Full/long title of study	The dynamics and clinical relevance of grey matter and periventricular white matter pathology in multiple sclerosis
Short title	GML03
Version and date of protocol	Version 2.0 10/05/2022

Sponsor:	University College London (UCL)
Sponsor reference number:	147293
Funder (s):	Medical Research Council MR/W019906/1
IRAS Number:	310565
UCL Data Protection Number:	Z6364106/2022/02/36

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PROTOCOL VERSION HISTORY

Version Stage	Versions Number	Version Date	Protocol updated & finalised by;	Reasons for Update
Current	2.0	10.05.2022	Dr Nitin Sahi & Dr Declan Chard	Revisions following HRA REC review: 1. Key study milestones updated 2. Updated section 5: study schedule 3. Updated section 8: Consent 4. Updated section 14: Risk, including addition of distress policy 5. Updated section 15.2: Incidental findings 6. Updated abbreviations list
Previous	1.0	18.03.2022	Dr Nitin Sahi & Dr Declan Chard	

DECLARATIONS

The undersigned confirm that the following protocol has been agreed and accepted and that the investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the U.K. Policy Framework for Health and Social Care Research 2017 (3rd edition) (as amended thereafter), the EU General Data Protection Regulation (2016/679) and the UK Data Protection Act (2018), Sponsor SOPs and applicable Trust policies and legal frameworks.

I (investigator) agree to ensure that the confidential information contained in this document will not be used for any other purposes other than the evaluation or conduct of the research investigation without the prior written consent of the Sponsor.

I (investigator) agree to ensure that no research activity or recruitment will commence at participating research sites until the appropriate regulatory approvals and NHS confirmations of Capacity and Capability have been issued, and Sponsor green light confirmed.

I (investigator) also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest, accurate and transparent account of the study will be given. Any deviations from the study as planned in this protocol will be explained and reported accordingly.

Chief Investigator:

Signature: ..... Date...../...../.....

Print Name (in full): .....

Position: .....

On behalf of the Study Sponsor:

Signature: ..... Date...../...../.....

Print Name (in full): .....

Position: .....

STUDY SUMMARY

IDENTIFIERS	
IRAS Number	310565
REC Reference No.	To follow
Sponsor Reference No.	147293
Other research reference number(s) (if applicable)	UCL Data Protection number - Z6364106/2022/02/36
Full (Scientific) title	The dynamics and clinical relevance of grey matter and periventricular white matter pathology in multiple sclerosis
Health condition(s) or problem(s) studied	Multiple sclerosis
Study Type i.e. Cohort etc.	Cohort
Target sample size	140
STUDY TIMELINES	
Study Duration/length	3 years
Expected Start Date	01/03/2022
End of Study definition and anticipated date	Scanning of the last participant 31/02/2025
Key Study milestones	Approvals to be finalised – 01/06/2022 First participant contact – 01/06/2022 First participant scanning – 1/07/2022 Participant contact completed – 01/08/2022 Scanning expected to be completed – 31/01/2025
FUNDING & OTHER	
Funding	Medical Research Council
Other support	Internal support provided within UCL Queen Square Institute of Neurology
STORAGE of SAMPLES / DATA (if applicable)	
Human tissue samples	N/A
Data collected / Storage	N/A
KEY STUDY CONTACTS	
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Funder(s)	Medical Research Council (UK)
Committees	N/A
Sub-contractors	N/A
Other relevant study personnel	Dr Ferran Prados Carrasco, Dr Arman Eshaghi, Dr Rebecca Samson, Dr Carmen Tur, Dr Nitin Sahi

KEY ROLES AND RESPONSIBILITIES

SPONSOR: The sponsor is responsible for ensuring before a study begins that arrangements are in place for the research team to access resources and support to deliver the research as proposed and allocate responsibilities for the management, monitoring and reporting of the research. The Sponsor also must be satisfied there is agreement on appropriate arrangements to record, report and review significant developments as the research proceeds, and approve any modifications to the design.

FUNDER: The funder is the entity that will provide the funds (financial support) for the conduction of the study. Funders are expected to provide assistance to any enquiry, audit or investigation related to the funded work.

CHIEF INVESTIGATOR (CI): The person who takes overall responsibility for the design, conduct and reporting of a study. If the study involves researchers at more than once site, the CI takes on the primary responsibility whether he/she is an investigator at any particular site.

The CI role is to complete and to ensure that all relevant regulatory approvals and confirmations of NHS Capacity and Capability are in place before the study begins. Ensure arrangements are in place for good study conduct, robust monitoring and reporting, including prompt reporting of incidents, this includes putting in place adequate training for study staff to conduct the study as per the protocol and relevant standards.

The Chief Investigator is responsible for submission of annual reports as required. The Chief Investigator will notify the REC and JRO of the end of the study (including the reasons for premature termination, where applicable). Within one year after the end of study, the Chief Investigator will submit a final report with the results, including any publications/abstracts to the REC and JRO.

PRINCIPLE INVESTIGATOR (PI): Individually or as leader of the researchers at a site; ensuring that the study is conducted as per the approved study protocol, and report/notify the relevant parties – this includes the CI of any breaches or incidents related to the study.

KEY WORDS

Grey matter, periventricular white matter, cortical atrophy, neurodegeneration, disease progression, multiple sclerosis

LIST OF ABBREVIATIONS

ASL – arterial spin labelling
BICAMS – brief international cognitive assessment test for multiple sclerosis
BVMT – brief visuospatial memory test
CIS – clinically isolated syndrome
CSF – cerebrospinal fluid
CVLT – California verbal learning test
DIR – double inversion recovery
DWI – diffusion weighted imaging
EDSS – expanded disability status scale
FLAIR – fluid attenuated inversion recovery
GM – grey matter
GP – general practitioner
HCP – human connectome project
MRI – magnetic resonance imaging
MS – multiple sclerosis
MSFC – multiple sclerosis functional composite
MTR – magnetisation transfer ratio
MTV – macromolecular tissue volume
PP – primary progressive
PSIR – phase sensitive inversion recovery
QSM – quantitative susceptibility mapping
REC – research ethics committee
RR – relapsing remitting
SDMT – symbol digit modalities test
SP – secondary progressive
WM – white matter

CONTENTS

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1 INTRODUCTION

About 130,000 people in the UK have multiple sclerosis (MS) and, other than head injuries, it is the commonest cause of neurological disability in young adults. MS is highly variable and unpredictable: some people with MS develop few neurological problems over decades, while others develop significant progressive disability. Overall, it is thought that the loss of nerve cells determines irreversible disability in MS, but we do not know what the main cause of nerve cell damage is. On magnetic resonance imaging (MRI) scans the most obvious sign of MS is the presence of lesions in brain white matter, and nearly all people with MS have an MRI scan looking for these lesions to help establish a diagnosis. However, these lesions appear to explain only a small amount of overall disability and nerve damage due to MS.

