2.5 [2 -3]	4 [3 - 4]	p=0.262

Intraclass correlation coefficient with two-way random effects model for absolute agreement was used to assess differences. WMH Fazekas score 0.988 95% C.I. 0.971 to 0.995, p<0.001, CMBs 0.926 [95% C.I. 0.863 to 0.960], p<0.001 and lacunes 0.816 [95% C.I. 0.577 to 0.913], p<0.001. Cohen's kappa was run to determine agreement between repeat readings of EPVS after an interval of 4 weeks. There was substantial agreement with $\kappa = 0.623$ (0.439 to 0.807), p<0.001

To minimise confounding factors and emphasise the difference from MRI field strength alone images were also reviewed simultaneously for comparison. In this case, there was excellent agreement for cerebral microbleeds and lacunes. CMBs ICC 0.992 (95% C.I. 0.977 to 0.997), $p < 0.001$ and lacunes ICC=0.926 (95% C.I. 0.819 to 0.971), $p < 0.001$.

Cohen's kappa was run to determine agreement between EPVS at low field-strength with high field strength. There was poor agreement with $\kappa = 0.111$ (-0.075 to 0.297) $p = 0.262$. Cohen's kappa was run to determine agreement between EPVS at 1.5T with 7T. There was poor agreement $\kappa = 0.153$ 95% C.I. (-0.08 to 0.388). Cohen's kappa was run to determine agreement between EPVS at 3T with 7T. There was poor agreement $\kappa = 0.00$ 95% C.I. (-0.139 to 0.139).

Figures 9.1 to 9.5 show examples of SWI and T2TSE imaging at 1.5, 3 and 7-Tesla to demonstrate differences in imaging quality for CMBs and numbers of EPVS detected.

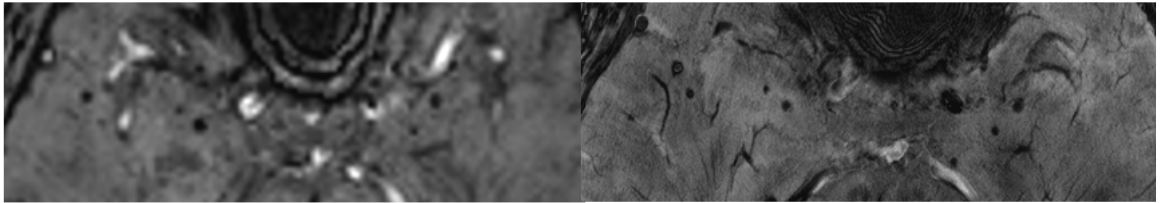


Figure 9.1 3-Tesla (left) and 7-Tesla (right) SWI imaging of cerebral microbleeds

