Title: GEM Study: Prevalence of genetic diseases in ME/CFS patients. IRAS ID: 313068. Date: 10/01/2023. Version Number: 3.0.

Study title: How common is late onset Pompe disease and Limb Girdle Muscular Dystrophy 2A in children and young people and adults treated for Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS): A cross-sectional study.

Short Title: GEM Study: Prevalence of genetic diseases in ME/CFS patients

Sponsor Name: Adam Taylor, Head of Research Governance,
2nd Floor, St Augustine's Courtyard, Orchard Lane
University of Bristol, Bristol, BS1 5DS.
Email: research-governance@bristol.ac.uk

Research Team Contact details:

Main Applicant & Chief Investigator:
Professor Esther Crawley
Professor of Child Health, and Consultant Paediatrician.
The Centre for Academic Child Health (CACH), Bristol Medical School, Canynge Hall, 39 Whatley Road, University of Bristol, Bristol, BS8 2PS.
Email: esther.crawley@bristol.ac.uk

Co-applicants:
Prof Volker Straub
Harold Macmillan Professor of Medicine
John Walton Muscular Dystrophy Research Centre
University of Newcastle, International Centre for life
Newcastle upon Tyne, NE1 3BZ

Dr Ana Töpf,
Senior Research Associate
John Walton Muscular Dystrophy Research Centre
Institute of Translational and Clinical Research
University of Newcastle, NE1 3BZ

Dr Susan Ring
Head of Laboratories and Executive Director ALSPAC
Bristol Bioresource Laboratories, Oakfield House, Oakfield Grove, University of Bristol, BS8 2BN

Alix Groom
Head of Biological Sample Collection
Bristol Bioresource Laboratories, Oakfield House, Oakfield Grove, University of Bristol, BS8 2BN

Contents

Abbreviations

CFS	Chronic Fatigue Syndrome
CYP	Children and Young People
CK	Creatine Kinase
DBS	Dried Blood Spot
LGMD2A	Limb girdle muscular dystrophy 2A
ME	Myalgic Encephalomyelitis

1.SUMMARY

Myalgic Encephalomyelitis or Chronic Fatigue Syndrome (ME/CFS) is relatively common in adults and children and young people (CYP). To receive a diagnosis, CYP and adults must have: debilitating fatigue made worse by activity, worsening symptoms after activity, and sleep problems. Those with ME/CFS are disabled and use significant health care resources over a considerable period prior to accessing ME/CFS treatment.

Pompe disease (also named glycogen storage disease type II, acid maltase deficiency, OMIM #232300) is a rare metabolic myopathy caused by a deficiency of alpha-glucosidase. This results in the intra-lysosomal accumulation of glycogen. Fatigue is common in those with late-onset Pompe disease. It affects over 66% of those with the condition and is the presenting symptom in 25% of patients.

Limb girdle muscular dystrophy 2A (LGMD2A) also known as Calpainopathy is an autosomal recessive form of limb girdle muscular dystrophy. It is caused by mutations in the Calpain-3 (CAPN3) gene which gives instructions to produce a protein important to the muscle fibres. The age of onset of muscle weakness is extremely variable; the most common being between 8 and 15 years. Common symptoms include fatigue.

Many of the symptoms used to make a clinical diagnosis for ME/CFS overlap with the symptoms experienced by patients with Pompe disease or LGMD2A. Anecdotal reports suggest that some patients with Pompe disease have been treated in ME/CFS clinics for many years before the correct diagnosis is made. These patients are unlikely to get better with ME/CFS treatment approaches. A diagnosis of Pompe disease is important as it enables access to treatment that improves quality of life and life expectancy. A diagnosis of LGMD2A also enables patients to access to appropriate disease management.

2. BACKGROUND

2.1 Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS)

Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS) is common in adults (prevalence 0.76%), children and young people (CYP).[1] Symptoms for ME/CFS include debilitating fatigue, post-exertional malaise (i.e., the symptoms get worse after activity) and unrefreshing sleep or sleep disturbance and cognitive activities.[2,3] Symptoms need to be present for three months. CYP and adults with ME/CFS are disabled, and use significant health care resources over a considerable period prior to accessing ME/CFS treatment.[4-6]

2.2 Diagnosis of ME/CFS

The diagnosis of ME/CFS is a clinical diagnosis. Other causes of fatigue should be excluded prior to making a diagnosis and therefore the clinical assessment requires the following screening blood tests to be normal: full blood count; inflammatory markers (CRP, plasma viscosity/ESR); thyroid function; liver function; renal function; glucose; creatinine kinase; coeliac screen; ferritin (CYP only) and vitamin D (housebound only).[3]

2.3 Pompe disease

Pompe disease (also named glycogen storage disease type II, acid maltase deficiency, OMIM #232300) is a rare metabolic myopathy caused by a deficiency of alpha-glucosidase (GAA). This results in the intra-lysosomal accumulation of glycogen.