About a decade ago we undertook a research study investigating grey matter (the part of the brain which contains nerve cells) abnormalities in MS. The study provided much needed insights into previously overlooked aspects of MS, and in particular highlighted that grey matter changes in some people with MS could be substantial and associated with neurological function, memory and thinking. Practically, it has led to at least one new MRI method being used in trials of treatments for MS. Building on our previous study, we now aim to see if any of the MRI scan measures we previously looked at can predict how people with MS are now, and how these MRI features have changed over a decade. We hope that this work will help us to identify targets for treatment long before they have had a chance to cause disability.

The original research study was funded by a 3-year project grant from MS Society of Great Britain and Northern Ireland. (Ref: 917/09) Grey matter abnormalities on 3 Tesla MRI and their functional effects in multiple sclerosis. Ethical approval was obtained by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Research Ethics Committee (REC): 09/H0716/59 and REC: 09/H0716/77.

2 BACKGROUND AND RATIONALE

Most people with MS initially run a relapsing-remitting (RR) MS course but, after a decade or more, most develop progressive neurological disability (secondary progressive (SP) MS). About 15% of people with MS present with progression from onset (primary progressive (PP) MS). Most disability in MS occurs due to progression without relapses [1]. We now have multiple highly effective treatments that reduce the risk of relapses, but only two treatments have been approved in the UK for progressive MS, and only when there is evidence of ongoing relapses or white matter (WM) lesion activity. ***Ultimately neurodegeneration independent of WM lesions is thought to be the principal cause of irreversible disability in MS [1]. We do not know what the main cause of neurodegeneration is in MS.***

MS has long been thought of as an essentially inflammatory demyelinating WM disease, characterised by WM lesions seen both histopathologically and *in vivo* on MRI. However, ***WM lesion numbers and volumes explain less than half the disability people with MS develop*** [2], and in the longer-term brain atrophy is more closely linked with clinical outcomes. MS brain atrophy is mainly attributable to GM volume loss [3], which in turn is due to GM neurodegeneration (synaptic, axonal and neuronal loss all contributing) [4]. While cortical demyelination in extensive in MS, and particularly so in progressive MS (~26.5% of cortical GM compared with ~6.5% of WM by area [5]), ***neuronal loss is not confined to GM lesions***, with similar reductions seen in extra-lesional cortex [6]. *In vivo* studies have also shown cortical atrophy and lesions only partly overlap [7]. If GM lesions are not the main driver of GM neurodegeneration, then what is? Do we need to consider multiple contributing factors?

WM pathology could lead to GM neurodegeneration through tract-mediated processes. In our previous *in vivo* work using magnetisation transfer ratio (MTR) mapping and diffusion weighted imaging (DWI), we found in PPMS that WM lesion formation within a tract was associated with subsequent abnormalities within the rest of the tract, and that abnormalities within WM tracts preceded abnormalities in associated cortical GM [8]. However, with the data available we were only able to assess this over two years, in five tract-cortex pairs, and in PPMS. Further, it has recently been shown that ***some WM lesions are chronically active,*** with ongoing inflammation seen at their edges, particularly in people with progressive MS. *In vivo* a rim may be seen around chronically active lesions using iron-sensitive MRI [9]. Another potentially relevant factor is proximity to the surface of the brain, as both demyelination and neuronal loss occur more towards brain surfaces [5,6]. In *post-mortem* studies of GM, both ***demyelination and neuronal loss have been linked with meningeal inflammation, and more recently with cerebrospinal fluid (CSF) cytokines [10].*** *In vivo*, MRI has revealed links between cortical GM and periventricular WM disease (for example [11]) suggesting a common factor, such as proximity to CSF, unites them too.

Histopathological work suggests that ***GM neuronal loss may be due to a combination of tract-mediated and oxidative damage*** (particularly in demyelinated cortex), associated with microglial activation, and compounded by hypoxia in areas with low blood perfusion, and age-related iron accumulation [12]. Recent *in vivo* MRI work using source-based morphometry, a method capable of disentangling spatially overlapping components contributing to regional atrophy, also suggests that multiple processes combined may explain cortical atrophy [13]. MRI can assess perfusion using arterial spin labelling (ASL) [14] and iron deposition using quantitative susceptibility mapping (QSM) [15], with sufficient resolution to undertake regional cortical measurements. Previous work has already shown regional hypo-perfusion in the cortex, but (in 26 people with RRMS) it does not appear to overlap with regional atrophy [14]. However, atrophy in RRMS will be less substantial than in progressive MS, and so this negative result may be due to limited sensitivity. Previous work has also revealed iron deposition in deep GM, linked with clinical disease progression, modestly correlating with regional atrophy [16].

Robustly assessing GM pathology, and linking it with clinical outcomes, has proven challenging as it has been very difficult to detect using MRI. In particular, detecting GM demyelination and neurodegeneration *in vivo* has been very difficult, since both conventional, and quantitative MRI methods (e.g. MTR) were limited in terms of resolution. ***In 2010 we initiated a study to refine GM MRI techniques*** and undertake a detailed MRI and clinical assessment of a large and diverse cohort of people with MS. We recruited 148 people with MS, 9 with a CIS, and 52 healthy controls, and of that cohort we followed up 77 people approximately 18 months later (limited by funding available at the time). We implemented high resolution (0.5 x 0.5 x 2 mm) phase sensitive inversion recovery (PSIR) imaging, which compared with double inversion recovery (DIR, also developed to look for GM lesions) enabled three times as many intra-cortical GM lesions to be seen [17] and much more confident separation of GM from WM lesions [18] (nearly 40% of lesions were misclassified on DIR). Using PSIR, in a longitudinal assessment over ~6 months we found that GM lesions formed more frequently in SPMS than RRMS, independent of WM lesion accrual [19]. We also found that over time GM lesions extended into adjacent WM, but WM lesions did not appear to spread into GM [19]. ***This, in concert with other studies at the time, highlighted potentially significant unrecognised inflammatory activity in the GM.*** Beyond an association with a SPMS course, it is still unclear if GM lesion formation predicts SPMS or disability progression.

Recalling work showing a surface-in gradient in MS pathology [7], we implemented high-resolution MTR mapping (1x1x1mm) and assessed the cortical ribbon split into inner and outer bands. We showed *in vivo* a preferential disease effect on the outer cortex consistent with *post mortem* findings [20]. Histopathological studies drew mainly on tissue from people with long-standing progressive MS,

but we were able to show less marked but still detectable gradients in people with RRMS [20] and in people clinically isolated syndromes (CIS) who went on to develop MS [21]. With the follow-up data available, we were not able to determine if these gradients predicted conversion from RRMS to SPMS, or disability. Considering the possibility that a CSF-mediated factor was influencing GM pathology, we developed a method that also revealed gradients in abnormalities in WM MTR, finding greater disease effects towards the ventricles. This was independent of WM lesions [22]. We demonstrated this gradient soon after a CIS and that it predicted the development of MS independently of WM lesions [23]. Using alemtuzumab trial data, we also found that this gradient improved after treatment, and the degree of baseline abnormality predicted the likelihood of a further relapse after treatment [24]. Together this suggests that ***surface-in gradients in WM and GM abnormalities reflect clinically significant processes***. Beyond predicting treatment response following alemtuzumab, we have yet to determine if these gradients predict the longer-term risk of relapses and disability.