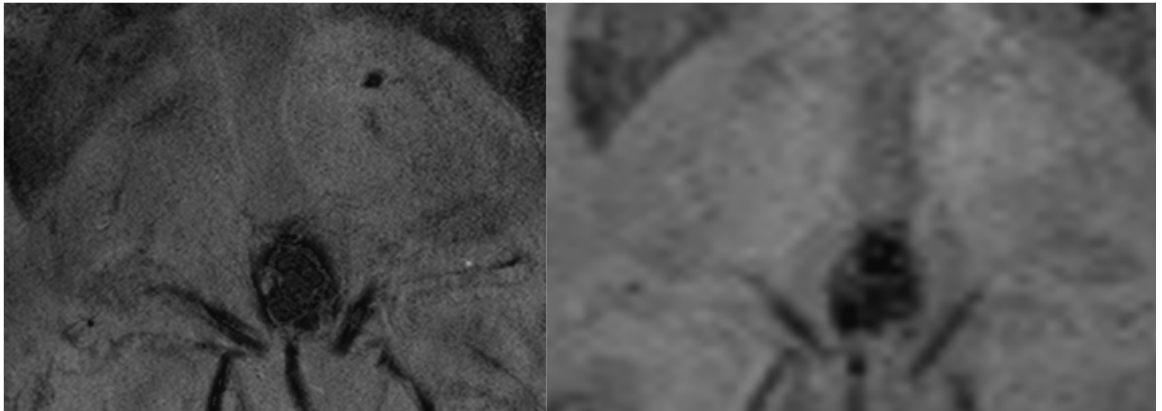


Figure 9.2 7-Tesla (left) and 1.5-Tesla (right) SWI MRI

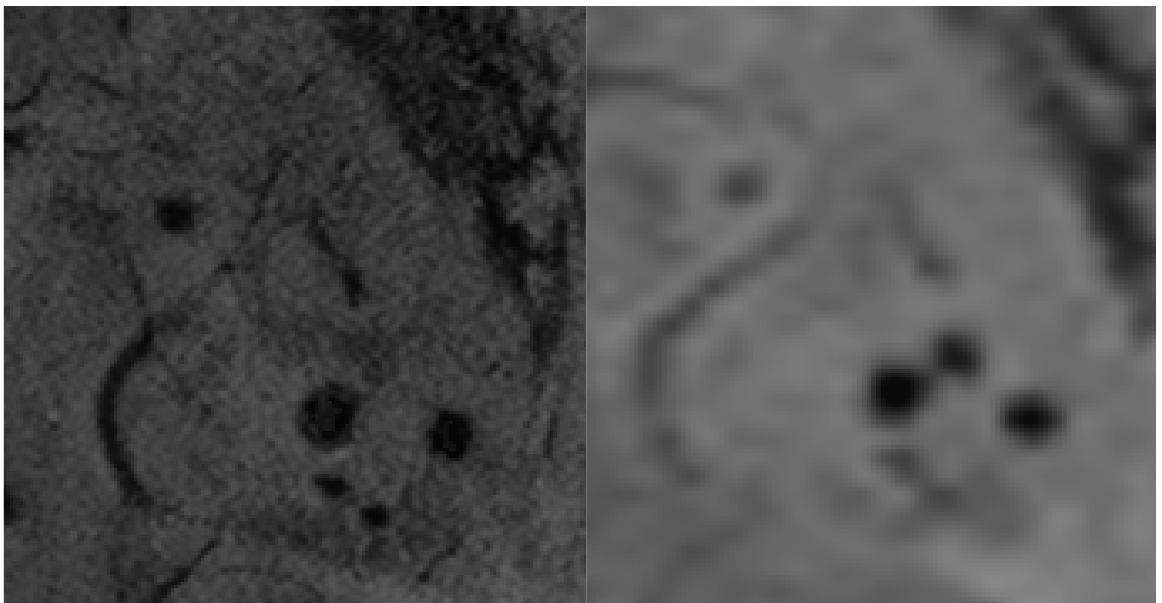


Figure 9.3 7-Tesla (left) and 3-Tesla (right) SWI imaging of cerebral microbleeds

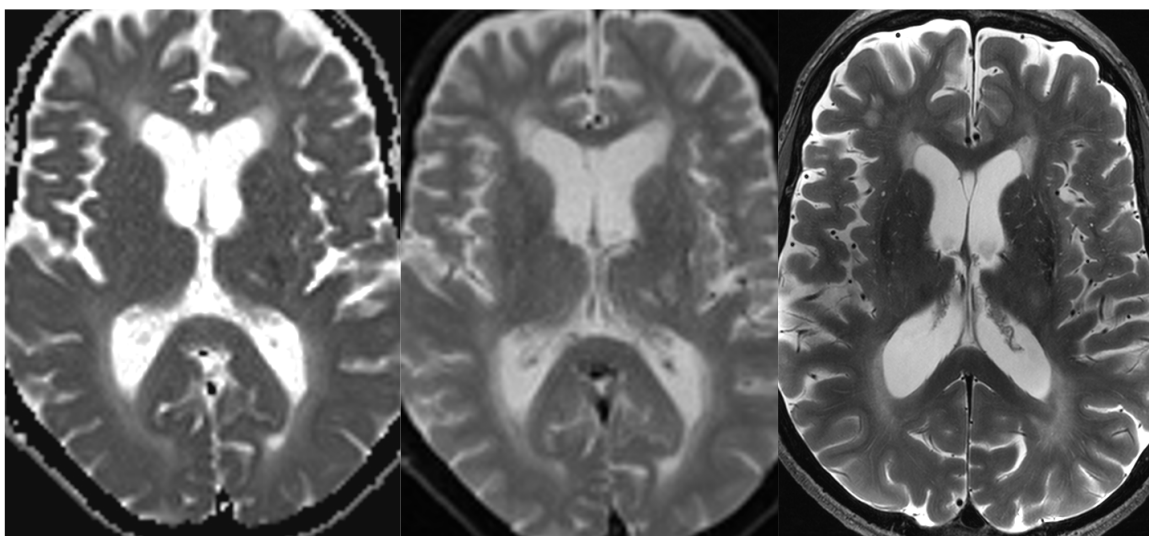


Figure 9.4 T2-TSE imaging two 3-Tesla images and one 7-Tesla image (right) for EPVS