Fatigue is common in those with late-onset Pompe disease. It affects over 66% of those with the condition and is the presenting symptom in 25% of patients.[7-9] Although axial and limb girdle weakness is present, it is not always the presenting symptom. Symptoms are frequently insidious, serum creatine kinase (CK) levels can be normal and there can be variation in age of onset, rate of progression and sequence of muscle involvement. [9,10]

2.3.1 Genetic mutations

Pompe disease is an autosomal recessive lysosomal disorder caused by a deficiency of the acid alpha-glucosidase (GAA) enzyme. The *GAA* gene is located on chromosome 17 and spans over ~20 Kb. To date, over 580 mutations, both exonic and intronic, have been identified in all ethnic and racial groups.[11] The carrier frequency has been estimated in China and Taiwan as between 0.01-0.005.[12] In the USA, 453,152 newborns were screened for enzyme deficiency. Of these, 18 were identified as having Pompe disease giving a birth prevalence of 1:25,000 of which the majority (11/18) were thought to be late onset Pompe disease. This prevalence is higher than current prevalence estimates suggesting a relatively large number of adults and young people may have symptoms that are consistent with late-onset Pompe disease but remain undiagnosed (and therefore untreated) even though effective treatment is available.

2.3.2 Investigations to confirm diagnosis

In the UK, patients with a possible clinical diagnosis of Pompe disease have a dried blood spot (DBS) test to measure *GAA* enzyme activity levels. If these levels are low, the test is repeated. If levels are still low, then the patient DNA is screened for *GAA* mutations to obtain a genetic diagnosis.

2.3.3 Treatment

Patients with Pompe disease have improved outcomes with Enzyme Replacement Therapy. One placebo controlled randomised controlled trial has been conducted in adults with late onset Pompe disease which demonstrated improvement in walking distance on the 6 minute walking test, and stabilization of pulmonary function.[13] Further improvements that were consistent with these findings were seen with an open label extension study.[14] Observational cohort data (N=283 adults with Pompe disease) described increased survival rate in patients offered Enzyme Replacement Therapy.[15] A systematic review which included 22 publications from 19 studies, described five-fold lower mortality rate, improved ambulation and respiratory function in those treated with enzyme replacement therapy compared to untreated controls. European consensus is that adults with late onset Pompe should be offered Enzyme Replacement Therapy.[16,17] Factors associated with improved response to treatment include better clinical status at baseline, female sex and younger age.[13,14,18] This suggests that earlier diagnosis and treatment could improve survival.

2.4 Limb girdle muscular dystrophy 2A (LGMD2A)

Limb girdle muscular dystrophy 2A (LGMD2A) also known as Calpainopathy is an autosomal recessive form of limb girdle muscular dystrophy (LGMD). It is one of the most common forms of LGMD. The age of onset of muscle weakness is extremely variable; the most common being between 8 and 15 years, although it can range between 2 and 50 years. The symptoms can include poor sleep, nightmares, tiredness or headaches after waking up in the morning, lack of appetite and falling asleep during the day. LGMD2A is caused by mutations in the Calpain-3 gene, which gives instructions to produce a protein important to the muscle fibres.

2.4.1 Treatment

To date there are no specific treatments for LGMD2A, however careful management of the symptoms of the condition can improve a person's quality of life. For instance, keeping mobile is important for

all people affected by muscular dystrophy. There are no guidelines about the type or intensity of activities however it is recommended that any exercise undertaken is done within an individual's limitations. Joint contractures (tightening) can occur in LGMD2A and therefore regular physiotherapy is recommended. In the case of severe contractures, minor surgical procedures may be necessary. With progression of the muscle weakness, people with LGMD2A are at risk of developing breathing difficulties. Therefore, regular monitoring of respiratory function (forced vital capacity – FVC) is recommended. Sometimes overnight studies are indicated (pulse oximetry). Regular cardiac assessment is usually not required because there is no involvement of the heart muscle in this condition.

2.5 Symptom overlap

The diagnosis of ME/CFS is a clinical diagnosis and many of the symptoms used to make a diagnosis overlap with the symptoms experienced by patients with Pompe disease or LGMD2A. It is therefore conceivable that patients with Pompe disease or LGMD2A may present to ME/CFS clinics with a primary symptom of fatigue. Anecdotal reports suggest that some patients with Pompe disease have been treated in ME/CFS clinics for many years before the correct diagnosis is made.

