This protocol has regard for the HRA guidance and order of content

FULL TITLE OF THE STUDY

Muscle MRI as a tool to detect glycogen in skeletal muscles of patients with adult onset Pompe patients

SHORT STUDY TITLE

Muscle MRI in Pompe disease

PROTOCOL VERSION NUMBER AND DATE

Protocol version 1.0 dated 20.10.2021

RESEARCH REFERENCE NUMBERS

IRAS Number:	305745
SPONSORS Number:	R&D reference: 09750
FUNDERS Number:	SGZ-2018-12028

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirements.

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

I 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; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature:
.....

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

Name (please print):
.....

Position:
.....

Chief Investigator:

Signature:
.....

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

Name: (please print):
.....

LIST of CONTENTS

KEY STUDY CONTACTS

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Sponsor	The Newcastle upon Tyne Hospitals NHS Foundation Trust, Freeman Hospital, Freeman Road, High Heaton, Newcastle upon Tyne, NE7 7DN, UK
Funder(s)	Sanofi 410 Thames Valley Park Drive, Reading, Berkshire, RG6 1PT, UK

STUDY SUMMARY

Study Title	Muscle MRI as a tool to detect glycogen in skeletal muscles of patients with adult onset Pompe patients
Short title	Muscle MRI in Pompe disease
Study Design	Prospective observational study
Study Participants	Patients with genetically confirmed pre-symptomatic or early symptomatic late onset Pompe disease (started showing symptoms after the age of 3) aged 12 and older Controls that are age and sex matched with Pompe patients
Planned Size of Sample	10 patients with Pompe disease and 10 age and sex matched controls
Follow up duration (if applicable)	Patients will be followed for 1 year, healthy controls will attend one visit only with no follow up
Planned Study Period	Patients with Pompe disease will attend two visits: baseline and year 1; controls will attend one visit (baseline) visit only
Endpoints	• To investigate if carbon spectroscopy, an MRI sequence able to identify and glycogen in tissue, can identify the presence of glycogen in muscles of patients with Pompe disease that are pre-symptomatic or early symptomatic;

	• To identify is there are changes in the amount of glycogen in the muscles of these patients after one year of follow-up • To identify if the amount of glycogen in the muscles correlates with the results of muscle function test at baseline and year 1 visit

FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this study)	**FINANCIAL AND NON FINANCIALSUPPORT GIVEN**
Sanofi 410 Thames Valley Park Drive, Reading, Berkshire, RG6 1PT, UK	Sanofi agrees to provide to the Research Grant in accordance with and subject to the terms of the study agreement. The Sponsor and the Principal Investigator shall disclose to the Funder any other support or benefit (financial or otherwise), including support from a public or government body, received in connection with the study agreement or the participation of such a body in the study.

ROLE OF STUDY SPONSOR AND FUNDER

The Sponsor is responsible for the conduct, management, monitoring, audit and closure of the study in accordance with applicable laws and regulatory requirements. The Sponsor is the sole sponsor of the study.

The Sponsor is responsible for developing the protocol. The Sponsor will seek the opinions of the Funder and will take into account comments provided by the Funder, before finalising the protocol.

The publication and dissemination of the results of the study will be the responsibility of the Sponsor. However, in order to ensure that the Funder is able to make comments and suggestions, where pertinent, the data, literature abstracts concerning the study, reports of the study, proposed manuscripts concerning the study and any materials for public dissemination concerning the study will be submitted to the Funder for review prior to submission for publication and/or public dissemination.

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

1H – MR	Proton spectroscopy
13C MR	Carbon Spectroscopy
AE	Adverse Event
AR	Adverse Reaction
ERT	Enzymatic replacement therapy
GAA	acid alpha-glucosidase
GDPR	General Data Protection Regulation
HRA	Health Research Authority
LOPD	Late onset Pompe disease
MRC	Medical Research Council
MRI	Magnetic resonance imaging
NJRO	Newcastle Joint Research Office
REC	Research Ethics Committee
R-Pact	Rasch-built Pompe-specific activity
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T1w	T1 weighted imaging