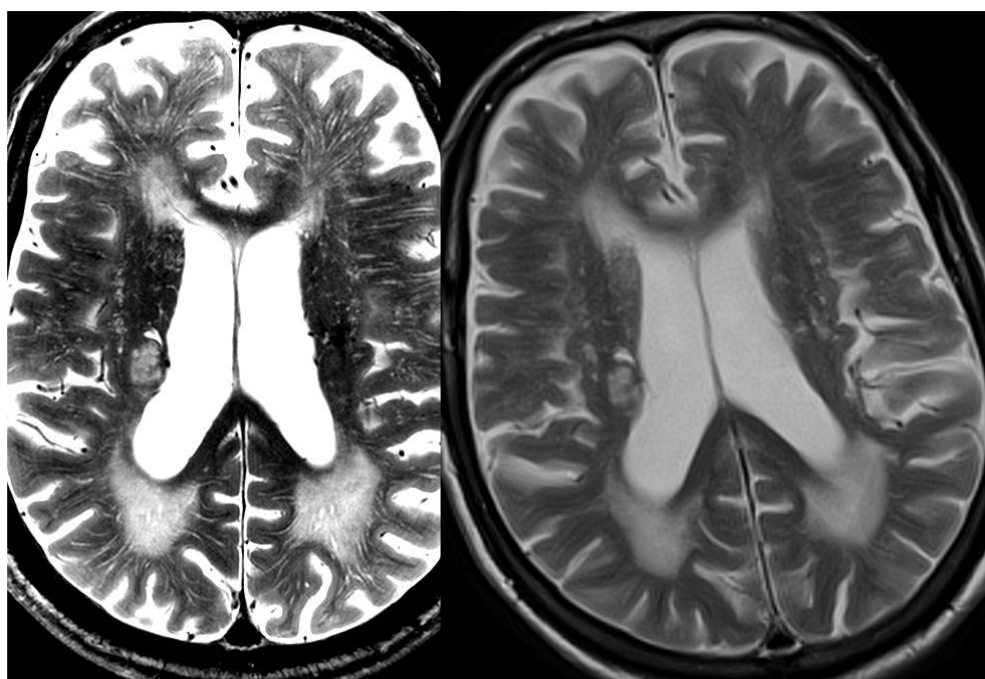


Figure 9.5 7-Tesla (left) and 3-Tesla (right) T2-TSE MRI for EPVS

9.4 Discussion

The main result of this study is that the EPVS rating scale used to visually assess extent of EPVS gives a different outcome at 7-Tesla when compared to the same study participant at 1.5 or 3-Tesla. The explanation for this is that the enhanced and exquisite detail of the images are allowing direct visualisation of more enlarged perivascular spaces. The EPVS rating scale divides the EPVS in basal ganglia and centrum semi-ovale into groups with selected cut-offs namely: 0 (absent), 1 to 10 (mild), 11 to 20 (moderate), 21 to 40 (frequent) and >40 (severe) (230). This allows for straightforward measurement and classification but has the limitation of leading to inconsistencies on the basis of field strength. This has potential implications for multi-centre studies where SVD imaging is rated locally, and imaging may be undertaken at differing field strengths. This highlights the importance of reporting the MRI field strength used in each study, as well as the MRI imaging sequences and other factors that might affect interpretation, such as number of raters, experience and speciality of raters (82). The increased sensitivity of 7-Tesla MRI already demonstrated for cerebral microbleeds (131, 249) and EPVS (252) should alert us to this possibility, and it is important to quantify this affect and to update scoring systems in common practice as novel technologies develop and alter the limits of detection.

There are limitations to the study. The main limitation is that the imaging review was completed by one rater and blinding to data was not possible when assessing the effect of field intensity as the quality of image itself is revealing. Attempts to minimise these factors included reviewing both field strengths simultaneously and marking with arrows each CMBs and lacune on both images to avoid double counting and reduce chance of missing subtle cases. There are plans to repeat this process with multiple staff in the radiology department to validate this finding.

10 Imaging technical development study at 7-Tesla

10.1 Introduction

Small vessel disease is a cause of nearly 25% of acute stroke and is associated with the development of dementia. Vascular cerebral blood flow is likely to have an impact on the development of vascular dementia. Accurate measurement of cerebral blood flow and flow velocity within the intracranial small vessels is useful to study potential links between blood flow in these vessels and the development of correlates of clinically relevant imaging endpoints including lacunes and abnormal white matter hyperintensities. Limitations of spatial resolution at lower field strengths of 1.5-Tesla and 3-Tesla present challenges in direct visualisation of small vessels including the lenticulostriate arterial branches from the middle cerebral arteries that are responsible for many lacunar infarcts. Movement artefact and other imaging limitations made visualisation impossible until recently but the improved image quality of 7-Tesla MRI has enabled the direct visualisation of very small intracranial vessels (160).

Phase-contrast imaging is a technique for measuring flow in blood vessels. It has been used for many years to study larger blood vessels throughout the body including in cardiac flow imaging, large aneurysms and in renal vascular flow. Commercial packages have been developed to enable analysis of flow patterns in these fields. Until recently the very small diameter of the relevant intracranial small vessels had precluded the use of phase contrast imaging in cerebral small vessel disease, but ultra-high field strength imaging and development of novel processing techniques have now enabled use of phase-contrast imaging intracranially both in healthy volunteers and in CADASIL patient groups (161). There have been different imaging and image processing techniques used to measure flow velocity incorporating direction of blood flow (136). Two-dimensional phase-contrast imaging has been used to assess flow velocity and pulsatility (153).

The purpose of this study was to undertake a human volunteer study to develop our local protocols and ability to perform 7-Tesla phase-contrast imaging of lenticulostriate vessels and use complex imaging-analysis techniques to convert these images into measurements of flow velocity. We use different encoding velocities and trialled a work-in-progress (WIP) compressed-sensing time-of-flight magnetic resonance angiography (TOF-MRA) to compare this with conventional TOF in the measurement of flow velocity.

10.2 Methods

Ethics

We were granted ethical approval for a volunteer study at local clinical research imaging facility research and ethics committee (GN21ST033).

Study Cohort

Healthy volunteer adults with no contra-indications to 7-Tesla MRI.

Imaging protocols

Volunteers were imaged on the CE-labelled 7-Tesla research MRI Siemens (Erlangen, Germany) using a 32 channel Nova coil. The purpose of this study was to develop the protocols and ensure that we had the capabilities to perform phase-contrast imaging of adequate quality, so each volunteer underwent different combination of sequences using different encoding velocities (VENCs) and with attempts to incorporate a novel compressed-sensing sequence and a parallel transmit T2-TSE WIP under development at Siemens with appropriate permissions.

For anatomical baseline and structural reference, we performed a TOF-MRA focusing on the lenticulostriate vessels, using repetition time (TR) of 15ms, echo time (TE) of 3.57 ms, and flip angle (FA) 20 degrees. The 3-dimensional phase-contrast imaging used TR 43.45ms, TE 7.14ms, with FA 20 degrees, and VENC variably 15 / 50 /100 depending on expected velocity of target vessels, namely 15cm/s for lenticulostriate arteries (LSA) and between 50 and 100cm/s for more proximal middle cerebral artery (MCA). The resolution was 0.35 x 0.35 x 0.40 mm³.

We consulted scientists working for Siemens in China due to their prior experience with 7-Tesla phase-contrast imaging and set-up a collaboration with researchers in Beijing who were able to provide recommendations and code to assist in analysis of flow velocity within the small vessels.

Imaging analysis

The post-imaging analysis methods involved preparation of custom-built software programming using MATLAB R2019b.