This may partially explain the poor recovery rates for patients with ME/CFS. Adults with ME/CFS are unlikely to recover (only 22% will recover at 6 months with treatment), and at least 15% of CYP have not fully recovered after 12 months. For some patients, it is possible that the low recovery rate is because they do not have ME/CFS but another, undiagnosed explanation, for their fatigue such as Pompe disease or LGMD2A. Making the correct diagnosis will enable CYP and adults to access appropriate treatment.

2.6 Hypothesis

A proportion of adults and CYP who are given a diagnosis of ME/CFS have undiagnosed Pompe disease or LGMD2A which explains their clinical symptoms.

2.7 Justification of research

Earlier diagnosis and treatment/management of Pompe disease or LGMD2A could improve survival. If patients with Pompe disease or LGMD2A are currently being treated in specialist ME/CFS clinics, we need to develop a method to identify these patients and offer appropriate treatment. This study will provide answers to the following questions:

1. Are patients with Pompe disease or LGMD2A being treated for ME/CFS in either adult or paediatric specialist ME/CFS clinics?
2. Are there clinical features that could alert clinicians that a patient with chronic disabling fatigue should be screened for Pompe disease or LGMD2A?

3. AIMS AND OBJECTIVES

3.1 Overall Aim:

Accurately determine the prevalence of Pompe disease and LGMD2A in adults and CYP with ME/CFS.

3.2 Objectives:

1. Use two large clinical ME/CFS services (one adult and one paediatric service) to recruit approximately 2000 participants with a diagnosis of ME/CFS.
2. Collate and utilise routine collected data including length of illness, symptoms, and demographic data.
3. Collect saliva samples, extract and store DNA.

4. Sequence genomic DNA from participants with ME/CFS in order to identify those who carry variants in the *GAA* gene and may have late onset Pompe disease.
5. Sequence genomic DNA from participants with ME/CFS in order to identify those who may have LGMD2A.
6. Develop predictive algorithms for ME/CFS clinicians to identify characteristics (e.g., symptoms) associated with Pompe disease or LGMD2A in the ME/CFS cohort (provided there sample size is sufficient)

4. METHODS

4.1 Study design

This is cross-sectional study of both CYP and adults with ME/CFS. In this study, we will collect patients' samples (i.e., saliva) to screen for *GAA* and Calpain-3 gene mutations as this can be done for large numbers of asymptomatic patients at home. All participants will have a clinical diagnosis of ME/CFS and will be screened for Pompe disease and LGMD2A using genetic testing.

4.2 Setting

The largest UK Specialist ME/CFS services are in Bristol and Bath. Both the adult (North Bristol Trust) and paediatric service (Royal United Hospital Bath) provide assessment and treatment for over 500 new cases of adults and CYP with ME/CFS each year (total 1000 new patients a year) and a similar number of follow up patients (overall total 2000 patients with ME/CFS a year). Both services collect routine clinical data including demographic data and self-completed questionnaires on fatigue (Chalder Fatigue Scale), pain (visual analogue scale), disability, mood (Revised Children's Anxiety and Depression Scale for CYP, and Hospital Anxiety and Depression Scale for adults) and quality of life (EQ5D). The paediatric service collects Patient Reported Outcome Measures online using REDCap. Both services also collect symptom data (i.e., headaches, cognitive dysfunction, muscle pain, joint pain, general malaise, post exertional malaise, dizziness, palpitations, sore throats, swollen glands) during the assessment. Therefore, these two settings are well placed to invite individuals to the study.

University College London Hospitals (UCLH) and Newcastle Upon Tyne Hospitals (NUTH) are other NHS sites which specialise in ME/CFS. UCLH has one of the most extensive portfolios of services for CYP in the UK. The children and young people's specialist services (particularly the treatment and rehabilitation of adolescents and children with complex conditions service, known as TRACCS) are trained to care for 13-18 year olds and have over 25 years of experience. The service often sees paediatric patients with possible ME/CFS. NUTH has a regional Chronic Fatigue Syndrome service which sees approximately 200-250 adult patients a year with possible ME/CFS.

Newcastle University has a long history as a centre of international excellence in muscle disease diagnosis, care and research. The University's John Walton Muscular Dystrophy Research Centre (JWMDRC) provides ongoing lifelong care for around 2000 patients with inherited neuromuscular disease from the North of England. The team coordinates diagnosis, care and management via specialised clinics for paediatric and adult disease and since 2001 has led the nationally commissioned service for rare neuromuscular diseases. The centre specifically provides the diagnostic and advisory service for limb girdle muscular dystrophies (LGMD) and overlapping diseases like Pompe disease, based on a multidisciplinary approach including specialised muscle biopsy analysis, directed genetic testing and clinical assessment. The centre has responded to the promise of next generation sequencing technologies with the introduction of extended gene panels and contributes in this area to Genomics England.