Muscle MRI in Pompe disease

STUDY FLOW CHART

Tasks	Months									
	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27	28-30
1. Set up of the protocol										
2. Baseline visit: patients and controls										
3. Analysis of baseline data and interim report I										
4. Year 1 visit: patients										
5. Analysis of first year data										
6. Manuscript and interim report (2)										

Muscle MRI in Pompe disease

STUDY PROTOCOL

Muscle MRI as a tool to detect glycogen in skeletal muscles of patients with adult onset Pompe patients

1 BACKGROUND

Pompe disease, also known as glycogenosis type II or acid maltase deficiency, is a genetic disorder produced by mutations in the GAA gene that codify for the lysosomal enzyme acid alpha-glucosidase (1). This enzyme is essential for degradation of glycogen to glucose in lysosomes. The absence or deficiency of the enzyme leads to an accumulation of glycogen in the lysosomes of several tissues particularly cardiac, skeletal and smooth muscle cells (2).

Pompe patients can be classified into infantile or adult onset (2, 3). The onset of the disease in infantile patients is during the first year of life (4). These patients have a rapidly progressive disorder characterized by muscle weakness, hypotonia, cardiomyopathy and hepatomegalia. Without treatment, they die in the first two years of life due to hypertrophic myocardiopathy or to respiratory problems.

Adult Pompe patients have completely different courses of disease (5). The age of onset is variable, from the teen years to late in adulthood, but most patients notice their first symptoms during the third or fourth decade of life. In the case of adult patients, the skeletal muscle is the main tissue affected (6). Patients develop a slowly progressive weakness involving proximal muscles of the upper and lower limbs, axial and respiratory muscles (7). Although there is a notable variability among patients' symptoms, natural history studies published showed that Pompe patients develop muscle weakness leading to a variable degree of motor disability. Most of the patients need canes for walking during the fourth decade of life and probably a wheelchair in the fifth or sixth decade of life. Respiratory symptoms characterized by effort dyspnea progressing to orthopnea and respiratory failure requiring non-invasive ventilation are common. Recent studies have suggested that adult onset Pompe patients have an increased risk of premature death due to respiratory problems (8, 9).

Enzyme replacement therapy (ERT) with acid alfa-glucosidase has changed the natural history of the disease both in infantile and adult onset patients. ERT reverses cardiac hypertrophy, improves muscle weakness, reduces the need of supporting ventilation and decreases the mortality ratio in infantile onset patients. The age at which the treatment is started in these cases seems to be critical, and recent studies suggest that earlier treatments are associated with better outcomes. In the case of adult onset patients, ERT produces a mild improvement in motor function during the first 6-12 months of treatment followed by a stabilization of both motor and respiratory function (10-15). However, the moment at which treatment should be started in adult onset patients is still a matter of discussion. Guidelines recommend starting the treatment only in symptomatic patients with muscle weakness involving skeletal or respiratory muscles (16). It is not recommended to start the treatment in asymptomatic patients or in patients with fatigue, muscle pain or other symptoms in which muscle weakness is not demonstrated using clinical examination or muscle function tests.

However, there is compelling evidence suggesting that earlier treatment in adult onset Pompe patients can result in better outcomes. From a pathological point of view, accumulation of glycogen inside muscle fibres leads to disruption of myofibrils, accumulation of autophagic vesicles and eventually muscle fibre loss and substitution of muscle by fatty tissue (17). The process of fibro-fatty expansion is irreversible and can lead to permanent weakness and disability (18). Therefore, therapeutics effort should be committed to avoiding or slowing the process of muscle fibre degeneration and fatty tissue

expansion. Although muscle fibre loss and fatty expansion produces muscle weakness, glycogen accumulation per se is probably also associated with a decrease in muscle strength and endurance. Accumulation of glycogen inside muscle fibres disrupts the normal function of the myofibrils and leads to muscle weakness. This is, in theory, a process that can be reversed by treatment with ERT. In fact, it has been demonstrated that glycogen vesicles are reduced in muscles of ERT treated adult onset patients using repeated muscle biopsies (19). Therefore, earlier treatment of adult patients in which glycogen deposition is present but fatty infiltration is not yet detected should delay muscle degeneration and avoid permanent motor and respiratory disability. However, this fact should be demonstrated through a clinical trial. One of the main problems we have in conducting this type of clinical trial is that at this moment we do not have any non-invasive test capable of identifying and/or quantifying skeletal muscle glycogen. There is pressing need for a non-invasive, repeatable test for initial assessment and follow-up of patient muscle glycogen content in a clinical trial.

