Full Title:	Leigh Syndrome Roadmap Project: A Natural History Study (UK)
Short Title:	LSRP
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Data Coordinating Centre (DCC):	The Children's Hospital of Philadelphia (CHOP)

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The Leigh Syndrome and Mitochondrial Disease patient community was instrumental to the final design of this study. The Leigh Syndrome Roadmap Project was designed by a convened panel of content experts that included mitochondrial disease professionals, research professionals, and representatives of patient advocacy groups (PAGs). The PAGs participants were all either parents of affected individuals or leaders of PAGs with direct insights into the needs of the community. They provided advice and feedback at all stages of design, including on the disease-relevant signs and symptoms, the length and scope of proposed outcome measures, and the overall perception of study burden. We extend our thanks for their valuable and ongoing contributions to research

PROTOCOL AUTHORISATION SIGNATURES

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ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
BADS	Barry Albright Dystonia Scale
BRICS	Biomedical Research Informatics Computing System
CHOP	The Children's Hospital of Philadelphia
CI	Chief Investigator
eCRF	electronic Case Report Form
GCP	Good Clinical Practice
GP	General Practitioner
GUID	Global Unique Identifier
HRA	Health Research Authority
ICF	Informed Consent Form
ISF	Investigator Site File
LSS	Leigh Syndrome Spectrum
MD-CRS	Movement-Disorder Childhood Rating Scale
MFIS	Modified Fatigue Impact Scale
NHS	National Health Service
NMDAS	Newcastle Mitochondrial Disease Adult Scale
NPMDS	Newcastle Paediatric Mitochondrial Disease Scale
ObsRO	Observer Reported Outcome
PedsQL	Paediatric Quality of Life Inventory
PEDI CAT	Paediatric Evaluation of Disability Inventory Computer Adaptive Test
PI	Principal Investigator
PIC	Patient Identification Centre
PIS	Participant Information Sheet
QoL	Quality of Life
R&D	Research & Development
REC	Research Ethics Committee
SAE	Serious Adverse Event
SARA	Scale for the Assessment and Rating of Ataxia
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

Contents

STUDY SUMMARY

Study Title	Leigh Syndrome Roadmap Project: A Natural History Study (UK)
Short Title/Acronym	LSRP (UK)
Summary of Design	Observational prospective cohort study evaluating the natural history of disease. Outcome measures in disease-relevant domains will be administered to patients with Leigh Syndrome at 3-6 month intervals. Retrospective medical history-based objective outcomes will also be evaluated.
Summary of Participant Population	Participants aged 0 to 75 years old with a genetically confirmed diagnosis of a Leigh syndrome spectrum (LSS) disorder.
Study Setting	This study will be conducted at the Newcastle upon Tyne Hospitals NHS Foundation Trust and via remote telephone/video follow-up.
Planned Sample Size	20 participants
Per Participant Duration	Up to 3 years
Proposed Overall Duration	3 years
Objective	To define the natural history of Leigh Syndrome through objective and subjective assessments of symptom involvement over time including objective clinician assessments, subjective patient or parent-reported outcomes, and patient medical history.
UK Sites	The Newcastle upon Tyne Hospitals NHS Foundation Trust (Newcastle Hospitals)
Data Collection	De-identified study data, using a unique global participant identifier (GUID) will be sent by each site to the project database hosted by The Children's Hospital of Philadelphia (CHOP)

BACKGROUND

INTRODUCTION

Leigh Syndrome (LS) is a rare, progressive neurodegenerative condition caused by genetic mutations in mitochondrial or nuclear DNA. It typically presents in infancy with variable symptom presentation resulting from mitochondrial dysfunction. Leigh Syndrome is often characterized by bilateral CNS lesions, specifically bilateral symmetric T_2-weighted hyperintensities in the basal ganglia and/or brain stem on brain MRI. Symptom involvement can include motor and intellectual developmental delay, developmental regression, ataxia and other movement disorders, hypertonia, dysphagia, and failure to thrive.

There are currently no proven treatments for LS. Due in part to the rarity of disease, multisystemic involvement, and variable phenotypic presentations, the natural history of LS is not well understood and has not been rigorously studied. To improve outcomes for patients and develop robust clinical trials for the treatment of this disease it is important to study the natural history of the disease, including typical symptom progression through objective and subjective assessments, and to identify and validate appropriate outcome measures for use in clinical trials.