In recent years, JWMDRC has seen great progress in the understanding of the genetic, genomic, molecular and physiological underpinnings of many neuromuscular disorders. This has led to the first clinical trial based on molecular and genetic mechanisms of disease. Prof Straub has been chief investigator in the UK for interventional trials in Pompe disease for the past 15 years. In addition, he established the JWMDRC largest sequencing effort, the MYO-SEQ project[19], where the prevalence of Pompe patients in a cohort of 2000 neuromuscular patients with unexplained muscle weakness was investigated.[20] Therefore, the JWMDRC will play an important role in the genetic testing component of the study.

4.3 Population

In this study, all patients with a diagnosis of ME/CFS attending either the adult or the paediatric service based in Bristol or Bath will be offered the opportunity to take part in this study.

4.3.1 Adults Inclusion: Age 18-70 with a diagnosis of ME/CFS

4.3.2 CYP Inclusion: 8-17 with a diagnosis of ME/CFS

4.3.3 Inclusion: Individuals who live in the UK

4.3.4 Exclusion: Recovered or unable to provide informed consent

4.4 Recruitment and Consent

4.4.1 Provision of study information: Recruitment is two-fold.

Retrospective recruitment:

An administrator from the adult services (Bath and Newcastle) will email and/or telephone patients that have been seen in the last two years and ask if they are interested in taking part in the study. If they are interested, they will be provided with the adult patient information leaflet and asked to provide consent to be contacted by a member of the research team to discuss their potential participation. At this point, consent to contact will be obtained and recorded on REDCap.

Retrospective recruitment will also take place in the paediatric services (Bath and London). A member of the care team will identify eligible patients who have been seen in the service in the last two years, and are currently not being seen by a clinician. If they continue to meet the criteria/eligibility to join the study, they will be contacted by a member of the clinical team and invited to take part in the study. If the patient is interested in taking part, they will be asked to complete the consent to contact form via REDCap.

Prospective recruitment:

Potentially eligible patients will be identified when they attend the specialist clinics for either a new assessment or a follow up appointment. Patients will be provided with a brief explanation about the study from a clinician. If a patient is happy to participate, the clinician will provide the patient with a patient information leaflet for them to read before asking them to complete the consent/assent forms. If a patient requires more time to read the patient information leaflet and/or think about their participation, the clinician will either obtain verbal consent and record this on REDCap or ask the patient to complete a consent to contact form on REDCap. For those who provide consent to contact, a research nurse will contact the patient to discuss participation within 1-2 weeks. The majority of adults and CYP are attending clinics online using video platforms. The information will therefore be provided via video online or in person, for those being seen face-to-face. In the Bristol adult service, patients are seen in either individual or group appointments. The adult service holds an introductory 'Foundation Phase' live webinar once a month for ME/CFS patients to attend to discuss self-management techniques. A member of the research team will attend these monthly webinars to introduce the study to patients as a potential source of recruitment.

In all NHS services, an informative poster/flyer about the study will be displayed or shared within the service (e.g., in clinic rooms and on service websites) as an additional source of information for potential participants. In due course, the study will have a webpage affiliated with the University of Bristol and include details about the study, the research team and how to get involved for potential participants.

4.4.2. Consent and assent: We will obtain consent from CYP aged 16-17 years and from adults. We will obtain assent from CYP aged 8-15 years and consent from the parents of CYP aged 8-15 years.

Retrospective recruitment:

A research nurse will phone potential participants, check they have read the patient information leaflet and provide further information about the study. They will explain the potential risks and benefits, listen to concerns and answer questions. Those willing to take part will provide consent with the support of the research nurse using REDCap (i.e., an online system for obtaining consent remotely). We anticipate that some adult patients may read the patient information leaflet and wish to participate without discussion with a research nurse. In this case, they will be provided with an online consent form to complete if they do not wish to have further discussion.

Prospective recruitment:

The clinician will offer the patient the opportunity to consent to take part in the study or consent to contact from a research nurse if they require more time to think about the study. If patients decide on the latter, they will be given 1-2 weeks to think about their potential involvement in the study. Consent to contact will be collected verbally (and the clinician will document this on REDCap) or provided by the participant in writing.

If patients wish to provide consent to study in the clinical appointment, the specialist clinician will provide information about the study, discuss the potential benefits and risks, and obtain consent.

If patients provide consent to contact, a research nurse will phone participants at home within 1-2 weeks, check potential participants have read the patient information leaflet and provide further information about the study. They will explain the potential risks and benefits, listen to concerns and answer questions. Those willing to take part will provide consent with the support of the research nurse using REDCap for obtaining consent remotely.