In addition to assessing the cortex with MTR, we acquired diffusion weighted imaging (DWI) to characterise changes in neuronal architecture. We implemented a novel analysis measure, diffusion orientation complexity, showing that this correlated more closely with cognitive outcomes than conventional DWI measures [25].

Building on our earlier work, the present study will leverage the detailed MRI of GM and WM, and neurological and cognitive data, we obtained a decade ago to assess the longer-term evolution of GM and WM pathology, their prognostic and ongoing clinical relevance

3 AIM(S) AND OBJECTIVES

Our primary research aim is to investigate the pathogenesis of neurodegeneration and to achieve this we will test four core hypotheses:

1. *Cortical GM atrophy is essentially unrelated to cortical GM lesion formation;*
2. *WM-tract mediated abnormalities lead to regional GM atrophy;*
3. *Gradients in cortical GM and periventricular WM abnormalities predict cortical atrophy;*
4. *Iron accumulation and hypo-perfusion promote cortical atrophy.*

In addition to testing these *a priori* hypotheses, we will comprehensively assess potential links between WM and GM abnormalities using multivariate models. We will use structural equation models (dynamic models) to establish the trajectory of changes in each of the MRI measures, and determine their most likely temporal relationship. We have previously used structural equation models to investigate regional progression of MS GM atrophy [26], and the likely mechanism through which simvastatin slows brain atrophy in MS [27]. These analyses have been limited by the MRI measures available, which for GM has meant only atrophy. This project will provide a much more comprehensive set of GM and WM measures (including regional, cortical and WM tract).

We also aim to assess the clinical relevance of new MRI markers of GM and WM pathology by testing the following two hypotheses:

5. *GM compared with WM MRI features better predict a transition from RR to SPMS, and better predict disability accrual.*
6. *Early gradients in cortical GM and periventricular WM abnormalities predict a transition from RR to SPMS, and disability accrual, and do so independently of GM and WM lesions.*

Each of these hypotheses has direct implications for the targeting and timing of MS treatments. ***Investigating pathways that lead to neurodegeneration, that can be observed using MRI and are***

demonstrably linked with clinical outcomes, will enable the assessment of treatment effects on the processes leading to neurodegeneration, rather than waiting for neurodegeneration to occur. The cohort we propose to follow-up is well suited for this project. It is the detail of the early MRI and clinical data, and length of follow-up, rather than the cohort size (although more than sufficient), that are major strengths. This will allow us to efficiently address multiple hypotheses simultaneously, and do so now, rather than trying to found a larger cohort, potentially compromising on the range of MRI and clinical data we obtain, and delaying research objectives by many years.

4 STUDY DESIGN & METHODS OF DATA COLLECTION

This is an observational single-site cohort study, during which we will reassess participants who enrolled in our original study 10 years ago.

Participants

Members of the original cohort of 148 people with MS (69 with RR, 48 with SP and 31 with PP MS), 9 with a CIS, and 52 healthy controls will be contacted and invited to take part in this follow-up study provided they have given permission to be contacted about further research. They will be traced based on contact details we already hold, and where these are out of date or unavailable via UCLH records, the NHS Tracing Service or publicly available resources. If a member of the cohort has died, to determine if MS was a contributing factor, a copy of their death certificate will be sought.

Those members of the cohort we are able to contact will be able to take part in this study in two ways, either at the UCL NMR Research Unit's scanning facility, by attending for a clinical assessment and MRI scan, or by telephone interview.

Sample Size Determination

This is based upon:

1) Our previous study on the same cohort [19], where we were able to find significant differences between RRMS (N=27) and SPMS (N=22) participants in terms of cortical lesion number (p=0.043), and we expect these differences to increase over time.

2) A sample size calculation based on our 30-year CIS studies [28-30], indicated that, to find differences between established RRMS and SPMS cortical lesion counts and GM atrophy we would need group sample sizes between 20 and 30.

3) The minimum sample size required to be able to capture (significant) moderate-strong correlations, i.e. rho=0.40 or higher, considered as clinically relevant, is 50 people.

4) Finally, under the reasonable assumption that at least a third of all patients may have progressed clinically since the original study baseline until now [28, 31] our sample size of ~70-75 MS patients will allow us to capture significant and clinically relevant associations, i.e. odds ratios ≥2.0 [28,31], between baseline MRI metrics an the risk of clinical progression.

During a feasibility evaluation, 50 members of the original cohort were contacted by letter and were each sent a draft patient information sheet. Within ~8 weeks 32 (64%) said they would be willing to take part, and a further 4 (8%) would provide clinical information without an MRI. Based upon this and our recent experience with a 30-year follow-up of people with a CIS and MS [28], in which ~70%

were reassessed, we aim for 140 of the cohort to be reassessed in this 10-year follow-up study. This will allow us to capture more subtle, but potentially still clinically relevant, associations between MRI-visible pathological processes.

Data Collection

Participants will be asked to attend the NMR Research Unit at the UCL Queen Square Institute of Neurology. They will then complete an MRI safety checklist on two separate occasions. The first will be undertaken by clinical members of the study team during screening. The second will be undertaken by radiographers from the NMR Research Unit immediately prior to MRI.

If a consent form has not already been signed and returned to the study team, written consent will be obtained by clinical members of the study team at the start of the study visit.

Once consent has been given, the clinical and cognitive assessments will then be undertaken, and will take around 45-50 minutes. This will be followed by the MRI scan, which will require approximately 90 minutes of scanning time with comfort breaks for participants as necessary.

Clinical and cognitive assessment

Participants with MS will be assessed to determine their MS phenotype, EDSS and MS functional composite scores, and have a cognitive assessment including all elements of BICAMS [31]. These clinical and cognitive assessments will be undertaken by the clinical research fellow, Dr Nitin Sahi or other clinical members of the study team. The BICAMS battery includes the Symbol Digit Modalities Test (SDMT) for information processing speed), the California Verbal Learning Test (CVLT) for verbal memory immediate recall and the Brief Visuospatial Memory Test (BVMT) for visual memory immediate recall. The BICAMS battery takes about 15 minutes, does not require neuropsychological expertise and covers the main cognitive domains affected by MS.