Muscle MRI has become a useful tool to study patients with muscle disorders (20-22). There are at present several imaging sequences that allow the study of different components of the muscle degeneration process (23). For example, T1 weighted imaging (T1w) demonstrates the presence of fatty infiltration (23-25). This sequence has been widely used to study patterns of muscle fatty infiltration in patients with muscle dystrophies, which are characteristic of mutations in specific genes (26). However, T1w does not provide a quantitative measure of fat content and is not useful to follow-up muscle degeneration in patients with muscle disorders (27). To address this, Dixon-method sequences quantify the exact amount of fat present in a region of interest of the muscles using a software. Dixon is able to identify significant changes in the amount of muscle fatty infiltration in several muscle disorders including Duchenne muscle dystrophy or facio-scapulo-humeral muscle disease (28-31). We have also demonstrated its use in patients with adult onset Pompe disease. Using repeated Dixon studies, we have established that fat infiltration increases by a mean of about 2% in thigh muscles of ERT treated LOPD patients per year of follow-up (32, 33). However, we did not detect changes in fat infiltration in 8 asymptomatic patients after a 4 year period of time (34). Moreover, although two of these patients developed muscle weakness during the follow-up, Dixon did not show an increase in fatty infiltration in their skeletal muscles. These two patients have reversed their muscle weakness after being treated with ERT. In our opinion, this fact supports our idea that an increase in glycogen accumulation inside skeletal muscle fibres disrupts muscle function. Therefore, to identify patients in which glycogen accumulation progresses quickly in a short period of time is crucial to start treatment early in order to avoid muscle degeneration and, consequently irreversible fatty tissue expansion associated with permanent muscle weakness and disability.

In this study we aim to use an MRI sequence known as ^{13}C-spectroscopy to identify glycogen in the skeletal muscles of patients with LOPD who are still pre-symptomatic or who have minor symptoms. ^{13}C-spectroscopy has previously demonstrated identification of glycogen in different organs of the body, including skeletal muscle. There are only a few studies in patients with glycogenosis, which are diseases characterized by the accumulation of glycogen inside the muscle fibres. There is only one study that has included LOPD patients. This study was published in 2003 and included 11 patients with LOPD. The mean levels of glycogen in muscles were higher than controls and glycogen was considered higher than the mean in 7 out of the 11 patients. However, that study did not include pre-symptomatic patients and was transversal, not allowing the investigators to know if there were differences in the amount of glycogen over time.

We aim to use a second MRI sequence known as T_2-weighted imaging that provides information about composition of the skeletal muscles. Glycogen accumulation in muscles may be associated with a change the T_2 relaxation properties of water in muscle. We will assess whether muscle T_2 is different in patients compared to healthy controls.

Muscle MRI in Pompe disease

Additionally, we will use a series of muscle function tests to confirm that patients still do not have any sign of muscle damage, and can be considered pre-symptomatic, or these are minor with patients scoring higher than 30 points in the RPact scale (see above). We are selecting this population of patients with LOPD as they are typically non-treated with ERT following the accepted guidelines. As discussed before, it is crucial to have a biomarker that can help us to identify patients at the earliest stages of disease progression who are starting to develop muscle damage because of the accumulation of glycogen in their muscles and can respond better to ERT. Our main hypothesis is that both ^{13}C-spectroscopy and T_2 imaging can be useful to identify accumulation of glycogen in the muscle tissue of these patients and be used to monitor changes in the concentration of glycogen in the tissue.