Samples will be collected with informed consent using the participant information leaflets and consent forms approved by an NHS REC. The study will be responsible for ensuring consent is collected in line with HTA guidelines and will confirm consent is in place for all samples to be processed and stored by Bristol Bioresource Laboratories (BBL). BBL will send a report to the study team on a monthly basis detailing samples received. The study team will verify consent status of samples received, including if consent for future use was obtained. If consent was not obtained and can't be verified, the samples will be disposed of in line with University of Bristol and Human Tissue Authority guidelines. If consent has not been given for future use, and once the genetic testing has been undertaken, samples will be disposed of.

The participant's General Practitioner (GP) will be informed via letter/email about the study after consent is provided, and later informed of the participant's genetic testing result.

4.5 Planned Recruitment

4.5.1 Sample size. We aim to recruit approximately 2000 adults and CYP with ME/CFS over two years to this study.

4.5.2 Planned recruitment rates. We assume that the percentage of eligible CYP and adults that can be recruited will be fairly consistent over the two year time period with a lead in time (with lower recruitment).

4.6 Data Collection

Data will be entered into REDCap which is a secure system used by multiple institutions for large multicentre studies. Assessment data that has already been collected for paediatric patients at the Bath service will be transferred to the research database with consent. For the adults and some CYP, participants will enter their own Patient Reported Outcome Measures (PROMs). Paediatric patients will all need to enter their symptom data.

4.6.1 Data collection using REDCap: The Bath paediatric service uses online systems to collect assessment and outcome data in CYP. The research team at the service have developed, tested and use online consent to enable participants to take part in randomised controlled trials. Therefore, all participants will use REDCap to record their answers. Only members of the research team will have access to the participant's data recorded in REDCap.

4.6.2 Demographic Data: we will collect the following data: Date of birth, gender, ethnicity, NHS number, and contact details.

4.6.3 Symptom data: We will collect the following data at assessment (yes=frequently present): cognitive problems, headaches, muscle aches, joint aches, sore throats, tender lymph nodes, nausea, dizziness, palpitations, respiratory problems.

4.6.4 Patient Reported Outcome Measures (PROMs): We will collect the following Patient Reported Outcomes Measures routinely collected at assessment in the ME/CFS clinics: Fatigue (Chalder fatigue scale, 11 items; Physical Function (SF-36 physical function subscale); pain (visual analogue scale); Anxiety and Depression (Adults: Hospital Anxiety and Depression Scale, CYP: Revised Childrens Anxiety and Depression Scale).

At Royal United Hospital Bath, demographic data and the PROMs are collected using REDCap and we will therefore obtain consent to use this data, rather than ask the CYP to complete these questionnaires again. We will therefore only collect symptom data on REDCap for CYP. At the other NHS sites, participants will be asked to provide all the data using REDCap.

If REDCap detects partial completion of one of the forms (e.g., consent to study or the questionnaire), the research team will contact the participant 1-2 weeks after the form was opened and ask the participant if they would like to proceed with completing the form. This will also allow the research team to ask if there have been any technical difficulties with completing the form, and support the participant if necessary.

4.7 Sample Collection and Storage

4.7.1 Sample collection: Saliva samples will be collected from all consenting participants. They will be labelled with barcode ID numbers and personal information will not be passed to individuals processing or analysing the samples. Participants can choose to collect saliva at home or in clinic (if they are being seen face to face). If participants chose to collect saliva at home, we will send them an Oragene saliva collection kits (https://www.dnagenotek.com) with a returned address envelope, sample pot and instructions. The instructions include a link to a video describing how to collect the saliva. Previous feasibility work has demonstrated that adults with ME/CFS have found these easy to use at home and have produced saliva from which good quality DNA can be extracted.

If participants prefer, samples can be collected in the specialist ME/CFS clinics with help from the research nurse (or the recruiting clinician). All samples will be posted to the Bristol Bioresource Laboratories (BBL), Oakfield House, University of Bristol.

Three weeks after the research team have sent the Oragene kit to the participant, the participant will be contacted and reminded to complete the saliva sample. At this point, the research team will check that the participant is happy with the instructions they've been provided and if they have any questions.

If there is indication of Pompe disease and/or LGMD2A, these participants will undergo further testing to confirm the diagnosis of Pompe disease/LGMD2A. This is likely to include providing a second saliva sample for testing or a blood sample for DBS testing in a certified NHS clinical setting. The NHS laboratory will be responsible for collecting these additional samples.

4.7.2 Sample storage: When saliva samples arrive to the lab, DNA will be extracted and stored in freezers (set at -80° Celsius) at Bristol Bioresource Laboratories (BBL) in aliquots for analyses. Samples will be stored for future use provided appropriate consent has been obtained. All samples will be used, stored and disposed of in accordance with the Human Tissue Act 2004.