Telephone Assessment

Participants consenting for telephone consultation alone will be assessed using a telephone expanded disability status scale (EDSS) consultation. [33]

MRI

Scanning will be undertaken using a 3T Philips Ingenia CX scanner (Best, The Netherlands) and a 32-channel receive coil at the NMR Research Unit at the UCL Queen Square Institute of Neurology. We aim to obtain scans that are comparable with earlier imaging (from our 3T Philips Achieva scanner which has undergone a major hardware and software upgrade), using the higher signal-to-noise ratio of the current system to shorten scan times, increase resolution, and obtain additional measures. The scan protocol will include:

1. 3D sagittal T2-weighted and 3D fluid attenuated inversion recovery (FLAIR) images (both 1x1x1mm^3) for automatic WM lesion identification;
2. PSIR (~0.5x0.5x2 mm^3) for GM lesion identification;
3. 3D T1 sagittal volumetric scans (1x1x1 mm^3) for GM and WM tissue segmentation, and to measure brain atrophy;
4. 3D MTR sagittal (1x1x1mm^3) to assess intra-cortical and periventricular tissue demyelination and axonal loss;
5. Multi-shell axial DWI (2x2x2mm^3), providing a number of independent (of MTR) measures of GM and WM tissue microstructure;

6. 3D MTV sagittal (1x1x1mm^3) simultaneously allowing QSM [15] to assess iron deposition in GM and WM, and to detect 'rim-positive' WM lesions [9], while providing an additional (to MTR) measure of myelin;
7. Arterial spin labelling (ASL) axial (~3x3x3mm^3) to assess perfusion in GM and WM.

MRI scanning will take about 1.5 hours, allowing for participant transfer and comfort breaks. This was previously well tolerated by the people in this cohort, including those with more disabling MS. Participant comfort has been carefully considered, and we typically allow for at least one scheduled break, and more if needed, during MRI scanning protocols of this length. We expect that scanning will take place on the same day as the clinical assessment but will accept clinical assessments within two weeks of the scanning session, unless new neurological symptoms occur in the interim.

5 STUDY SCHEDULE

The planned commencement date for the study is 1st June 2022 following full sponsorship and ethic approvals being obtained.

Once the study has commenced invitation letters will be sent out to previous participants in the study. It is anticipated that all previous participants eligible to be contacted will receive correspondence by the end of June 2022. Approximately two to three weeks after postal date, participants will be contacted by telephone to confirm receipt and interest in the study as outlined above.

Study visits comprising clinical and radiological assessments are expected to commence from July-August 2022 and may continue until the study end date. Telephone consultations will run concurrently. Following acquisition of MRI scans, analyses involving lesion marking and tissue segmentation will commence.

The study end date will be defined as the last participant visit, and is anticipated to be no later than 31st January 2025, but it is expected that data collection will be completed before this date, and the REC and JRO will be informed when this occurs. Data analysis will continue beyond this date, but it is expected that the final report to the REC will be submitted within 12 months of the study end date.

6 ELIGIBILITY CRITERIA

6.1 Inclusion Criteria

- All participants must have been included in the original research study (Ref: 917/09).

Participants in the original study were:

- Aged 18-65 years (at time of original study)
- Able to undertake written informed consent in English
- Had a diagnosis of a CIS or clinically definite MS, or were healthy controls with no known neurological disease

6.2 Exclusion Criteria

- Involvement in other research studies or medical intervention which might have contraindications for this study
- Neurological disease other than CIS and MS that impedes study interpretation

Contraindication to MRI scanning is not an exclusion criteria for the study, however participants must meet safety criteria to take part in this element of the study. Participants will be asked to complete the NMR Research Unit's MRI safety checklist to determine whether or not it is safe to proceed with MRI scanning.

7 RECRUITMENT

No new recruitment will be required for this study, as only people who took part in the earlier study will be invited to participate. Where participants cannot be traced due to change in contact details, NHS or GP records may be used to update contact information. A screening log will be maintained of all participants including reasons for ineligibility and/or non-participation of eligible participants. Reasonable travel expenses will be paid for participants (typically not exceeding £50 per participant) attending the research site for clinical and MRI assessments.

8 CONSENT

Written informed consent will be obtained from all participants attending for clinical and MRI assessment on the research site immediately prior to their participation. This will involve:

- The presentation of written materials (participant information sheet and consent documents) as approved by the REC.

- Discussion between the potential participant and a clinical member of the study team knowledgeable about the research, the nature and objectives of the study and possible risks associated with participation.

- Consent will be sought by a clinical member of the study team, who is experienced in undertaking capacity to consent assessments if required.

For study participants undertaking telephone EDSS assessment alone consent will be sought either by asking them to return a signed paper consent form in a prepaid stamp addressed envelope, or alternatively by providing consent online using an inhouse digital consenting process. The online consent form would be materially the same as the paper consent form but allowing digital signatures to be recorded.

9 DATA ANALYSIS

MRI Analysis

New GM lesions will be identified on PSIR scans. GM lesions have already been marked and quality assured at baseline. We will use a semi-automatic contouring technique to segment GM lesions [34] and generate lesion probability maps [35]. Both methods have already been developed and applied in this cohort. For WM lesion accrual, automated difference imaging has proven sensitive to subtle changes [36], and we will apply this to the T2-weighted and FLAIR scans. Rim-positive lesions will be identified using the QSM images. Brain tissue will be segmented into GM and WM, and parcellated into cortical regions, using GIF [37] or an equivalent segmentation method. This will be used to measure atrophy and extract masks for regional measures. WM tracts will be masked using the DWI scans, registered to a recently developed WM tract atlas based on Human Connectome Project (HCP) data [38]. MTR, multi-shell DWI (enabling neurite orientation dispersion and density imaging analysis,

which has been developed and applied in MS since our original study [39]), MTV and QSM measures will be assessed in the whole brain, in cortical regions, in WM and GM based on distance from the surface of the brain, and in WM tracts.

Baseline MRI data for this cohort was obtained using a Philips Achieva, now upgraded to Philips Ingenia CX system. We had on-going longitudinal studies, including drug trials, at the time of the scanner upgrade and so planned for this issue. A cohort of healthy controls underwent scanning before and after the scanner upgrade, and we continued our routine quality assurance scanning throughout. Further, as the upgrade will affect all participants, even a measurable step change will not prevent us from assessing differences between groups or associations with clinical outcomes, or applying longitudinal dynamic models (for example [40]).

Pathogenic hypothesis testing

1. Cortical GM atrophy is essentially unrelated to GM lesion formation;

We will look for spatial correlations between whole brain and regional cortical GM lesion accrual and volume loss. We will test this using source-based morphometry to look for regional cortical atrophy [13] and cortical lesion probability mapping [41], and their co-localisation. With longitudinal data on cortical atrophy and cortical lesions, we will be able to build longitudinal mixed-effects models, making use of all available data, to assess the temporal trajectories of these two pathological features and whether they influence each other. This will provide complementary information to the co-localisation analyses described above.