2 RATIONALE

There is an approved treatment for LOPD that consists of repeated infusions of the recombinant enzymatic therapy with alpha-glucosidase (Myozime) that is considered to be standard of care. Experts in the field recommend starting the treatment in patients that have weakness identified on clinical examination. However, in several cases patients have lost a considerable amount of muscle tissue when they start manifesting muscle weakness as it has been shown in studies using muscle MRI to quantify muscle lost and replaced by fat in large cohorts of patients with LOPD. There is not any treatment yet able to recover muscle fibres lost and replaced by fat and the accumulation of fat in the muscle tissue is associated with permanent weakness and disability. Taking into account that accumulation of glycogen in the tissue is thought to be the main cause behind muscle damage leading to muscle degeneration and replacement by fat, having a non-invasive test able to measure glycogen content could identify patients at risk of muscle degeneration, even before they start presenting muscle symptoms or coinciding with the presence of minor muscle weakness. This could lead to earlier treatment avoiding or delaying considerably permanent weakness and disability.

Here we will use ^{13}C-spectroscopy to identify and quantify glycogen content in muscle tissue. We will use this sequence to quantify the presence of glycogen in pre-symptomatic or early symptomatic patients and correlate the results of muscle function tests with the accumulation of glycogen in the muscles. We will also measure the T_2 of muscle water to gauge whether this parameter is different in patients compared to controls. T_2 imaging is widely available in conventional hospitals across the world, while ^{13}C-spectroscopy is commonly not available in conventional hospitals as it uses hardware and scanner capabilities not common on hospital scanners.

3 THEORETICAL FRAMEWORK

Muscle glycogen content has traditionally been measured by invasive biopsy methods, though **^{13}C-magnetic resonance spectroscopy** offers a non-invasive measurement by direct MRI-based detection of a unique signal from natural abundance ^{13}C in glycogen. This method has been used in clinical research studies of energy storage in health (e.g. exercise physiology) and disease (eg. glycogen storage diseases such as Pompe and in studies of diabetes) (35-37). ^{13}C-MR spectroscopy requires hardware that is non-standard and thus unavailable on the majority of clinical MRI scanners (38), yet available and well established at the Newcastle Magnetic Resonance Centre. An additional aim is to measure the T_2 of muscle water to gauge whether this parameter is different in patients compared to healthy controls. Water T_2 is increased in the presence of free water and it has been

found to be related with muscle inflammation but also with necrosis and denervation (39-41). In Pompe disease, T_2 has been found to be increased in some patients, both pre-symptomatic and symptomatic.

The MRI sequences could be useful to identify and quantify glycogen in skeletal muscles in patients with late onset Pompe disease. We are especially interested in using this technology in patients that are still pre-symptomatic or who have started with symptoms recently, to know if ^{13}C-MR sequences could be useful to monitor disease progression in this group of patients, which are commonly not presenting relevant clinical symptoms.

4 RESEARCH QUESTION/AIM(S)

Identification of glycogen accumulated inside skeletal muscle fibers using MRI can be challenging. We hypothesize that ^{13}C spectroscopy can quantify skeletal muscle glycogen content in patients with Pompe disease.. If so, muscle MRI could be useful as a biomarker of the progression of the disease in early stages. It is important to point out that many patients are not aware that they are progressively losing skeletal muscle until they have clear weakness that in many cases is irreversible and leads to permanent disability. If we succeed, muscle MRI could be considered a reliable biomarker which is useful as a follow-up tool of the progression of the disease and to monitor the effect of therapies in its progression.

4.1 Objectives

The primary objective of the study is to determine the difference in skeletal muscle glycogen content between patients with Pompe Disease and healthy controls by ^{13}C-MR spectroscopy.

The secondary objectives are:

1) to determine whether change in muscle glycogen content can be observed in Pompe disease patients over a 1 year period by ^{13}C-MR spectroscopy
2) to measure the T_2 of muscle water to gauge whether this parameter is different in patients compared to healthy controls.

4.2 Outcome

1. Confirm the capacity of ^{13}C spectroscopy to identify and quantify glycogen in the muscle fibres of patients with Pompe disease.
2. Identify muscle water T_2 is different in patients compared to healthy controls.
3. Find out if there is a correlation between levels of muscle glycogen quantified by ^{13}C spectroscopy and results of muscle function tests.