The analysis pipeline has been created and shared by Professor Zhang and colleagues at the State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing. Further details are published in the online supplement (161).

There are five stages in the analysis pipeline for measuring flow velocity from the base magnitude (mag) and phase difference MRI images.

1. Perform an intensity bias correction using a version of the publicly available popular non-parametric non-uniform intensity normalisation algorithm (N3) (253).
2. Extract the relevant LSA region using the maximum intensity projection images.
3. Perform a threshold-based noise masking.
4. Perform velocity reconstruction using the phase-contrast data.
5. Put a region of interest (ROI) round the dominant LSA branches and calculate the mean flow velocity.

Figure 10.1. Diagrammatic representation of pipeline for imaging analysis for flow velocity in LSA (161).

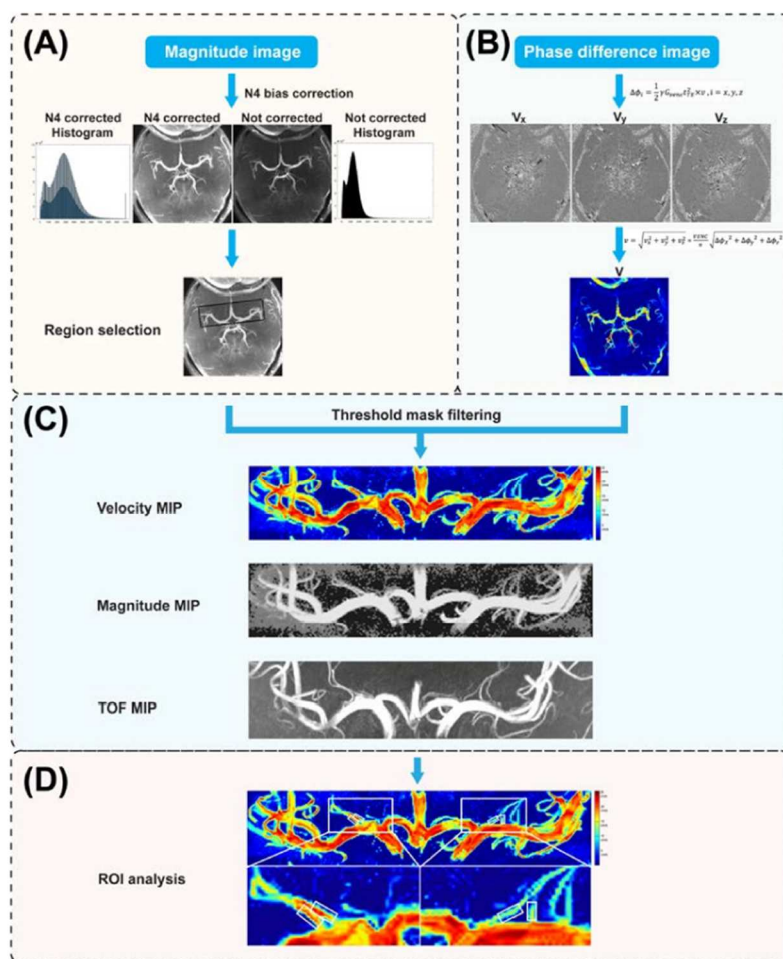


Figure 10.1 Image analysis processing pathway (161)