RELEVANT LITERATURE AND DATA

Leigh syndrome is an extremely heterogeneous neurodegenerative condition and one of the most common forms of paediatric mitochondrial disease [1]. There are now more than 90 known genes that are encoded by both the nuclear or mitochondrial genomes that are causally associated with Leigh syndrome [2]. The Clinical Genome Resource (ClinGen) Gene Curation Expert Panel provides a fluid resource for current gene-disease associations reflecting the ongoing discovery of disease-causing genes for Leigh syndrome and primary mitochondrial disease [2].

Survey tools and clinical assessments validated in mitochondrial disease or diseases with similar clinical presentations, as well as tools that assess clinical features of mitochondrial disease, will be used to collect data on the natural history and progression of LS over time. These measures will be further evaluated for their utility

as outcome measures for clinical trials in mitochondrial disease. These assessments are described below.

The Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) and the Newcastle Adult Mitochondrial Disease Scale (NMDAS) are clinical rating scales developed and validated in mitochondrial disease to measure the extent of disease burden. The NPMDS and NMDAS are objective, multi-dimensional, reproducible, and encompass a diverse and broad scope of disease [3, 4]

The Barry Albright Dystonia Scale (BADS), a 5-point ordinal severity scale for secondary dystonia, has shown to be reliable and responsive to change with high inter-rater, intra-rater and test-retest reliability [5].

The Movement Disorder Childhood Rating Scale has been found to successfully detect changes in various types of movement disorders independent of age [6]. It has been validated in children 0 to 18 years old.

The Scale for the Assessment of Ataxia (SARA) has been validated in spinocerebellar ataxia as a successful tool for measuring severity of ataxia. It has high inter-rater and test-retest reliability and high internal consistency [7].

The Modified Fatigue Impact Scale (MFIS) has been validated in multiple sclerosis (MS) [8], which shares many clinical features of mitochondrial disease, particularly regarding the impact of disease on fatigue. In MS, the scale has excellent concurrent and content validity [8], and the physical and cognitive subscales are valid for independent interpretation of physical and cognitive fatigue [9].

The Paediatric Quality of Life Inventory (PedsQL) Generic Core scales measure health-related quality of life and have demonstrated reliability and validity in both healthy populations and populations with acute and chronic health conditions [10].

The Caregiver Burden Scale was developed to assess caregiver burden and has shown reliability and validity across populations of caregivers and patients [11].

The Observer Reported Outcome (ObsRO) survey is a tool for measuring at-home functionality through daily sign and symptom observation. It has been validated for daily use in children with pyruvate dehydrogenase complex deficiency, a relatively common cause of Leigh syndrome [12].

The Paediatric Evaluation of Disability Inventory Computer Adaptive Test (PEDI-CAT) is a computerized assessment of functional domains including daily activities, mobility, social/cognitive and responsibility. It is designed for children and youth from birth through 20 years old with a variety of physical and behavioural conditions [13].

STUDY OBJECTIVES

The purpose of the study is to define the natural history of Leigh Syndrome.

PRIMARY OBJECTIVE (OR AIM)

The primary objective of this study is to define the natural history of Leigh syndrome spectrum disorders (LSS) through objective and subjective assessments of symptom involvement over time including:

- Objective clinician assessments of movement, dystonia, ataxia and mitochondrial disease burden.
- Subjective measures of fatigue, quality of life (QoL), function, daily sign and symptom observation, and caregiver burden.

SECONDARY OBJECTIVES (OR AIM)

The secondary objective is to evaluate medical history-based objective outcome measures including respiratory function, cardiac function, acute decompensation and infection history, growth, hospitalizations, and other disease symptoms

STUDY DESIGN AND SETTING

This is a multi-centre observational prospective cohort study of natural history of LSS.

Funded by the Leigh Syndrome International Consortium, institutions across multiple countries are collecting similar data. While each institution follows separate protocols and regulatory permissions, the study designs are standardised to ensure compatibility in the data collected. Data from all participating countries will be combined for analysis, enabling a comprehensive and consistent approach to understanding Leigh syndrome

This protocol relates to the UK study and data collection only.

The study will take place in UK secondary and tertiary care services. It will enrol 20 participants aged 0-75 years with LSS. Each participant will be followed-up for (up to) three years. Follow up will be via in-person visits and remote assessments (video/telephone calls).

Objective outcome measures in disease-relevant domains will be administered to participants at six-month intervals.

Subjective outcome measures will be collected (via online questionnaires) every three months. These online questionnaires will take approximately 35 minutes for participants and/or caregivers to complete.