5 STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYSIS

5.1. Overall Study design

This prospective observational study will consist of: clinical assessments, collection of blood samples, a series of muscle function tests and collection of patient reported outcome measures, performed at the Newcastle Clinical Research Facility. MRI scans will be performed at the Newcastle Magnetic Resonance Centre. The schedule of events is included in Appendix 1.

The participants with Pompe disease will attend two visits over one year (baseline and year 1). During the visit, we will perform a clinical assessment, a series of muscle function tests, we will collect patient reported outcome measures, collect blood samples and perform an MRI scan. Muscle function tests will include muscle strength and muscle performance.

Healthy controls will attend a single study visit only at baseline which will include clinical assessment, a series of muscle function tests, collection of a participant reported outcome measure, collection of blood samples and an MRI scan. Muscle function tests will include muscle strength and muscle performance.

Muscle strength will be studied using the Medical Research Council Scale (MRC) and Hand Held Dynamometry for specific pelvic, paraspinal and thighs muscles. Muscle performance will be tested using the 100 metre timed test, the North Star Assessment for limb girdle type muscular dystrophies and the Timed Up&Go test. We will specifically evaluate pelvic muscles which are commonly affected from earlier stages in Pompe using the time stand up from a kneeling position, squatting, standing on one leg and standing up and down step tests. Rasch-built Pompe-specific activity (R-Pact) scale (47) test will be used as a patient reported outcome measures (appendix 2).

A blood analysis obtaining serum and plasma for further examination will be acquired and stored at -80º C.

5.2. Storage and Analysis of Samples

The study will include a collection and biobanking of blood samples. The samples will be used in the future to study potential biomarkers. The study site will ensure that samples are appropriately labelled in accordance with the study procedures to comply with the Data Protection Act and UK GDPR. Biological samples collected from participants as part of this study will be transported, stored, accessed, and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and associated Codes of Practice.

5.3. MRI methods

MRI Images will be acquired using a Philips 3T Achieva MR scanner equipped with a $^{13}C/^{1}H$ surface coil (PulseTeq Ltd, UK) and multinuclear MR capability. The scan protocol will comprise acquisition of survey, multiecho-T_2 and 3-point Dixon scans using Philips ^{1}H receive array coils, and a ^{1}H-decoupled ^{13}C spectroscopy scan for measurement of muscle glycogen content. The acquisition of the images will be centred on the thigh muscles, and the ^{13}C spectroscopy coil will be placed over the anterior surface of the thigh, with the main aim to measure glycogen content within the vastus lateralis muscle.

The MR scan session duration is approximately 45 minutes.

5.4. Post-processing and quantification of the MR images

Scan data will be anonymized before processing and analysis. Muscle MRI will be analysed by a blinded reader who will not have any clinical information regarding the patient. The following measurements will be performed:

- Quantitation of ^{13}C spectroscopy signals to determine glycogen content of the *vastus lateralis* muscle.

- Calculation of muscle water T_2 from T_2-weighted images acquired with two or more echo times. Pixel-wise maps of T_2 will be calculated. Quantitative measurements of T_2 will be obtained from each parametric map obtained by drawing a region of interest over the vastus lateralis to determine a spatially-localised measure of muscle water T_2.

- Fat infiltration measurements will be computed according to the content of water-fat Dixon images over the same regions analyzed using ^{13}C spectroscopy and T_2 measurements.

5.5. Statistical analysis

Mann-Whitney U test will be used to analyse if there are significant differences in the glycogen content identified using ^{13}C spectroscopy in patients with late onset Pompe disease and in controls in muscles of the thighs at baseline. Moreover, Wilcoxon test will be performed for repeated measures in order to identify significant changes from baseline to year 1 assessment in late onset Pompe patients. Differences in the distribution of signal among the three muscle compartments comparing controls and Pompe will be performed using ANOVA studies. Additionally, we will study if there are differences in the distribution of T_2 signal among the compartments at baseline and year 1 using Wilcoxon tests for repeated measures. The aim is to establish if there are correlations between muscle glycogen content and results muscle function using Spearman tests. The results of all statistical studies will be considered significant if P is lower than 0.05. Statistical analysis will be performed using IBM SPSS® Statistics software version 21.