10.3 Results

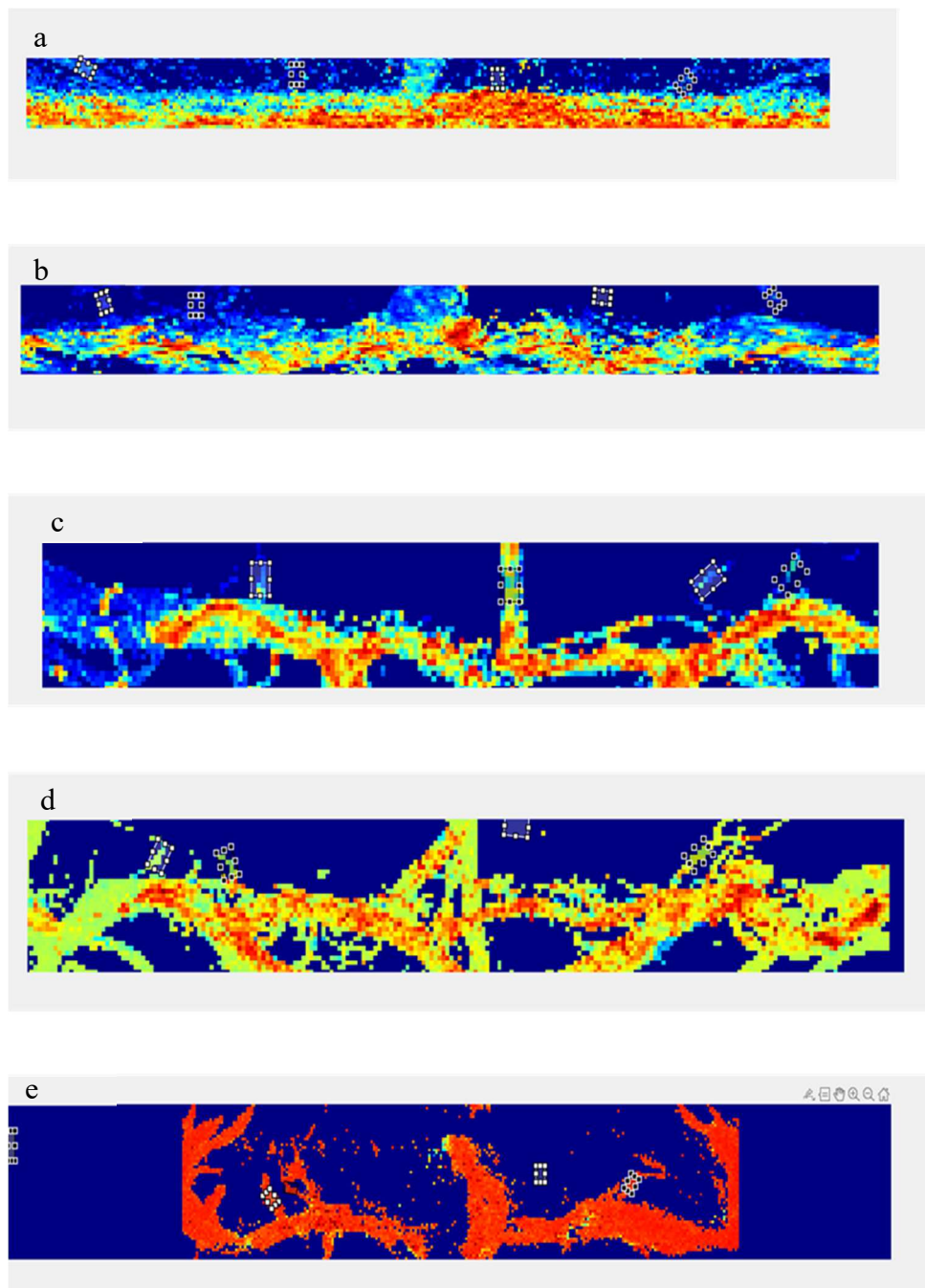


Figure 10.2 a to e Examples of output display of MCA and LSA with box ROI selecting vessels for flow velocity measurement. Boxes labelled 1 to 4 from left to right on each image.

Table 10.1 Flow velocity outputs in individual vessels shown in figure above ROIs from left to right

		Box 1 (left)	Box 2	Box 3	Box 4 (right)
1a	Mean flow velocity (cm/s)	5.5	2.6	4.2	3.2
1b	Mean flow velocity (cm/s)	2.1	2.9	0	2.8
1c	Mean flow velocity (cm/s)	3.4	12.7	2.1	2.2
1d	Mean flow velocity (cm/s)	6.9	7.0	0	8.9
1e Venc 100	Mean flow velocity (cm/s)	20.2	15.9	0	16.9

Reviewing the images on the left, 1.2 e was excluded as VENC 100cm/s is too high for measurement of lenticulostriate vessels. Movement artefact affected the initial imaging in figure 10.2 a and b so they were excluded. For approximate assessment of average velocity in LSA within this group I have included individual boxes 1c -1,3 and 1d 1,2 and 4. This gives a mean flow velocity in LSA 5.7 with standard deviation of 2.8.