2. WM-tract mediated abnormalities lead to regional GM atrophy;

Using a modified version of our tract-cortex methodology [8], we will determine if earlier changes in WM tracts precede atrophy in connected GM. While GM atrophy reflects MS-associated neurodegeneration [4], prior to atrophy, such neurodegeneration may also be seen as reductions in MTR and changes in DWI measures, therefore we will also assess associations of WM tract abnormalities with these cortical measures too.

3. Gradients in cortical GM and periventricular WM abnormalities predict future cortical atrophy;

After optimising for longitudinal measurements the methods we have already developed to assess gradients in cortical GM and periventricular WM abnormalities, we will determine how closely changes in GM and periventricular WM each correlate, and so how plausible it is that they are driven by the same underlying process. We will then determine their association with cortical atrophy at a whole brain level and, recognising the multiple factors that may influence atrophy in the same cortical region, with the source-based morphometry identified covarying regions of atrophy.

4. Iron deposition and hypo-perfusion promote cortical atrophy.

Whilst we do not have baseline data to assess iron accumulation and hypo-perfusion, we will test the plausibility of this based on whether or not regional atrophy measured longitudinally co-localises with QSM markers of iron and ASL measures of cortical perfusion. Within source-based morphometry identified regions of cortical atrophy we will assess iron deposition and hypo-perfusion.

Multivariate modelling of MRI-derived GM and WM features

In addition to testing specific hypotheses, recalling that multiple factors may overlap resulting in the ultimate loss of neurons, we will also assess associations across all of the GM and WM measures we obtain. To avoid large numbers of comparisons being undertaken, reducing that risk of spurious associations being found or overzealous adjustments for multiple comparisons obscuring pathologically significant links, we will use structural equation models to establish the dynamic relationship of changes in each of the MRI measures and determine their most likely temporal relationship [27]. This will produce one or more (causal) models that can plausibly explain the dynamic changes, distribution, and connections between MRI markers of pathology. In contrast to earlier studies using structural equation models, which have been limited to measures of WM lesions and brain atrophy, we will include WM tract specific, regional cortical and deep GM measures sensitive to focal inflammatory demyelination, more widespread demyelination and neuroaxonal disruption (MTR, MTV and DWI), iron deposition (QSM) and hypo-perfusion (ASL). As such, we will be able to produce much more comprehensive, and testable, models of possible pathogenic mechanisms.

Although structural equation models will be the primary multivariate modelling approach, other multivariate models will be considered using a model comparison to capture a broad range of mechanistic theories and to identify the best performing and most realistic models.

Clinical hypothesis testing

5. GM compared with WM MRI features better predict a transition from RR to SPMS, and better predict disability accrual (assessed using the EDSS, MSFC, and cognitive measures);

6. Early gradients in cortical GM and periventricular WM abnormalities predict a transition from RR to SPMS, and disability accrual, and do so independently of GM and WM lesions.

We will undertake a survival analysis, based on time to development of SPMS. We will also assess correlations between gradients in cortical GM and periventricular WM abnormalities, at baseline and changes over time, with measures of disability progression (both neurological and cognitive).

Main statistical approaches to test the study hypotheses

1. *Assessing regional atrophy:* Source based morphometry will be used to disentangle spatially overlapping components contributing to regional atrophy. To obtain comparisons between groups adjusted for confounders (e.g. age and sex), the loadings will initially be regressed on these confounders and the residuals of such regressions compared between groups through independent sample t-tests.

2. *Assessing co-localisation between spatially independent components of GM atrophy and cortical lesions:* We will assess atrophy and lesions in parcellated GM, and correlations between them.

3. *Assessing longitudinal changes and gradients:* Linear mixed-effects models will be used to assess changes in cortical atrophy, cortical lesions, cortical and periventricular WM abnormalities over time, and to investigate their relationships.

4. *Causal models of dynamic changes:* Multiple linear regression (i.e. univariate, multivariable models) and structural equation modelling (i.e. multivariate, multivariable models) will be used to determine if earlier changes in WM tracts precede atrophy in connected GM.

5. *Prediction of clinical outcomes*: Both multiple linear regression and logistic regression models will be used to test the clinical hypotheses, i.e. to predict either disability scores, or binary clinical outcomes (such as a disability threshold) over time.

Statistical analyses to address potential biases and other potential sources of error

1. *Sensitivity analyses to assess the risk of selection bias:* We will assess whether participants followed up in this study are comparable (based on their baseline clinical and MRI features) with the full cohort originally recruited.

2. *Investigating and tackling missing data:* We will apply MRI methods that have already proven sensitive to cross-sectional differences in the present cohort and determine longitudinal changes 10 years later. Based on cross-sectional differences already seen in subsets of this cohort, and typical rates of atrophy and clinical progression, we will be able to address all our main questions if we reassess ≥50% of the original cohort [19, 24, 28, 42 and 43]. There will be missing data, and we will use longitudinal mixed-effects linear regression models account for data missing at random, making use of all the available data, even if only present for some time points. Sensitivity analyses will be undertaken to look for any bias in the follow-up cohort, and if found we will consider multiple imputation techniques.

3. *Potential role of clinical and MRI variability in our results:* Participant variability, both at baseline and over time, powers rather than hinders our analyses. We need participants to be different at baseline (and we have already detected significant phenotypic differences in the MRI measures at baseline) and for enough time to have elapsed for clinical and MRI outcomes to diverge (for example in our 30-year MS and clinically isolated syndrome study clear differences in EDSS progression were seen over a decade [28]). With regard to MS treatments, at baseline only 26% of the relapsing-remitting group, and 12% of the secondary progressive and 3% of the primary progressive groups were on treatment, and it is unlikely that this will have increased substantially over a decade due to NHS prescription criteria. However, we will also look for treatment effects in our statistical models.

10 PATIENT AND PUBLIC INVOLVEMENT (PPI)

Previous study participants have been actively involved in evaluating the recruitment feasibility of our study. They were also asked to provide feedback on our research proposals, the information provided and the relevance of our study to people with MS. The patient information sheet has subsequently been edited to answer queries raised in the feedback received.

We hope to involve patients and the public in the dissemination of our research findings. We will host an end of study meeting (in person or virtual, dependent on circumstances at the time) to which all participants will be invited. At this meeting the research team will present the findings, answer questions, and seek participants views on how they think the results of our work could benefit their clinical care, and how they think we should pursue this. Our team also regularly contributes to events run by the MS Society, which provide further opportunities to share our work with people affected by MS. Rebecca Samson, one of the co-applicants, is also co-leading a new patient and public involvement programme at our centre, which will include regular events (both held at Queen Square and online). This is being developed with advice and support from the UK MS Society, and specifically the Society's Research Network of people affected by MS.

11 FUNDING AND SUPPLY OF EQUIPMENT

The study funding has been reviewed by the UCLH/UCL Joint Research Office, and deemed sufficient to cover the requirements of the study. NHS costs will be supported via UCLH and/or the Local Clinical Research Network.