Retrospective medical history and demographic data will be collected and added to the research record upon enrolment into the study. Disease-relevant outcomes will continue to be collected from medical records throughout the study.

We will also catalogue acute adverse events that occur during the study period. The latter include metabolic decompensation, seizures, strokes, bleeding, acute infection (enteritis, urinary tract infection, upper respiratory infection, pneumonitis encephalitis) and any untoward reaction to medications and serious allergic reactions.

Study data will be collected and collated via a REDCap [14] database hosted by the Children's Hospital of Philadelphia (CHOP).

ELIGIBILITY CRITERIA

Eligibility must be assessed by a suitably qualified and delegated member of the study team (either a medically qualified doctor or appropriately experienced research team member e.g., research nurse, physiotherapist, exercise physiologist), and this assessment documented in the participant's medical records. Only personnel formally delegated by the PI may assess eligibility.

INCLUSION CRITERIA

To be eligible to participate all participants must:

- Be aged ≤ 75 years at the start of study (Baseline).
- Have a genetically confirmed diagnosis of a Leigh Syndrome spectrum (LSS) disorder.
- Be able to provide informed consent (consent on behalf of participants aged <16 years will be obtained from a parent/individual with parental responsibility), or
- For adult participants lacking capacity, have an appropriate consultee whose opinion on participation can be sought.

EXCLUSION CRITERIA

Potential participants will not be eligible if they:

- Have a diagnosis of a primary genetic disease other than mitochondrial disease (e.g., Down syndrome), including genetic diseases with secondary mitochondrial dysfunction
- Are unable, in the opinion of the recruiting investigator, to complete study assessments.

Potential participants that do not meet all the eligibility criteria may not be enrolled.

RECRUITMENT AND CONSENT

PARTICIPANT IDENTIFICATION

Potential participants will be identified for the study by their direct clinical care team at site via the NHS clinic/service at which they are seen for their mitochondrial disease. This will be via screening of clinic lists and patient records.

Potential participants may also be identified via the Centre for Mitochondrial Research Patient Cohort: A Natural History Study and Patient Registry (MitoCohort) (previously known as the MRC Mitochondrial Disease Cohort), REC Ref: 13/NE/0326 (IRAS: 122433). The MitoCohort, which is a natural history study and patient registry, has > 2000 registered patients with extensive storage of

clinical and genetic information. Patients registered on the MitoCohort have consented to receive information about clinical trials and studies for which they may be eligible.
Several participant identification centres (PICs) will be set up in other secondary care centres in the UK (for example, Great Ormond Street Hospital for Children NHS Foundation Trust (GOSH) and Cambridge University Hospitals NHS Foundation trust, Addenbrookes). The appropriate permissions will be obtained from each participating PIC prior to any study related PIC activity there.

The study will also be publicised on a number of websites including the website of mitochondrial disease charities and partner organisations. In addition, it will be promoted at public and patient engagement events and conferences. Any website or study advertisement will advise patients who are interested in participating in the study to contact the study team for further information.

Potentially eligible patients will be sent an invitation letter along with a copy of the relevant Participant Information Sheet (PIS) or will be contacted (via telephone, email or in person) by their direct clinical care team and invited to participate.
Where potential participants are aged under 16 years- a parent/individual with parental responsibility will be approached on their behalf. The potential paediatric participant may also be provided with an age-appropriate PIS (if applicable).

For adults lacking capacity, a consultee will be identified and provided with a Consultee Information Sheet (CIS). They will be asked for their opinion on whether the potential participant would want to be included in the study. If considered appropriate, an information sheet designed for adults lacking capacity will be provided to the potential participant.

Each participating site will be asked to keep a study invitation/pre-screening log which will include de-identified details of all individuals who are invited to participate/are pre-screened for participation. This log will include the reason for declining the invitation or pre-screen failure (if applicable/provided).

INFORMED CONSENT

Written informed consent (or consultee declaration) will be obtained from all participants prior to the start of study assessments. This will be carried out by appropriately qualified, experienced, and delegated members of the research team at the study site.

Consent will be obtained through initialling to confirm agreement with each consent statement, followed by the participant's dated signature and the dated signature of the individual obtaining the consent.

As outlined above, parental consent will be sought on behalf of children aged under 16. Where appropriate, written assent for participation will also be obtained from the child. If a child is capable of providing assent but chooses not to, they will not be recruited into the study.

For participants who turn 16 during the study, re-consent will be sought as adults at the earliest opportunity, ideally at their next in-person study visit.