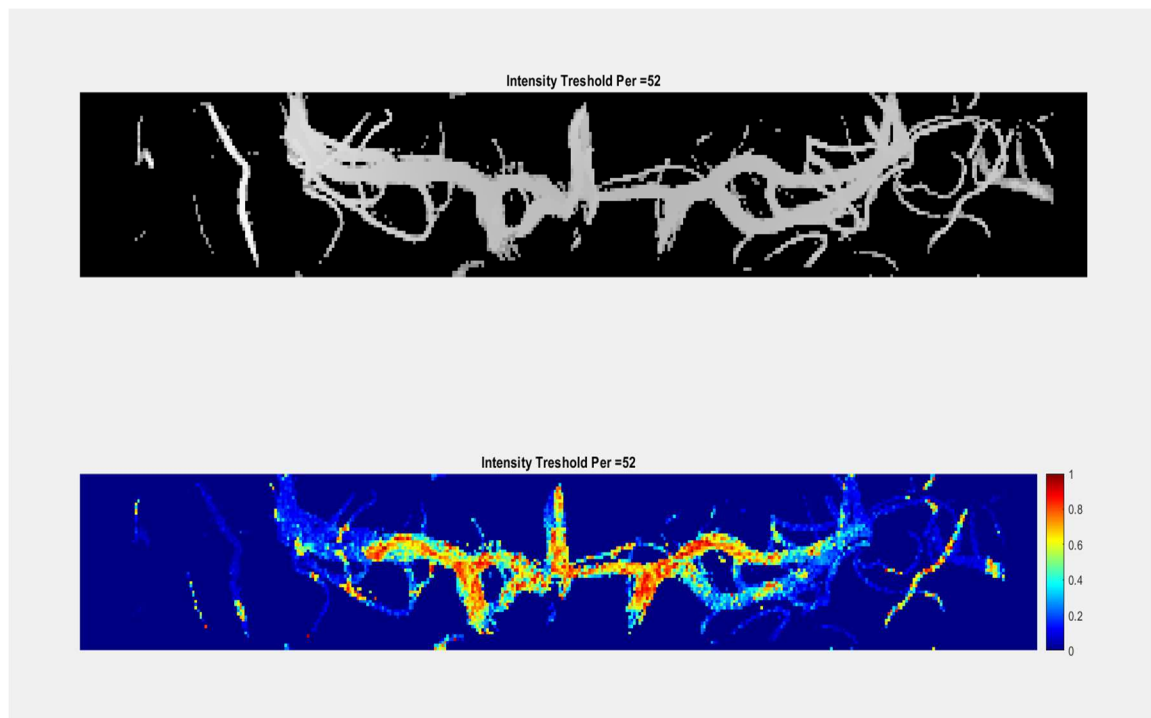


Figure 10.3 Example output from phase-contrast imaging at 7-Tesla

10.4 Discussion

The purpose of this study was to develop our imaging protocols to allow us to use 7-Tesla MRI phase-contrast imaging to measure flow velocity in the lenticulostriate vessels. We have achieved this and measured mean velocity to be 5.7 ± 2.8 cm/s. Through experience the quality of our images is improving, and we confirm 15 cm/s as the appropriate Venc for lenticulostriate vessels. The value 5.7cm/s may not be reflective of our final cohort and we are undertaking further study to calculate reliability of repeat measurements. This is somewhat smaller than the mean flow velocity reported using the same technique in which a cohort with CADASIL were imaged and found to have mean flow velocity between 10 and 11.5 cm/s (161). This value was higher than previously recorded 9.6 cm/s using similar analysis techniques for a group of healthy volunteers (136). And these values are, in turn, higher than previously reported studies measuring flow velocity and pulsatility using 2-dimensional phase-contrast imaging. The primary study to achieve this reported mean flow at the level of the basal ganglia of between 3.9 and 5.1 cm/s (153). A recent study compared differences between 1-dimensional and 3-dimensional encoding velocities and found little difference between the two ($<10\%$) and recorded mean LSA velocities ranging from 4.3 to 10.6 cm/s depending on the size of lenticulostriate vessels (254). This range of difference between minimum and maximum values is not unusual in the above

studies. Established normal values of flow in these vessels has been challenging as they are deep and too small to be measured using more established imaging techniques including transcranial doppler and digital subtraction angiography.

Our ability to achieve good image resolution improved as we continue to gain experience of the imaging techniques and we intend to complete this volunteer study. We have received funding and ethical approval for an observational study of phase-contrast in CADASIL to establish mean flow velocity in this cohort.

11 Discussion

11.1 Research problem and aims of thesis

CADASIL is the most common monogenic cause of small vessel disease. There remains no treatment for the condition, and it is progressive often resulting in early dementia and increased disability with a relatively preserved life expectancy. Within the disease spectrum of CADASIL there is much variability even within single families. This heightens the psychological impact of the condition to both the individual affected and their immediate family and necessitates an increased understanding of markers of disease progression and prognosis. Extrapolating from understanding gained from imaging endpoints including white matter hyperintensities, lacunes and cerebral microbleeds, an improved understanding of the function and dysfunction of the small vessels implicated in CADASIL provides a potential opportunity to better understand and potentially intervene with the underlying disease processes. Ongoing research into measures of function of small vessels may serve as sensitive correlates of disease progression that can be used to guide outcomes in much needed clinical trials.

11.2 Summary of key findings

Despite the recent findings of a large number of community dwelling individuals over 1 in 1000 people harbouring cysteine-altering NOTCH3 variants previously thought pathognomonic for CADASIL (15), this remains on a different scale from those affected clinically by CADASIL (8, 9). Although the presence of NOTCH3 is associated with increased WMH even in the absence of symptoms (16), there is evidence for an extended spectrum of mild disease even through to possible non-penetrance. The position of the pathogenic variant is an important determinant of disease spectrum. EGFr domain 1 to 6 is associated with more typical CADASIL with extensive WMH and more distal EGFr domains 7 to 34 predisposing to a less severely affected phenotype (58). Despite the increased awareness of the possibility of a much higher disease prevalence since 2016 the numbers attending clinical services for CADASIL appear to remain similar. We reviewed our national service and updated records to find that the prevalence of those currently attending clinic with CADASIL remains at a level that constitutes a rare disease at 2.76 per 100,000 people nationally. This is consistent with the predominance of symptomatic mutations in the clinical population. The imaging from our national cohort is markedly different from other sporadic small vessel disease, exhibiting much more extensive WMH at a lower age, more lacunes and a trend towards more CMBs. When divided into

previously suggested groups of mutations with differing prognosis and MRI abnormalities, our cohort appears to follow very similar patterns (44, 58, 81), with earlier onset stroke, and more extensive WMH, lacunes and CMBs in those with a proximal NOTCH 3 mutation. Also displaying similar clinical characteristics and the previously reported higher incidence of hypertension in those affected with more distal mutations (58, 59). Our retrospective analysis of routinely obtained imaging also suggested an increase in the rate of progression of WMH and lacunes over time although only 38 individuals were included in this study.

Ultra-high field strength MRI is becoming an important tool in small vessel disease research and is allowing previously unattainable degrees of precision and image quality, particularly for susceptibility-weighted images. The increased resolution comes at the cost of increased scan duration and movement artefact can be limiting in certain clinical situations. Novel image processing techniques may provide further avenues for research and potentially enable clinical use in small vessel disease. We undertook a study of recent lacunar stroke to assess 7-Tesla imaging and its ability to identify the culprit occluded small vessel and associated SVS which would consolidate the opinion that thrombotic occlusion is responsible for a proportion of lacunar stroke. Despite the appearance of SVS on axial SWI imaging it was very difficult to definitively confirm the presence of an associated occluded vessel. The imaging from this study allowed us to additionally review the sensitivity of 7-Tesla imaging for lacunes, CMBs and EPVS. While improved definition and additional detail of lacunes and CMBs boundary and structure was observed, the number detected remained very similar. This was not the case for EPVS for which a much larger number are visualised which results in a different category in the EPVS rating scale (251).

Finally, we were able to use 7-Tesla phase contrast MRI to assess the flow velocity in some of the very small lenticulostriate arteries to act as a correlate for blood flow to part of the basal ganglia. This will allow us to correlate this with other markers of flow from arterial-spin labelling and extend this to use apply it to a cohort affected by CADASIL.