4.7.3 Sample transfer: DNA samples will be sent to the University of Newcastle for genetic testing. BBL will be responsible for the transport of DNA to the Institute of Translational and Clinical Research, Newcastle University. DNA will be sent on dry ice with next day delivery to maintain sample integrity. A University of Bristol approved courier will be used that has a tracking system so that samples are traceable throughout delivery. The Newcastle laboratory will acknowledge receipt of samples upon delivery. A list of all samples within the shipment will be sent to the chief technician of the Newcastle laboratory. Any discrepancies will be reported to either BBL's chief laboratory technician or head of biological samples. The receiving laboratory will adhere to the required storage conditions of the DNA. Any material remaining after analysis will be returned to BBL and stored within the biorepository for future use, if the participant has consented to this.

4.8 Genetic Testing:

To identify likely pathogenic variants causative of Pompe disease or LGMD2A, the full size of the *GAA* (20 kb) and *CAPN3* (50kb) genes, including exons and introns, will be screened. The labs in Newcastle University will first amplify the whole length of the respective genes by long-range PCR amplification. This entails four overlapping amplicons for *GAA* and nine amplicons for *CAPN3*. Amplicons will be then pooled, indexed and sequenced using an Illumina DNA prep kit assuming total library size 1.1 Mb with 30-50X coverage, and run on a MiSeq v2 Nano at the Genomics Core Facility of Newcastle University. Bioinformatics pipeline, including alignment, variant calling and appropriate filtering to identify recessive disease causing-variants in either gene will be carried out at the Bioinformatics Support Unit, Newcastle University.

4.9 Outcome of genetic screening for participants:

A member of the clinical team from the Bristol and Bath service will notify participants of their genetic screening via email and/or telephone (participant preference). If a participant receives an inconclusive genetic result, they will immediately be offered support from our research nurses and referred to a local genetic service for further testing. This is likely to include providing an additional saliva sample and may include a blood sample (or DBS test) for those with ambivalent results. If a participant receives a positive genetic result (i.e., indication of Pompe disease/LGMD2A), confirmation from a certified NHS diagnostic lab will be needed following the collection of a new sample. Those who have a confirmed result of Pompe disease or LGMD2A will receive genetic counselling with their treatment. The participants' general practitioner will also be informed. For participants with confirmed LGMD2A, counselling about the diagnosis will be offered and provided by the team at Newcastle University.

Patients would be counselled about the autosomal recessive mode of inheritance of these conditions and genetic implications for other family members. Patients will be provided with prognostic implications and information about disease management.

5. ANALYSES

5.1 Identification of cases

Pompe: This study will identify participants with pathogenic mutations in both alleles of the GAA gene. To follow European guidance, participants will undergo further testing in a certified NHS setting to confirm reduced enzymatic activity. After sequencing and bioinformatics analysis, patients carrying two known pathogenic or likely pathogenic variants according to ACMG guidelines in the *GAA* gene will be considered preliminary genetically diagnosed. Cases carrying two variants of uncertain significance will be further tested in the NHS (e.g., using DBS). Those with reduced enzymatic activity and the ones with a preliminary diagnosis will be confirmed in a certified NHS lab.

LGMD2A: Similarly, patients carrying two known pathogenic or likely pathogenic variants according to ACMG guidelines in the *CAPN3* gene will be considered preliminary genetically diagnosed. Patients carrying variants of uncertain significance will be investigated further by the clinician team and, if required, the histopathology unit.

We will therefore screen participants for mutations in a research setting involving analysis of research samples. However, those identified as carrying Pompe disease or LGMD2A will require confirmation in a certified NHS clinical setting (i.e., diagnostic lab) with additional samples (i.e., saliva and blood). It is also possible that in some cases, family members may need to be tested.

5.2 Analyses of prevalence

Prevalence will be calculated as the number of patients diagnosed with Late Onset Pompe disease or LGMD2A as a percentage of ME/CFS patients.

When we know the number of cases of Pompe disease and/or LGMD2A, we will perform a sample size calculation and create a statistical analyses plan (prior to analyses) which will be deposited in the University of Bristol's open access repository to investigate if there is a relationship between phenotypic symptoms and the presence of Pompe disease and/or LGMD2A. If there are insufficient cases to provide enough power for a detailed analysis, we will perform a descriptive analysis on demographic details (e.g., sex, age) and using phenotypic symptoms focusing on symptoms known to be associated with Pompe disease and/or LGMD2A. If there are sufficient cases to provide adequate power, we will use logistic regression to investigate phenotypic symptoms associated with those identified as having late onset Pompe disease and/or LGMD2A compared to those who do not have late onset Pompe disease and LGMD2A.