For adults who lack the capacity to consent, a consultee declaration will be sought. The consultee, such as a family member or guardian, will confirm in writing that they believe participation is in the best interests of the participant. We will use the same questions from the informed consent form to guide this process, ensuring consistency in format. While the direct benefit to the individual may be minimal, inclusion is important for better representation of adults with learning difficulties in research. This approach is seen as beneficial for the population as a whole and may ultimately lead to improvements in care and treatment. Given the minimal risk and discomfort involved, the risk-benefit ratio is appropriate.
Once consent/consultee declaration has been completed and the relevant form countersigned, study activities can commence.

A copy of the signed Informed Consent/Assent/Consultee Declaration Form will be provided to the participant or their parent/consultee, with a copy filed in the participant’s hospital records. The original signed consent forms will be securely stored in the Investigator Site File.

A letter will be sent to the participant’s general practitioner to inform them of their patient’s participation in this study. A copy of this letter will be filed in the participant’s hospital records.

As part of the study, parents and caregivers of participants will be asked to complete a caregiver questionnaire at certain time points. This questionnaire is optional and participants can still take part in the study if the parent/carer choses not to. A separate information sheet will be provided to parents/carers outlining what they are asked to do and what will happen to their data. Completion of these questionnaires will be classed as their consent for participation. Identifiable data will not

be collected as part of these questionnaires and the responses will be linked to the relevant study participant by that participant's unique study ID.

Email address of the participant and/or parent/carer will be required if they opt to complete the questionnaire online (needed to send out a link to the questionnaire). Participant, parents/carers will be fully informed of this as part of the participant recruitment process.

If a participant loses capacity after entering the study, the opinion of a Consultee will be sought regarding the participant's continued involvement. No new data will be collected until a Consultee Declaration has been provided.
If an appropriate Consultee cannot be identified, or is unwilling for the participant to continue, the participant will be withdrawn from the study. All data collected up until the point that the participant lost capacity will be retained and used.

STUDY ASSESSMENTS AND PROCEDURES

STUDY VISITS

The following assessments will be conducted at study visits and during remote follow-ups (as applicable) for each participant.

Clinician/researcher assessments will take approximately one hour to complete and will be completed when participants are seen in clinic or during remote video visits.

Participant/ Parent-completed measures will take approximately 35 minutes to complete. These questionnaires will be sent to Participants/Parents electronically or via post and can be completed in their own time close to the time of their clinical visit/follow-up date.

Further details on the assessments can be found in the study evaluation and measurements section below.

Initial Visit (Baseline)

- Informed consent
- Medical record review and confirmation of eligibility
- Demographics (age, sex)
- General clinical assessment

- Clinician completion of the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) or the Newcastle Mitochondrial Disease Adult Scale (NMDAS)
- Clinician/researcher completion of the BADS
- Clinician/researcher completion of the Movement-Disorders-Childhood Rating Scale
- Clinician/researcher completion of the SARA
- Participant/Parent completion of the MFIS
- Participant/Parent completion of the PedsQL
- Parent/primary caregiver completion of the Caregiver Burden Scale
- Parent/primary caregiver completion of the ObsRO survey (to continue daily for one week)
- Parent/primary caregiver completion of the PEDI-CAT assessment.
- Clinical review of medical records and collection of previous/existing results

Questionnaire Follow-up (every 3 months)- Remote

- Participant/Parent completion of the MFIS
- Participant/Parent completion of the PedsQL
- Parent/primary caregiver completion of the Caregiver Burden Scale
- Parent/primary caregiver completion of the ObsRO survey (to continue daily for one week)
- Parent/primary caregiver completion of the PEDI-CAT assessment.

Clinician Assessment Follow-up Visits (every 3-6 months)*

- Confirmation of continued consent (verbal)
- Clinician completion of the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) or the Newcastle Mitochondrial Disease Adult Scale (NMDAS)
- Clinician/researcher completion of the BADS
- Clinician/researcher completion of the Movement-Disorders-Childhood Rating Scale
- Clinician/researcher completion of the SARA
- Collection of updated medical history and details of adverse events

*In person visit preferred however limited assessments may be completed remotely (i.e. via video consultation) if in-person assessments are not possible.