11.3 Interpretation of results and their meaning

Our current population estimate extrapolated from clinical attendance of 2.76 per 100,000 suggests that CADASIL remains a rare disease. The Neurovascular clinic set-up as a national service for people with CADASIL has been based in Glasgow since its inception

in 2002. The population distribution of the CADASIL clinic by geographical area suggests that attendance decreases further from greater Glasgow and Lanarkshire. This may reflect a degree of referral bias. Although the clinical genetics department are involved in communication and testing of family members, and there is now availability of online communications allowing people to attend clinic virtually, it seems likely that there are a proportion of people in northern and eastern Scotland who have CADASIL and do not attend the clinic. This group may remain well and do not seek medical input, or are managed by neurologists, geriatricians, or other health professionals in their local area. An alternative explanation extrapolated from isolated populations suggest that because of the autosomal dominant nature of the disease inheritance, there can be uneven geographical distributions with large representations of specific NOTCH3 mutations explained by the 'founder effect' (79, 218). We aim to set-up an observational national database to ensure most complete clinical and genetic data and gain more detailed information on this uneven geographical distribution.

We were able to compare MR imaging from a CADASIL cohort with a control cohort with presumed sporadic small vessel disease. The purpose was to compare the distribution and extent of WMH, lacunes, cerebral microbleeds and EPVS between groups. Abnormal WMH in the anterior temporal lobe, external capsule and corpus callosum have variably been described as specific or frequently present in CADASIL (88, 179). Our binary logistical regression analysis showed that again the anterior temporal pole (odds ratio 33:1) to be strongly associated with presence of CADASIL as well as WMH in the frontal lobe. Presence of lacunae in the temporal and frontal lobes were significantly associated with CADASIL.

Comparison of WMH distribution is challenging in these two populations due to lack of clear definition for anatomical boundaries and the large difference in WMH volumes in each case, but it is interesting to note the continued specificity of anterior temporal pole involvement for CADASIL. It was however present in 6% of the control group with presumed sporadic small vessel disease (226). Genetic testing was not required for involvement in this trial, and we cannot be sure if any of these presumed sporadic SVD cases with temporal pole involvement had a NOTCH3 mutation. Population studies would suggest that on average at least one case in this group may have done so (16). The finding of lacunes in the temporal lobe is a novel finding but fits with the overlapping pathological development and interconnections between abnormal WMH and lacunes, where it has been noted that lacunes preferentially develop around the wedges of WMH (255). Presence of cerebral microbleeds in the brainstem and thalamus were the significant indicators of

CADASIL over sporadic small vessel disease. A comprehensive recent review of CMBs in CADASIL had found a very similar distribution to our cohort and also found the thalamus to be the most frequently affected area of the deep subcortical region (44) in common with another study in a Korean cohort (256). A phenomenon of very extensive CMBs clustering in the thalamus has been reported with specific gene mutation R75P (228). This was not present in our cohort and those with large numbers of thalamic CMBs came from multiple EGFR domains including “distal” mutations.

There is increasing evidence of the importance of location of EGFR mutation on NOTCH3 for the severity of clinical condition and extent of imaging abnormalities. This was initially dichotomised into “proximal” mutations affecting EGFR domains 1 to 6 and “distal” mutations affecting EGFR domains 7 to 34 comprising a milder disease phenotype (58). Certain exceptions to this dichotomised system have been proposed and attempts have been made to refine this system investigating distal mutations thought to confer higher risk. A three-tiered approach is undergoing validation (81) and a separate group had found a lower risk of stroke associated specifically with EGFR domains 10 to 17 (78). Further work is needed to clearly delineate the precise risks associated with each EGFR domain and to establish external factors that impact on the inherited genetic risk.

The patterns of our CADASIL cohort followed those established in the literature with the proximal mutation group undergoing MRI at a younger age, while having a higher volume WMH, and earlier age onset of first stroke on Kaplan-Meier curve. These results were not statistically significant, but the cohort of distal mutation carriers was a small group, and the scatter plot is suggestive of a trend consistent with prior reports. When further subdivided into a three-tier risk score the pattern for age against WMH was maintained, although group size was very small, and the mid-tier group had experienced no strokes.

Longitudinal assessment of WMH volume, lacunes and microbleeds is a challenge in small vessel disease imaging and efforts are ongoing to establish reliable reproducible methods to do this in an automated manner. The cohort and routinely available imaging we used for this study were derived from multiple scanners with differing field strengths, sequences, and quality. There are limitations to accuracy using the visual and individual rating methods used, both in terms of diagnostic sensitivity with varying field strength and through observer variability. However, we observed numerically greater expansion of WMH volume in proximal mutations when compared to distal. This result could be validated using longitudinal imaging studies and would be well served with automated techniques. This may improve inter-observer reliability and process larger data sets more

time efficiently. They are however susceptible to false positive and negative errors and requiring ongoing validation with expert agreement.