6. PROJECT MANAGEMENT

The study will be led by Prof Crawley who will meet with the research team weekly or bi-weekly. The trial management group (TMG) will be responsible for overall trial management, monitoring trial progress and quality, and ensuring that the study protocol is adhered to and that participants are safe. They will meet every 6 weeks. The chief investigator, the research team, and co-applicants (as relevant) will join this meeting. The trial steering committee (TSC) will ensure milestones are realistic and achieved. The TSC will be responsible for reviewing the study. The TSC will include at least one patient, have an independent chair and include 2 clinicians and 4 methodologists. The TSC will meet prior to the start of the trial and then annually.

The Data Monitoring and Safety Committee (DSMC) will include 2 independent experts in ME/CFS, medical statistics and trials. The DSMC will meet at the start of the study, after recruitment has been running for 6-months and then 18-months after recruitment started. The DSMC will have unblinded access to data. DSMC meetings will be timed to provide reports to the TSC.

7. ETHICAL ISSUES

7.1 Risks and benefits

7.1.1 Potential benefits to participants. The main benefit will be to participants who are identified as having Pompe disease or LGMD2A as this will enable them to access effective treatment or management of their condition.

7.1.2 Potential Risks to participants. The main risk is if participants receive an uncertain result at the genetic testing (are told they might have Pompe disease or LGMD2A, but subsequent testing shows this is not the case). This is because a proportion of the identified *GAA* and *CAPN3* variants will be of unknown significance. The risk of this is very small. However, we want to be sure that all participants understand this before recruitment. We will ensure that the patient information leaflet (PIL) is clear on this risk, and that additional information is provided from the PIL. This additional information will provide similar information to that covered in genetic counselling. We will also ensure this risk is discussed in the recruitment discussions. Patients who have an inconclusive result will immediately be offered further testing, support from the research team, Association for Glycogen Storage Disease-UK, and offered referral to a local genetic service for further support. Those who have a confirmed positive result will receive genetic counselling with their treatment.

We have also considered the additional stress/anxiety whilst waiting for the results. We will batch samples for analyses regularly to minimise the wait and warn potential participants that the wait for results will be up to 6 months but hopefully significantly less. The time taken will depend on the recruitment rate as samples will be batched to extract DNA and for transport and testing.

7.1.3 Potential benefits to society. Understanding which patients may have Pompe disease or LGMD2A and not ME/CFS will enable the NHS to allocate resources appropriately.

8. DATA PROTECTION AND PARTICIPANT CONFIDENTIALITY

Data will be entered into REDCap which is a secure system used by multiple institutions for large multicentre studies. Assessment data that has already been collected for paediatric patients will be transferred to the research database with consent.

For the adults and some CYP, participants will enter their own Patient Reported Outcome Data. Paediatric patients will all need to enter their symptom data. Participants will be required to log in to the system and pass authentication before they can access their own data. There are several authentication methods available. The University of Bristol will use table-based authentication, which utilises the storage of username/password pairs in a database table. In this system, the password in the database table is encrypted as a one-way hash of the password. Participants will be sent a web link to REDCap which will only allow access to their data. They will create a password which they will use each time they log in. REDCap also has an auto-log out system that will log participants out after 30 minutes if they have stopped using the database.

Data will be managed according to the following procedure. Participants will be allocated a unique research identification number. A list of names and corresponding identification numbers will be kept separately and securely on a password protected University of Bristol server. Personal information will be kept on consent forms which will have contact details. Consent forms will then be password protected and stored securely online with the University of Bristol server.

9. COVID-19 RISKS AND MITIGATION

Participants will be recruited online as the majority of clinics are conducted online. Although some clinics are currently face to face, these will move to online clinics if required by the Department of Health. Sample collection will not require personal contact as can be posted to participants homes. Participants will be asked to only provide a sample if they do not have COVID-19 symptoms.

Use of Oragene collection kits minimises the risk of infection from SARS-COV-2 as the stabilising solution in the kits provides more than 99% inactivation of enveloped viruses (https://dnagenotek.com/ROW/pdf/MK-01430.pdf).

In addition, when samples arrive in the Bristol Bioresource Laboratories packages containing the kits will be opened in a microbiological safety cabinet and tubes wiped with ethanol. The samples will also be heat treated at 56°C for 30 minutes in their original tubes to ensure virus inactivation is complete before DNA extraction.

10. DISSEMINATION AND OUTPUTS

The main outputs will be a paper on the prevalence of Pompe disease and/or LGMD2A in patients diagnosed with ME/CFS. Secondary papers may include information on risk factors that clinicians should be aware of that would indicate that further testing should be conducted.
We will disseminate findings at international meetings for metabolic disorders as well as paediatric meetings in Europe and the USA.