6 STUDY SETTING

This is a single centre prospective observational study to be performed at Newcastle upon Tyne Hospitals NHS Foundation Trust involving patients with Pompe disease and controls.

Patients with Pompe will be identified through different strategies including:

a. Identification of potential participants from those that are followed up in the Highly Specialized Service on Limb Girdle Muscular Dystrophies in Newcastle.
b. Referrals by clinicians at other centres, including but not limited to the National Reference Centre for metabolic diseases in Manchester.
c. Advertising the study though the Pompe patient association in UK.

As controls, age and sex matched healthy volunteers will be included in the study.

Healthy controls will be identified through posters, leaflets, website advertisements and mail outs. This information will be distributed within Newcastle University and the Trust.

All participants included in the study will attend the Clinical Research Facility at the Royal Victoria Infirmary in Newcastle for screening, informed consent to participate, and clinical assessments, blood collection and muscle function tests. The acquisition of MRI scan data will take place at the Newcastle Magnetic Resonance Centre.

7 SAMPLE AND RECRUITMENT

7.1 Eligibility Criteria

7.1.1 Inclusion criteria

Patients with Pompe disease

Inclusion criteria for the study will be:

1) Diagnosis of Pompe disease based on recommendations recently proposed by the European Pompe Consortium (16): reduced enzymatic activity in leukocytes, fibroblasts or skeletal muscle and/or by the presence of two mutations in the in the GAA gene following the diagnostic (20);

2) Age: patients aged 12 years and older.

3) No contraindications to MRI;

4) No symptoms of muscle weakness or mild symptoms. Patients should score higher than 30 points on the RPact scale.

5) Willingness to complete all muscle function tests at baseline and year 1 visit.

Healthy Controls

1) Male and female age that matched with the patients included. Controls included in this study will be 12 years old and older.

2) No contraindications to MRI;

3) Willingness to complete all study assessments.

7.1.2 Exclusion criteria

1) Contraindications for MRI such as having a metallic protesis, pacemaker or any other device that makes the completion of an MRI impossible.

2) Not willing to complete all muscle function tests both at baseline and year 1.

3) Having claustrophobia or other condition that could limit the capacity of the patient for being located inside the MRI

4) Inability to lie supine during less than 45 minutes

5) Pregnancy (for female participants of child bearing age only)

6) Not being able to understand and speak English

7) Study team decision that it is not in the best interests of the patient to participate in the study

7.2 Sampling

Muscle MRI in Pompe disease

This is an observational study which will include 10 patients with genetic confirmation of Pompe disease and with minor symptoms of muscle weakness that fulfil the inclusion criteria, plus 10 controls who will be age and sex matched with Pompe patients.

7.2.1 Size of sample

We will study 10 pre-symptomatic or early symptomatic late onset Pompe Disease (LOPD) patients using 13C spectroscopy, multicomponent T2 and 3 point Dixon muscle MRI sequences. We will include 10 age and sex matched controls. LOPD patients will attend scans on two occasions, 12 months apart. Age and sex matched controls will attend for one scan only.

7.2.2 Sampling technique

This study is purely observational and there is no randomization into groups as there is no intervention to be tested. All patients eligible will be included in the study. The recruitment period will finish when we reach the number of 10 patients and 10 controls eligible for the study.

Taking into account that Pompe disease is a rare condition and that we are aiming to include patients that are pre-symptomatic or in early stages of disease progression, a sample of 10 is realistic in terms of availability of patients, and large enough to identify if ^{13}C spectroscopy able to detect glycogen inside muscle fibres of affected patients.

7.3 Recruitment

Patients with Pompe disease will be identified using local databases, national disease registries and professional networks. Identified patients will be contacted via telephone, letter or email and if they are interested in the study and they will be sent further information on the study to consider. We will ask patients included in the study for their permission to inform their GPs.
Healthy controls will be recruited locally if possible through posters, leaflets, website advertisements and mail outs. This information will be distributed within Newcastle University and the Trust. Anyone potentially interested in participation will be asked to contact the study team in order to establish whether they meet eligibility criteria and are potentially suitable to take part in the study. Once this is confirmed they will be sent further information on the study to consider.
Age appropriate information and consent / assent form will be provided to participants so that they have a thorough understanding of what the study involves and so that they are fully aware that participation is voluntary. Once potential participants have been given time to consider their involvement and express interest, they will be invited to come to the site where general procedures used during the study will be explained, and participants will be screened for any counter-indications to MRI (including a history of psychiatric or neurological disorders). Written informed consent or assent will be obtained from all the participants and legal guardians where appropriate.