The research costs for the study have been supported by an MRC (UK) project grant award of £956,080 dated 30th November 2021. The grant award includes provision of two new research workstations and a £5000 contribution to NMR Research Unit's data storage and computing environment. This offers a secure, fully backed-up service for the collection and analysis of neuroimaging data supported by a dedicated team with experience in MS research. All other equipment necessary for this project is available internally in the NMR Research Unit at the UCL Queen Square Institute of Neurology.

12 DATA HANDLING AND MANAGEMENT

The study is compliant with the requirements of General Data Protection Regulation (2016/679) and the UK Data Protection Act (2018). All investigators and study site staff will comply with the requirements of the General Data Protection Regulation (2016/679) with regard to the collection, storage, processing and disclosure of personal information, and will uphold the Act's core principles.

UCL is the data controller; the UCL Data Protection Officer is data-protection@ucl.ac.uk.

The data processors are the study team who are appointed to this project.

Data

The study will be collecting the following personal data:

- Clinical assessments, including neurological and cognitive test scores.
- Magnetic resonance imaging scans

Clinical data will be generated by history taking and examination by a clinically qualified person. Magnetic resonance imaging date will be obtained using a clinically approved scanner that is operated in accordance with current clinical safety standards by suitably qualified radiographers.

Clinical data will be obtained by a single clinician, trained to administer the neurological and cognitive tests. Magnetic resonance imaging data will be collected using a scanner that is regularly serviced by the manufacturer to ensure that it performs to its design specifications. The scanner is further monitored through a regular programme of phantom scanning.

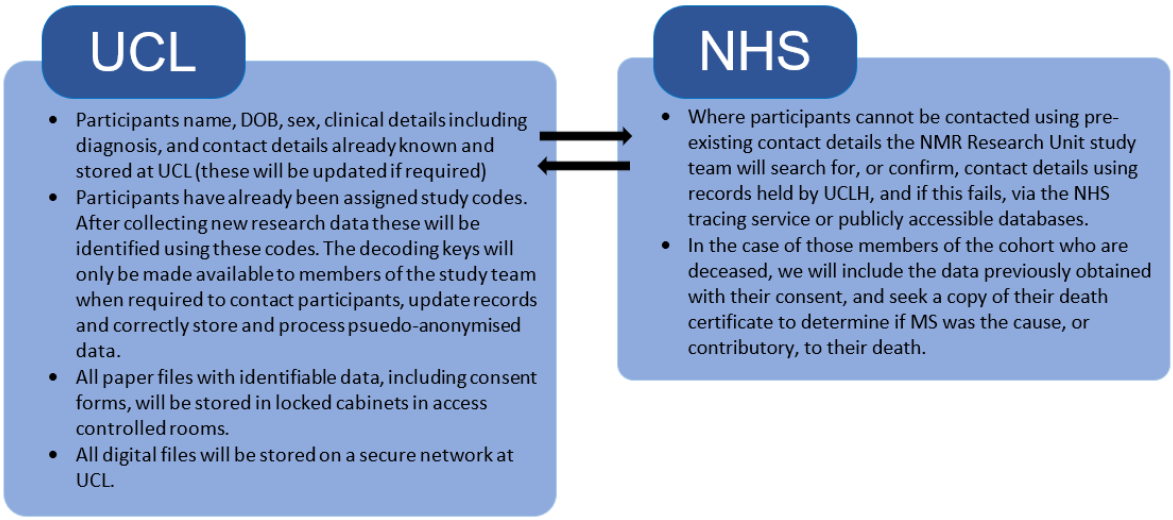
Storage

Data at the UCL Queen Square MS Centre is held using RAID10 storage, backup disk storage and tape archiving, to maximise availability and minimise the risk of loss.

Digital data are stored on servers that a physically secured. The data itself can only be accessed through a password protected network employing security measures to prevent external intrusion. Paper records are stored in locked cabinets, in access-controlled rooms.

Data will be stored for at least 10 years.

Data Flow Diagram



Data sharing

All processing for this project will be undertaken at UCL. However, the data we collect may be suitable for other studies. We will seek participants consent for sharing, including depositing fully anonymised MRI data on an open access platform such as https://openneuro.org/ or similar. Full anonymization of MRI scans requires some processing, for example this may include the removal of facial features from some scans which can be achieved using tools already freely available as part FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL), and we aim to incorporate such procedures into our processing pipelines where appropriate.

Where needed, data sharing will be authorised by the Principal Investigator (or their successor) for this study. Data sharing agreements are subject to review by UCL Business (https://www.uclb.com/for-researchers/material-transfer-agreements/). Data sharing is subject to the UCL Information Security Policy (https://www.ucl.ac.uk/information-security/informationsecurity-policy).

13 PEER AND REGULATORY REVIEW

The study has been peer reviewed in accordance with the requirements outlined by UCL

- The Sponsor considers the procedure for obtaining funding from the Medical Research Council (UK) to be of sufficient rigour and independence to be considered an adequate peer review.

The study was deemed to require regulatory approval from the following bodies: NHS REC Favourable Opinion and HRA Approval. **Before any site can enrol patients into the study,** the Chief Investigator/Principal Investigator or designee will ensure that the appropriate regulatory approvals have been issued, and NHS Confirmations of Capacity and Capability and Sponsor green lights are in place.

For any amendments to the study, the Chief Investigator or designee, in agreement with the Sponsor, will submit information to the appropriate body in order for them to issue approval for the amendment. The Chief Investigator or designee will work with sites (R&D departments as well as the study delivery team) to confirm ongoing Capacity and Capability for the study.

All correspondence with the Sponsor, REC and HRA will be retained. The Chief Investigator will notify the Sponsor and REC of the end of the study.

It is the Chief Investigator's responsibility to produce the annual progress reports when required; an annual progress report (APR) will be submitted to the Sponsor and REC within 30 days of the anniversary date on which the favourable opinion was issued, and annually until the study is declared ended.

If the study is ended prematurely, the Chief Investigator will notify the Sponsor and REC, including the reasons for the premature termination.

Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the Sponsor and to the REC and HRA.

14 ASSESSMENT AND MANAGEMENT OF RISK

This cohort has been involved in previous research studies and undergone MRI scans in the past. MRI is a safe technique, provided that participants with cardiac pacemakers, metal implants and other contraindications to MRI are excluded from scanning. Participants must meet safety criteria to take part in this element of the study and will be asked to complete the NMR Research Unit's MRI safety checklist on two separate occasions to determine whether or not it is safe to proceed with MRI scanning. Occasionally people undergoing MRI can experience tingling or twitching in the body or limbs. This is harmless but if it causes distress the participant can request that the scanning session be stopped.