11. RESEARCH GOVERNANCE

The study will be sponsored by the University of Bristol. HRA and HCRW Approval (including NHS REC review) will be sought and confirmation of capacity and capability will be obtained from the NHS Trusts.

12. INSURANCE

The insurance for this study will be arranged by the University of Bristol.

13. ADVERSE EVENTS

The study will not be recording or reporting adverse events.

14. ARCHIVING

Consent and questionnaire data will be uploaded to a RDSF (i.e., a university research data secure storage facility) for other researchers to use for 2-years after the study has stopped. We will not keep the study data open access longer than this as we do not currently have funding for long term storage and we consider that 2-years is sufficient time for other researchers to use the data.

15. REFERENCES

1. Lim EJ, Ahn YC, Jang ES, Lee SW, Lee SH, Son CG. Systematic review and meta-analysis of the prevalence of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). *J Transl Med.* 2020;18(1):100.
2. Royal College of Paediatrics and Child Health. *Evidence Based Guideline for the Management of CFS/ME (Chronic Fatigue Syndrome/Myalgic Encephalopathy) in Children and Young People.* London2004.
3. NICE. *Myalgic Encephalomyelitis (or encephalopathy)/chronic fatigue syndrome: Diagnosis and management (NICE guideline NG206).* London 2021.

4. Crawley E, Sterne JA. Association between school absence and physical function in paediatric chronic fatigue syndrome/myalgic encephalopathy. *Arch Dis Child.* 2009;94(10):752-756.
5. Collin SM, Bakken IJ, Nazareth I, Crawley E, White PD. Health care resource use by patients before and after a diagnosis of chronic fatigue syndrome (CFS/ME): a clinical practice research datalink study. *BMC Fam Pract.* 2017;18(1):60.
6. Collin SM, Crawley E, May MT, Sterne JA, Hollingworth W, Database UCMNO. The impact of CFS/ME on employment and productivity in the UK: a cross-sectional study based on the CFS/ME national outcomes database. *BMC Health Serv Res.* 2011;11:217.
7. Hagemans ML, van Schie SP, Janssens AC, van Doorn PA, Reuser AJ, van der Ploeg AT. Fatigue: an important feature of late-onset Pompe disease. *J Neurol.* 2007;254(7):941-945.
8. Hagemans ML, Winkel LP, Van Doorn PA, et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain.* 2005;128(Pt 3):671-677.
9. van der Beek NA, de Vries JM, Hagemans ML, et al. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis.* 2012;7:88.
10. Schuller A, Wenninger S, Strigl-Pill N, Schoser B. Toward deconstructing the phenotype of late-onset Pompe disease. *Am J Med Genet C Semin Med Genet.* 2012;160C(1):80-88.
11. Peruzzo P, Pavan E, Dardis A. Molecular genetics of Pompe disease: a comprehensive overview. *Ann Transl Med.* 2019;7(13):278.
12. Lin CY, Shieh JJ. Molecular study on the infantile form of Pompe disease in Chinese in Taiwan. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi.* 1996;37(2):115-121.
13. van der Ploeg AT, Clemens PR, Corzo D, et al. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med.* 2010;362(15):1396-1406.
14. van der Ploeg AT, Barohn R, Carlson L, et al. Open-label extension study following the Late-Onset Treatment Study (LOTS) of alglucosidase alfa. *Mol Genet Metab.* 2012;107(3):456-461.
15. Gungor D, Kruijshaar ME, Plug I, et al. Impact of enzyme replacement therapy on survival in adults with Pompe disease: results from a prospective international observational study. *Orphanet J Rare Dis.* 2013;8:49.
16. Schoser B, Stewart A, Kanters S, et al. Survival and long-term outcomes in late-onset Pompe disease following alglucosidase alfa treatment: a systematic review and meta-analysis. *J Neurol.* 2017;264(4):621-630.
17. van der Ploeg AT, Kruijshaar ME, Toscano A, et al. European consensus for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a 10-year experience. *Eur J Neurol.* 2017;24(6):768-e731.
18. de Vries JM, van der Beek NA, Hop WC, et al. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet J Rare Dis.* 2012;7:73.
19. Topf A, Johnson K, Bates A, et al. Sequential targeted exome sequencing of 1001 patients affected by unexplained limb-girdle weakness. *Genet Med.* 2020;22(9):1478-1488.
20. Johnson K, Topf A, Bertoli M, et al. Identification of GAA variants through whole exome sequencing targeted to a cohort of 606 patients with unexplained limb-girdle muscle weakness. *Orphanet J Rare Dis.* 2017;12(1):173.