SCHEDULE OF EVENTS

Assessments	**Initial Visit (Baseline)**	**Questionnaire Follow-up (every 3 months)**	**Clinician Assessment Follow-up Visits (3-6 months)**
Informed Consent	x		
Confirmation of Eligibility	x		
Demographics	x		
Medical Record Review	x		x
Confirmation of Continued Consent			x
NPMDS/NMDAS	x		x
BADS	x		x
MDCRS	x		x
SARA	x		x
MFIS	x	x	
PedsQL	x	x	
Caregiver Burden Scale	x	x	
ObsRO	x	x	
PEDI-CAT	x	x	
Adverse Event reporting	x	x	x

WITHDRAWAL CRITERIA

Participants have the right to withdraw from the study at any time without having to give a reason. Participants who choose to withdraw from the study will be advised that the data collected up to the point of their withdrawal will be retained. A member of the research team should try to ascertain the reason for withdrawal and document this reason within the participant's medical records.

The Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Participant withdrawal of consent
- Participant loss of capacity to provide informed consent and inability to identify a consultee who is happy for the participant to continue
- Investigator's discretion that it is in the best interest of the participant to withdraw
- An adverse event that renders the participant unable to continue in the study
- Termination of the study by the sponsor or funder

If participants withdraw, or are withdrawn, from the study, any data already obtained will be kept. Participants will be advised of this during recruitment and consent. Participants who withdraw after consent will not be replaced.

END OF STUDY

As this is a longitudinal study, the intention is to follow-up participants for as long as possible. However due to funding, a study duration of three years in total has been allocated. This means that the study will close after three years regardless of where each participant is within their individual timeline.

The overall end of study will be defined as completion of three years of data collection from the first participant first visit. However, analyses of study data will continue after this point.

The end of study for each participant will therefore be completion of three years of data collection, or the overall study closure date (if earlier).

STUDY EVALUATIONS AND MEASUREMENTS

Medical Record Review

The following variables will be collected from each participant's medical record (if available):

- Sex
- Age (current, age at clinical symptom onset, age at genetic diagnosis)
- Ethnicity
- Genetic Diagnosis (including methodology, year, tissues tested and heteroplasmy)
- Laboratory results: lactate, pyruvate, GDF-15 and/or FGF-21, glutathione, acylcarnitine profile, plasma amino acids, urinalysis, urine amino acids, urine organic acids, Creatine Kinase, Complete Metabolic Profile (electrolytes, BUN/Cr, LFTs), and potentially other testing results including but not limited to immune or hormone level testing when done for clinically indicated purposes
- Height
- Weight
- Head Circumference
- Organ system involvement
- Cardiac function
- Respiratory function
- Acute decompensation history (e.g. hospitalisations, A&E visits)
- Infection history
- Vaccinations
- MRI and MRS results
- Physical Therapy assessments
- Neuropsychological testing results if available clinically (no mental health information will be collected)
- Developmental assessments from intermediate unit/individual education plans, if available
- Medications
- Other therapies, including participation in therapeutic clinical trials (historic or current) and start and end dates as applicable

- Any other disease-related symptoms not specified above

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Outcome Measure Evaluations

Objective Clinician Administered Outcomes

- **Newcastle Pediatric Mitochondrial Disease Scale (NPMDS) and Newcastle Mitochondrial Disease Adult Scale (NMDAS)**– *Mito Disease Burden*. The NPMDS and NMDAS scales encompass all aspects of mitochondrial disease by exploring several domains: I) Current Function, II) System Specific Involvement, III) Current Clinical Assessment. Sections I-III of the NPMDS or NMDAS, as applicable, will be completed by a trained clinician through caregiver interview. Section IV (Patient Reported QoL) will be completed by the patient/primary caregiver. The NPMDS will be used for participants 0-17 years old [3] and the NMDAS will be used for participants 18 years and older [4]. The NPMDS has separate age appropriate versions for 0-24 month olds, 2-11 year olds and 12-17 year olds.
- The NPMDS uses a 4-point scale (0-3), while the NMDAS uses a 6-point scale (0-5). To maintain scoring consistency, participants who turn 18 during the study will complete both the pediatric NPMDS (12-18 years) and the NMDAS at their first visit after their 18th birthday. This dual assessment during the transition period helps anchor the change and improves data comparability across the transition. Both scales will be completed every six months. Barry **Albright Dystonia Scale (BADS)** – *Dystonia*. The BADS is designed for evaluation of secondary dystonia and presence of dystonia in eight bodily regions. The scale will be completed by a trained clinician every 3-6 months [5].

- **Movement-Disorder Childhood Rating Scale** – *Movement*. For assessment of primary and secondary movement disorders during developmental age. This scale is a composite patient and clinician-reported outcome assessment. The scale, along with the parental interview, will be completed by a trained clinician every 3-6 months [6].