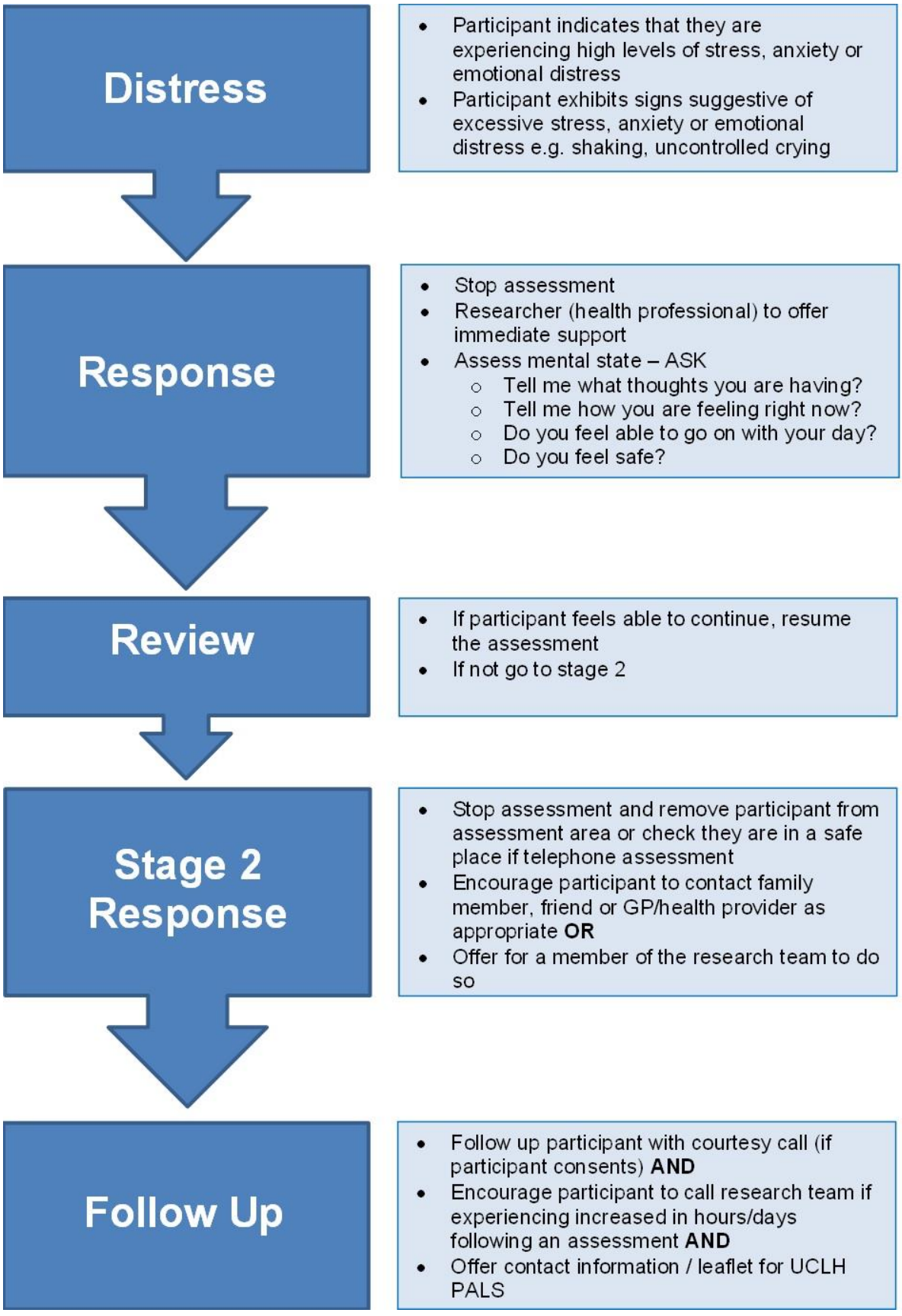
In addition to incidental findings, which are discussed in section 15.2, we will ask participants if they would like to know the findings of the MRI scans and clinical assessments where this may be relevant to their MS treatment. If a participant with MS has had new symptoms since they were last seen, or if the study assessments, including the MRI scan and cognitive evaluation, identify areas of concern, with the participant's approval, we will offer to share these results with their neurologist or clinical team managing their MS care.

Some of the participants who had a diagnosis of CIS when they first took part in this study may not have had further clinical review since then, and it is possible they may now fulfil criteria for a diagnosis of MS based either on clinical symptoms or MRI findings. It is routine in clinical practice, and was at the time this group of people were first recruited, to make people aware of this. However, we will remind any participants with a clinically isolated syndrome of this potential risk prior to them taking part in the study.

If findings from MRI scans or clinical assessments may be relevant to a participants' MS treatment, and they are not currently under the care of a neurology team, they will be offered the option of a clinic appointment with a neurologist at the National Hospital for Neurology and Neurosurgery. We would liaise with the person's GP and seek their approval before making any referral.

We recognise that taking part in MRI and clinical assessments, particularly where there may be unexpected findings, has the potential to cause distress for participants and we have therefore developed the following distress policy for this study.

Distress Policy



Adapted for Haigh and Witham (2015)[45]

15 RECORDING AND REPORTING OF EVENTS AND INCIDENTS

All events and incidents (and near misses) that occur to participants and/ or staff that are **unexpected** and directly **related** to the research study will be reported to the Sponsor via (UCL: research-incidents@ucl.ac.uk or UCL REDCAP incident reporting form) and host sites via their Trust reporting systems, and documented in the Trial Master File/Investigator Site File via study-specific incident logs (and related correspondence). This will be completed by the CI or PI. The Sponsor will be responsible for investigating, reviewing, or escalating to a serious breach if required.

15.1 Personal Data Breaches

- UCL sponsored studies: Personal data breaches will be immediately reported to the UCL Information Security Group (ISG) and the UCL Data Protection Officer [data-protection@ucl.ac.uk], (as per form and guidance: https://www.ucl.ac.uk/legal-services/guidance/reporting-loss-personal-data), and to the Sponsor via the UCL REDCAP incident reporting form (https://redcap.slms.ucl.ac.uk/surveys/?s=NE5dypTdFo). The following information will be provided: full details as to the nature of the breach, an indication as to the volume of material involved, and the sensitivity of the breach (and any timeframes that apply). Sites will additionally follow their Trust incident reporting mechanisms and will document this within their TMF/ISFs.

15.2 Incidental Findings in Research

Incidental findings may be identified by the study team. Participants will be advised about this possibility during the consenting procedure and in order for them to participate in this study we will require their consent to disclose findings to them and their general practitioner (GP) should an unexpected but potentially clinically significant finding be made. This is detailed in both the Patient Information Sheet and Consent Form.

The MRI scans performed in this study are not for diagnostic or clinical purposes. They will be reviewed by the research team but will not routinely be reviewed by a neuro-radiologist. If an incidental finding is not reported, it does not imply that no abnormality exists, but simply that no such abnormality was identified by the staff acquiring the scans.