7-Tesla MRI has enhanced our ability to view small vessels that was previously not possible due to scan resolution limitations. The purpose of the observational study investigating presence of SVS in lacunar stroke was to improve our understanding of the mechanism of vessel occlusion involved. The presence of small areas of axial hypointensity on blood-sensitive MRI sequences has been described as the SVS and noted to be present in lacunar stroke (142, 143), but there are alternative possible explanations for this imaging finding, including small areas of haemorrhage within the infarct or incidental microcalcifications. To confirm the presence of an occluded vessel on TOF-MRA at the same location as focal SWI hypointensity would support the hypothesis that the SVS was the result of thrombotic occlusion. Testing 7-Tesla MRI in clinical populations is important to establish feasibility, as achieving the gains in image quality may not be possible due to longer imaging duration. Our study did not provide confirmatory evidence of occluded vessels, possibly affected by movement artefact limiting resolution of TOF MRA at 7T. The only time we visualised consensus occluded lenticulostriate vessel, there was no evidence of SVS on SWI sequence. While it is disappointing to have not found evidence of small vessel occlusion in the acute lacunar stroke setting, current theories of mechanism for lacunar stroke include intrinsic disease of small arteries leading to endothelial damage and wall disruption (241). SVS and an occluded vessel may not be expected to be visible in this context. Our imaging was performed a median 5.5 days after the event, and it is also possible that visible vessel occlusion had resolved at time of imaging (247).

The higher image quality and visibility of smaller structures potentially has benefit for imaging of small vessel disease in the brain. As well as the ability to detect higher numbers of common findings such as cerebral microbleeds there is potential to gain additional insights into structural detail (131). It is important to continuously review the benefit of these additional imaging findings and to differentiate pathological findings from normal findings, now more clearly visible as a result of the increased field strength. Although the quality of images was higher with clearer definition using 7-Tesla MRI the number of microbleeds and lacunes were similar at both field strengths. EPVS on the other hand were clearly visible to a much greater degree at 7-Tesla. This is perhaps unsurprising, but the current scoring system categorises EPVS into 5 categories based on the number of EPVS seen (230). If 7-Tesla MRI detects both enlarged perivascular spaces and normal perivascular spaces that were not visible at lower field strengths, this may result in

assignment of a higher score based on EPVS category. While the significance of EPVS for prognosis in small vessel disease is not certain, this finding highlights the importance of including details such as field strength while reporting imaging outcomes in small vessel disease, and the need to calibrate risk prediction appropriate to different imaging approaches.

We have developed a protocol for performing phase-contrast imaging at 7-Tesla using a locally developed radiofrequency coil and analysis techniques developed by collaborators in China. The mean flow velocity in the lenticulostriate vessels is lower than that published recently (161), but higher than that previously demonstrated using 2-dimensional phase contrast imaging (153). We will further investigate this in our planned volunteer study. There has been quite broad range of velocities reported in other studies. Comparison between flow velocity of the lenticulostriate vessels and cerebral blood flow quantified by arterial spin-labelling which has previously been shown to be reduced in CADASIL and also to decrease over time (167) may provide another clinical correlate of potential value in clinical trials.

11.4 Limitations of studies

There are several limitations to the studies performed as part of this thesis. For our population estimate we are relying on retrospective review of clinical documentation of current and historical attendance record at the neurovascular clinic. People who have CADASIL in Scotland may not have been diagnosed or referred for multiple reasons. Some patients with a confirmed diagnosis may be managed locally in other regions of Scotland and have no relatives under review at the clinic. Through pedigree information it is also clear that many family members who are at risk of carrying, or who must carry, CADASIL-causing NOTCH3 mutations have not sought medical referral. We have no reliable method for accurately calculating an estimate of this population.

The retrospective imaging reviews have involved training and repeated measurement by one rater. Although the intraclass correlation coefficients were good and the inter-rater reliability acceptable for the purposes of these studies, further validation with additional external assessors and for longitudinal absolute measurements would be important to establish generalisability. The measurement of WMH itself, although clearly described in the STRIVE criteria, is in practice challenging since clear distinction between normal white matter and WMH can be difficult to ascertain and, small differences in the diffusely

spread multiple areas of WMH typical of CADASIL can make a large difference in volume measurement depending on precise location and this in turn could impact on longitudinal results. Measurement error is proportionately higher with small lesions. Machine learning and automated methods may achieve greater consistency in defining WMH and thereby reduce variation in measurement error. Likewise with lacunes and EPVS, there were initial size criteria to make this distinction (82), but with examples of both that did not fit neatly into these distinctions, additional features including anatomical location are required for interpretation (257). Our results for number of CMBs, lacunes and volume of WMH fit reasonably within the range published elsewhere (44, 99).

The review of 7-Tesla MRI for both SVS and to establish sensitivity to lacunes, CMBs and EPVS again confirm that variation is likely to arise from different field intensities; confirmation and quantification will be important with a wider range of observers in order to address the limitation of bias due to lack of blinding in a single observer study. Subjectivity in interpretation of SVS will always be present and our addition of TOF-MRA aimed to provide confirmation of relevant vessel occlusion. The absence of visible vessel occlusion may be attributable either as a result that axial SWI imaging alone is inadequate to differentiate occluded vessel from small haemorrhage as both these pathologies have very similar imaging appearance.

We are indebted to our collaborators in China who have provided us with software code to facilitate our analysis and assessment of flow velocity in LSA in the study volunteers. The size of these vessels is very small, and the image quality remains subject to movement artefact. The results we obtained are reasonable and velocity values are in keeping with previous literature, but we will have to undertake further study to ensure internal validity and to provide baseline values in our CADASIL cohort.

11.5 Future directions

The human volunteer study has allowed us to improve the sequences and imaging analysis for phase-contrast imaging at 7-Tesla. We have secured funding and recently received positive ethical approval to undertake a prospective observational study of 20 patients with CADASIL. The purpose of this is to compare the phase-contrast flow velocity in the lenticulostriate vessels with the cerebral blood flow as measured by arterial spin-labelling at 3-Tesla and compare these with conventional small vessel disease imaging features measured at 7-Tesla. Recruitment is due to start in March 2024.

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