- **Scale for Assessment and Rating of Ataxia (SARA)** –*Ataxia*. SARA is a clinical scale that is based on a semi-quantitative assessment of cerebellar ataxia on an impairment level. The scale will be completed by a trained clinician every six months [7].

Subjective Observer (Caregiver/Parent) Reported Outcome Measures

- These assessments can be posted or emailed to the participant/ caregiver/parent

- **Modified Fatigue Impact Scale (MFIS) –** *Fatigue*. The MFIS is a tool that doctors use to evaluate how fatigue affects someone's life. It is a 21-item questionnaire that includes physical, cognitive, and psychosocial fatigue subscales [8, 9]. The MFIS will be sent to all participants two years of age and older for completion every three months. The MFIS will be completed by the participants' parent/primary caregiver.

- **Paediatric Quality of Life Inventory (PedsQL) Generic Core Scales –** *Quality of Life.* The PedsQL Generic Core Scales measure health-related quality of life. They measure the core dimensions of health as delineated by the World Health Organization (physical, emotional and social functioning) as well as role (school or work) functioning, as applicable [10]. The PedsQL generic core scales consist of seven versions based on age. The PedsQL will be sent to all participants for completion every three months. The PedsQL will be completed by the participants parent/primary caregiver.

- **The Caregiver Burden Scale** – *Caregiver Burden*. A scale to assess caregiver burden. It has shown reliability and validity across populations of caregivers and patients [11]. It will be sent to a parent or primary caregiver for completion every three months.

- **The Observer Reported Outcome (ObsRO)** – *Quality of Life*. The ObsRO survey is a tool for measuring at-home functionality. It has been validated for daily use in children with pyruvate dehydrogenase complex deficiency, a type

of Leigh Syndrome [12]. The ObsRO will be sent to a parent or primary caregiver daily for completion for one week every 3 months.

- **The Paediatric Evaluation of Disability Inventory (PEDI-CAT)** – *Function*. PEDI-CAT is a computer adaptive test measuring the functional domains of daily activities, mobility, social/cognitive and responsibility. It is designed for children and youth from birth to 20 years old with a variety of physical and behavioral conditions [13]. Participants who turn 21 while in the study will continue to complete PEDI-CAT assessments. PEDI-CAT will be administered electronically every 3 months through Pearson's web-based Q-Global platform. PEDI-CAT requires a date of birth for scoring purposes; we will be using a pseudonymized date of birth to avoid the sharing of identifiable data.

DATA COLLECTION AND MANAGEMENT

SOURCE DATA

As the Sponsor and site are the same NHS Organisation, a combined Trial Master File/Investigator Site File (TMF/ISF) will be established and held at site.

Completed study consent forms will be held in the TMF/ISF. Copies will be held in each participant's medical record.

Source data for this study will consist of:

- Annotations in the participant medical records;
- Study specific researcher assessment tools and worksheets;
- Participant completed questionnaires and assessment tools (including ePROs recorded via REDCap);

Participants will be identified on all source data other than data recorded in medical records or the local REDCap instance via their unique study ID number rather than by name.
Completed researcher/clinician assessment tools and worksheets will be stored in the TMF/ISF.

Any participant questionnaires completed on paper will be stored in the TMF/ISF. Where participants complete questionnaires via REDCap- the local REDCap database will be the source data.

Data collected on paper or via the medical record will be transcribed from the source data directly onto REDCap.

All efforts should be made to ensure that the data provided in the source documents is as complete as possible. Regular review of data completeness and data cleaning activities should be undertaken. These activities may include contacting participants to obtain missing information. Any activities which involve contacting study participants should be conducted by delegated members of the site team who are known to the participant (i.e. study research nurse).

REDCAP

REDCap is an internet-based Electronic Case Report Form (eCRF) system. The REDCap system utilised for this project is maintained and supported by the Data Co-ordinating Centre (DCC), CHOP, on behalf of the Leigh Syndrome International Consortium. A site REDCap instance hosted by Newcastle Hospitals will also be created.

This study will use the NIH Biomedical Research Informatics Computing System (BRICS) Global Unique Identifier (GUID) tool to generate unique study numbers for participants; participant identifiers are processed locally on a secure, governance-compliant device, and are not stored or transmitted.

GUID-coded study data will be shared with the Children's Hospital of Philadelphia (CHOP) Leigh syndrome research project team for the purpose of aggregating natural history study data across multiple sites for further analysis. No identifiable information will be shared by any participating site with CHOP; identifiable data will be held on site level REDCap instance and de-identified before transfer.