If an incidental finding is made, we will ask that the MRI scan be reviewed by an experienced neuro-radiologist at UCLH, who together with clinical members of the research team will provide an expert opinion on the importance of the finding to the participant's health. Incidental findings considered to be normal variants or unlikely ever to cause symptoms or affect a participant's health will not be disclosed. Where it is judged that an incidental finding may significantly impact on the current or future health of a participant, this information will be disclosed to them and they will be appropriately counselled by the clinical members of the team. Subsequently their GP will be informed and the participant may be referred to a relevant medical specialist.

15.3 Protocol deviations and notification of protocol violations

Protocol deviations are usually an unintended departure from the expected conduct of the study protocol/SOPs, which does not need to be reported to the Sponsor. The CI will monitor protocol deviations, and if found to frequently recur, will discuss in the first instance with the Sponsor to determine re-classification and reporting requirements.

A protocol violation is a breach which is likely to effect to a significant degree: –
(a) the safety or physical or mental integrity of the participants of the study; or
(b) the scientific value of the study

The CI and Sponsor will be notified immediately of any case where the above definition applies via [UCL: research-incidents@ucl.ac.uk or UCL REDCAP incident reporting form].

15.4 NHS Serious Incidents and near misses

A serious incident or near miss is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a. It is an accident or other incident which results in injury or ill health.
b. It is contrary to specified or expected standard of patient care or service.
c. It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
d. It puts the Trust in an adverse position with potential loss of reputation.
e. It puts Trust property or assets in an adverse position or at risk.

Serious Incidents and near misses will be reported to the Sponsor and Trust Quality & Safety department as soon as the study team becomes aware of them.

15.5 Complaints from research participants

In the first instance, research participant complaints (patients or healthy volunteers) will be reported to the CI/PI to investigate, as documented in the patient information sheet(s), and to the Sponsor via research-incidents@ucl.ac.uk, following the *UCL Complaints from Research Subjects about UCL Sponsored Studies and Trials* policy; for participants who are NHS patients, complaints will be reported to the NHS Complaints Manager at the Trust where the recruitment and study procedures was undertaken. Complaints from NHS patients are handled under NHS complaints policies and procedures, with involvement from PALS and the Sponsor where necessary.

16 MONITORING AND AUDITING

The Chief Investigator will ensure there are adequate quality and number of monitoring activities conducted by the study team. This will include adherence to the protocol, procedures for consenting and ensure adequate data quality.

The Chief Investigator will inform the Sponsor should he/she have concerns which have arisen from monitoring activities, and/or if there are problems with oversight/monitoring procedures.

17 TRAINING

The Chief Investigator will review and provide assurances of the training and experience of all staff working on this study. Appropriate training records will be maintained in the study files

18 INDEMNITY ARRANGEMENTS

University College London holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this clinical study without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should be advised to do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this clinical study shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London upon request.

Additionally, UCL does not accept liability for sites such as GP surgeries in primary care; investigators/collaborators based in these types of sites must ensure that their activity on the study is covered under their own professional indemnity.

19 ARCHIVING

UCL and each participating site recognise that there is an obligation to archive study-related documents at the end of the study (as such end is defined within this protocol). The Chief Investigator confirms that he/she will archive the study master file at UCL for the period stipulated in the protocol and in line with all relevant legal and statutory requirements. The Principal Investigator at each participating site agrees to archive his/her respective site's study documents in line with all relevant legal and statutory requirements. Study documents will be archived for a minimum of 10 years from the study end, and no longer than 20 years from the study end.

The study master file will be archived at UCL, in accordance with the UCL Retentions Schedule and Policy. It will be archived for a minimum of 5 years from the study end, and no longer than 20 years from study end.

NB: UCL do not archive student projects and therefore, the length of storage is not subject to the standard Sponsor requirements.

20 PUBLICATION AND DISSEMINATION

Our primary aim is for scientific and clinical exploitation. This work builds on a series of scientific themes developed during our first study with this cohort and will provide much needed insights into the evolution of GM abnormalities in MS, their relationship to WM disease and disability progression, and their prognostic significance. The development of new methods is not the main goal of our proposal, but we aim to further refine our image analysis methods, specifically incorporating machine learning, for use in longitudinal studies. Given that our work has been, and will be, undertaken using a 3T MRI scanner that is widely available in clinical practice, applying methods that can be

implemented using different MRI scanner manufacturers hardware, it will be relatively straightforward to translate our work into methods for clinical trials. We have a long track record of sharing our findings, collaborating with other groups to utilise the MRI acquisition and analysis methods we develop, and through the Queen Square MS Centre Trials Office, make them directly available for use in clinical trials. For example, the periventricular MTR method developed at this study's inception has been adopted as an early phase treatment trial outcome. We anticipate following the same process of replication in independent cohorts [23] validation as clinically relevant and sensitive to treatment effects [24] and then adoption in clinical trials (for example GNbAC1 [44]) for new methods we develop.

Beyond publication in peer reviewed journals, we plan to disseminate our results as widely as possible through presentation at conferences, and through our collaborations with other researchers in the UK, Europe and North America. The Principal Investigator for this study already has collaborations with research colleagues in Cambridge, Amsterdam and Montreal, and through the MAGNIMS (www.magnims.eu) group of European centres. As with our previous work, we fully anticipate sharing our results and developing further studies through these networks. We also aim to make anonymised source data freely available to other researchers.

Funding for this study has been provided by the MRC, and will be acknowledged within any publications generated as a result of this study. In concordance with the MRC project grant award, we will submit our research outputs on a regular basis to the MRC and UK Research and Innovation (UKRI) from early in our study until at least 5 years after the study ends. We will publish the results of our research in accordance with normal academic practice and MRC (UKRI) policy on Open Access (https://www.ukri.org/wp-content/uploads/2021/08/UKRI-090222-UKRIOpenAccessPolicy-4.pdf). All the outcomes (once submitted) will be made visible in the public domain through the UKRI 'Gateway to Research' (http://gtr.ukri.org/) site. Any publications and/or abstracts resulting from this study will also be emailed to the JRO.

We want to engage study participants in the future direction of this research. With this in mind, we will host an end of study meeting (in person or virtual, dependent on circumstances at the time) to which all participants will be invited. At this meeting the research team will present the findings, answer questions, and seek participants views on how they think the results of our work could benefit their clinical care, and how they think we should pursue this. Our team also regularly contributes to events run by the MS Society, which provide further opportunities to share our work with people affected by MS. Rebecca Samson, one of the co-applicants, is also co-leading a new patient and public involvement (PPI) programme at our centre, which will include regular events (both held at Queen Square and online). This is being developed with advice and support from the UK MS Society, and specifically the Society's Research Network of people affected by MS.

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22 APPENDICES

- GML03 Data Flow Diagram
- GML03 Data Protection Registration Form
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- GML03 GP Letter
- GML03 Participant Consent Form
- GML03 Participant Information Sheet
- GML03 Participant Invitation Letter
- GML03 SoECAT form