7.3.1 Sample identification

Patients will be identified by the Principal Investigator of the study, using different strategies, including:

a. Reviewing clinical records of the cohort of patients followed at the Newcastle site

b. Contacting colleagues across UK who follow patients with Pompe disease, especially colleagues working at national reference centres for metabolic diseases

c. Contacting Pompe disease patient association in UK to advertise the study

As controls age and sex matched healthy volunteers will be included in the study. Healthy controls will be identified through posters, leaflets, website advertisements and mail outs. This information will be distributed within Newcastle University and the Trust.

Potential study participants that fulfil the inclusion criteria and are interested in participating will be first contacted by phone, email or videoconference by the Principal Investigator or a delegated member of the study team and an overview of the study will be discussed. If potential participants are interested in taking part in the study they will be invited to come to Newcastle to discuss the imaging protocol and the different tests to be performed during the visits. If they are not willing to come to Newcastle and prefer a video call, this option will be made available for them and will be performed through the "Attend anywhere platform" which is used by the NHS for remote assessments. There will be time for questions from potential study participants and their relatives and the protocol will be reviewed carefully. If the person agrees to participate, they will be invited to a screening visit that will take place in Newcastle.

7.3.2 Consent

Informed consent will be obtained prior to the participant undergoing any activities that are specifically for the purposes of the study.

Written informed consent will be taken by the principal investigator or a trained and delegated member of research team, in accordance with GCP guidelines for clinical research.

Potential participants or their parent/guardians will be given a copy of the participant information sheet outlining what their participation in the study will involve. They will be advised to review this information and to discuss the study with their family, friends or their GPs.

If the potential participant is interested in taking part in the study, the study will be explained to them in full by a member of the study team.

The participant will be assured that consent is entirely voluntary and they can withdraw consent at any time without providing a reason and their usual care will not be affected. The participant or their parent/guardian will be given the opportunity to ask questions.

Once all questions are answered to participant's satisfaction and they agree to take part in the study, they will be asked to sign and date the informed consent form. The participant will be given a copy of the information sheet and consent form for their records.

Children under the age of 16 will be provided with an alternative copy of the Participant Information Sheet that is suited to their age. If they and their parent/guardian are interested in taking part in the study, the participant will be asked to sign an Informed Assent Form, with the assistance of their parent/guardian. The parent/guardian will also sign a dedicated informed consent form. A copy of both forms will be provided to the participant and their parent/guardian.

8 SAFETY REPORTING

8.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death • Is life-threatening* • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect • Other important medical events that jeopardise the participant or require intervention to prevent one of the above consequences * - life-threatening refers to an event in which the participant was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

8.2. Assessment of Adverse Events

Each adverse event will be assessed for severity, causality, seriousness and expectedness as described below.

8.2.1 Severity

Category	Definition
Mild	The adverse event does not interfere with the participant's daily routine, and does not require further procedure; it causes slight discomfort
Moderate	The adverse event interferes with some aspects of the participant's routine, or requires further procedure, but is not damaging to health; it causes moderate discomfort

Severe	The adverse event results in alteration, discomfort or disability which is clearly damaging to health

8.3. Recording and Reporting AEs and SAEs

Chief Investigator/Principal Investigator or an appropriately delegated member of the study team will check adverse events (AEs) as part of the clinical assessment when participants attend for visit. They will ensure that all serious adverse events (SAEs) are recorded and reported to the Sponsor and the Funder within 24 hours of becoming aware of the event. All SAEs must be recorded on a serious adverse event (SAE) form. The Chief Investigator/Principal Investigator or designated individual will complete an SAE form and the form will be emailed to the Funder and the Sponsor within 1 working day of becoming aware of the event.