The study will comply with all relevant data protection legislation.

DATA SECURITY

Data collection and data entry to REDCap will be performed by trained and delegated members of the research team at site.

Access to the study REDCap eCRF to view/enter data will be restricted to site research team members and associated DCC administrators only. Permission to access the REDCap database at a site level will be issued by the Principal Investigator and will be allocated via the DCC.

Any other data storage systems (including the TMF/ISF) used at site will also be appropriately secured (e.g., stored in locked cabinets, password protected, backed-up) and accessible only to members of the research team with authorisation from the site Principal Investigation.

The link between a participant's unique study ID number and their name, will be via the study enrolment/recruitment log (also known as the Participant Identification Log) which will be held in the TMF/ISF. An electronic version of this log may also be held securely on NHS computer systems at site.

Access to final datasets will be restricted to members of the study team and collaborators involved in data analyses. Such final datasets will be pseudo-anonymised and will not contain any direct personal identifiers.

Following completion of the study, pseudo-anonymised sets of raw data may be made available for third party research purposes with the appropriate data transfer procedures. The data will also be made available as open research data. Consent for this will be obtained from all participants.

ACCESS TO DATA

Direct access to study data including source data contained in the participant medical records and personal identifiable data contained in the TMF/ISF will be granted to authorised representatives of the Sponsor, Newcastle University, or regulatory

authorities, for the purposes of monitoring, audit or inspection. Consent for this will be obtained from participants during recruitment.

ARCHIVING

Archiving will be authorised by the study Sponsor following submission of the end of study reports to REC and funder. Study documentation will be archived for a minimum of five years (or longer if mandated by Sponsor SOPs) following study closure.

Non-identifiable data will be retained by Newcastle University and CHOP for longer (minimum of 20 years) for analyses purposes.

The Sponsor will be responsible for archiving the TMF/ISF.

Data held on REDCap will be archived according to relevant DCC policies and procedures.

STATISTICS

STATISTICAL CONSIDERATIONS

The research activities conducted will consist of descriptive natural history study data collection. Statistical power for the natural history study analyses will be achieved through the aggregation of de identified data across multiple sites and protocols. A range of statistical methods will be used: Descriptive statistics to summarise baseline characteristics; longitudinal analyses, such as mixed-effects models, to assess disease progression over time. Associations between clinical, genetic, and subjective outcomes will be evaluated using correlation and regression analyses, with adjustments for potential confounders.

We also hope to perform subgroup analyses to explore variations in disease progression, and time-to-event analyses for episodes such as hospitalisations or acute decompensations.

CONTROL OF BIAS AND CONFOUNDING

We will implement several strategies to control bias and confounding. Selection bias will be minimised by using clear inclusion/exclusion criteria and leveraging the MitoCohort database to identify an appropriate representative cohort. Confounding variables such as age, disease severity, and genetic factors will be controlled through multivariable statistical adjustments. Observer bias will be reduced by training clinicians and using standardised assessment protocols. Missing data will be handled through multiple imputation and sensitivity analyses. Temporal effects should be minimised by using consistent personnel where possible, and consistent outcome measures, throughout the three year study period (apart from age appropriate corrections).

RISK ASSESSMENT

RISK ASSESSMENT

The risks associated with the proposed study are minimal. The clinician assessments may include the risk of discomfort, pain or fatigue. Importantly, this should not exceed the threshold experienced during routine clinical examinations that are standard of care. When completing questionnaires participants might experience momentary embarrassment or discomfort. They do not have to answer any questions that make them too uncomfortable.

There is also risk of breach of confidentiality of patient data. It is important that the confidentiality of each participant's information is carefully maintained, and only the minimal number of identified collaborators needed to participate will have access to research data.

POTENTIAL BENEFITS OF STUDY PARTICIPATION

There may be no direct benefit to participants from taking part in this study. The knowledge gained from this study may benefit the larger LSS community. The data will be a powerful tool toward development of precision clinical trials, treatments, and improved care for LSS disorders.

RISK-BENEFIT ASSESSMENT

The risk associated with participation in this study is minimal. The prospect of benefit to the greater mitochondrial disease community is in defining the natural history of LSS disorders and informing the development of clinical trials and ultimately treatments and therapies for patients. The potential benefit for the mitochondrial disease community outweighs the minimal risk to participants.

MONITORING, AUDIT & INSPECTION

The study may be subject to audit or monitoring by representatives of the Sponsor and Newcastle University, or inspection by regulatory authorities. Each investigator will permit study-related monitoring, audits and regulatory inspection including access to all essential and source data relating to the study. The site research team will follow local standard regulatory and quality assurance practices.

ADVERSE EVENT REPORTING

Information on each participant's general health throughout the study will be collected and recorded in their medical records as part of the medical history data collection. This will include acute medical events, untoward reaction to medications and serious allergic reactions which will be catalogued for analyses purposes. However, due to the non-interventional nature of this study, only adverse events which directly relate to study procedures, or occur during study assessments will be logged as study adverse events.

Such adverse events will be documented in the participant's medical records recording causality and severity. They will also be recorded on the study adverse event log in the TMF/ISF. AEs will be followed up until resolution or until stabilisation (if complete resolution is not anticipated).

ABNORMAL RESULTS OR ISSUES OF CONCERN

Any abnormal results or issues of concern identified during any study visit will be documented and referred to the Principal Investigator. The PI, or their delegated representative, will liaise with the participant's routine clinical care team.

ETHICAL AND REGULATORY CONSIDERATIONS

REGULATORY COMPLIANCE AND RESEARCH ETHICS COMMITTEE REVIEW AND REPORTS

The study will be conducted in accordance with sponsor SOPs and ICH GCP.

The CI will obtain a favourable ethical opinion from an NHS Research Ethics Committee (REC) prior to the start of the study. All parties will conduct the study in accordance with this ethical opinion.

The CI will notify the REC of all required substantial amendments to the study. Substantial amendments that require a REC favourable opinion will not be implemented until this REC favourable opinion is obtained. The Sponsor will notify the REC of any serious breaches of GCP or the protocol that occur during the study.

The CI will notify the REC of the early termination or end of study in accordance with the required timelines.

DEVIATIONS

The Chief Investigator and PI at site will be responsible for ensuring the study is conducted according to the protocol and GCP.

Protocol deviations, non-compliances and breaches are departures from the approved protocol. Any deviations from the protocol and GCP should be documented on the study deviation log (filed within the TMF/ISF). This log will be reviewed by the PI on a regular basis. Where necessary, Corrective and Preventative Actions (CAPA) will be implemented. These will also be documented and reported to the CI and Sponsor.

If the deviation constitutes a violation, this must be recorded on the log and reported to CI and Sponsor in a timely manner (within 3 working days).

Deviations found to frequently recur at a site are not acceptable and could be classified as a serious breach.

NOTIFICATION OF SERIOUS BREACHES TO GCP AND/OR THE PROTOCOL

A serious breach is a breach which is likely to effect to a significant degree –

a) the safety or physical or mental integrity of the subjects of the study; or
b) the scientific value of the study

The Sponsor must be notified immediately of any incident that may be classified as a serious breach. The Sponsor will notify the NHS REC within the required timelines in accordance with the Sponsor SOP.

INDEMNITY

The NHS has liability for clinical negligence. NHS Indemnity covers NHS staff and medical academic staff with honorary contracts for potential liability in respect of negligent harm arising from the conduct of the study.

As Sponsor, the Newcastle upon Tyne Hospitals NHS Foundation Trust will provide indemnity in respect of potential liability and negligent harm arising from study management.

Indemnity in respect of potential liability arising from negligent harm related to study design is provided by Newcastle University.

This is a non-commercial study and therefore there are no arrangements for non-negligent compensation.

AMENDMENTS

It is the responsibility of the Research Sponsor to determine if an amendment is substantial or not and study procedures must not be changed without the mutual agreement of the CI and Sponsor.

Substantial amendments will be submitted to the REC and Health Research Authority (HRA) and will not be implemented until approvals from both are in place.

Non-substantial amendments will be submitted to the HRA and will not be implemented until authorisation (if applicable) or acknowledgement is received.

POST-STUDY CARE

Following the study, participants will not have any further study assessments or procedures but will continue to receive standard care from their clinical care team.

COMMUNICATION AND DISSEMINATION OF RESULTS

Publications resulting from this research will involve the aggregated analysis of data from multiple institutions collecting LS natural history study data. Participant data will remain confidential in any scientific publication or presentation.

Findings from the study may be reported at local, national and international meetings, on social media platforms (including but not limited to the Leigh Syndrome International Consortium and associated members, Newcastle University, , charity partners), as well as in peer-reviewed journals.

Study participants will be advised in the Participant Information Sheet that they can contact the research team to request a lay summary of the overall research results once the study is complete.

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