Chief Investigator/Principal Investigator or an appropriately delegated member will ensure that AEs and ARs are recorded in the case report forms and reported to the Sponsor in line with the requirements of the protocol.

9 ETHICAL AND REGULATORY CONSIDERATIONS

9.1 Assessment and management of risk

9.1.1. Potential risks

There are no invasive procedures involved in this study.

MRI scan

MRI scanning is a safely repeatable medical imaging method that does not involve use of ionising radiation. On arrival at the MR Centre the study participant will be screened for MRI contraindications by a member of the radiography team. The scan involves lying still within a noisy confined space, which some participants find uncomfortable. Efforts will be made to ensure that the patient is positioned properly and comfortably. Patients will be given ear protection to wear during the scan, and may also listen to music. A parent will be allowed in the scanning room if the participant is under 16. The scan will be performed without use of intravenous contrast agents or sedation.

Acquisition of 13C spectroscopy scans involves modifications to the MRI scanner's software and the use of a custom sensor (an "RF coil", purchased from a commercial supplier) for ^{13}C measurements. These modifications are performed under a research agreement with the MRI scanner manufacturer, who provide the tools and scanner capabilities necessary to make these modifications. The introduction of these changes to deliver advanced scanner capabilities represents a modification to a CE-marked device, and the resultant scan data are used only for research purposes and are not for diagnostic use. The modifications to scanner software or hardware have been risk assessed and tested to ensure scanner operation is within regulatory limits, and we have performed numerous ^{13}C spectroscopy measurements in this manner in studies under NHS HRA ethical approvals over the past 15 years.

Personal information collected by the study plan will be strictly confidential, except as required by law, but will be made available to the subject and his/her physician in response to a specific request from the subject. There will be no personal identification of subjects in scientific outputs from the study. The consent form will be kept in the patient research file and stored inside a secured filing cabinet accessible only to the investigators. Data will be stored in a confidential manner both through the use of a numbering system (a number will be assigned to the data from a given subject instead of the subject's name) and through the security of the files and computer systems.

The MRI scans are being collected for research purposes and are not routinely reviewed by a radiologist. However, in the event that an abnormality is suspected by the supervising radiographer, a radiologist's report will be sought and provided to the Chief Investigator/Principal Investigator for further action. The study participant information sheets state that MRI scans are for research purposes and are not routinely reviewed by a radiologist.

Blood collection

Blood collection does not pose a significant risk. There is a small risk that the participant may experience pain and/or bruising at the site on the arm where blood is taken. The use of a needle can carry some discomfort and a small risk of bruising, bleeding, fainting, and a very small risk of infection where the skin is broken. In case of faintness, participants will be instructed to lie down immediately to avoid possible injuries and then notify the study personnel.

Muscle function tests

Muscle function tests do not pose a significant risk. Patients and controls will be asked to perform a series of activities such as walking or stretching a joint again a resistance and will be monitored by physiotherapists experts in the field throughout the whole duration of the test. None of the muscle function tests are related to potential adverse events, however as patients are requested to walk there is an increased risk of fall when completing the muscle function tests.

9.1.2. Potential Benefits for Participants

Participants are not expected to directly benefit from this study. All travel and meal expenses will be reimbursed for the patient and one accompanying person.

9.2 Research Ethics Committee (REC) and other Regulatory review & reports

A favourable opinion will be sought from Research Ethics Committee (REC) and approvals from the NHS Health Research Agency and sponsor (Newcastle upon Tyne Hospitals NHS Foundation Trust) will be in place prior to study start.

Substantial amendments that require review by REC will not be implemented until that review is in place and other mechanisms are in place to implement at site. All correspondence with the REC will be retained. It is the Chief Investigator's responsibility to produce the annual reports as required. The Chief Investigator will notify the REC of the end of the study. An annual progress report (APR) will be

submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended. If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination. The Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC within the required timeframe.

9.2.1. Regulatory Review & Compliance

Before the site can enrol patients into the study, the Chief Investigator/Principal Investigator or designee will ensure that appropriate approvals from participating organisations are in place. Specific arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance.

9.2.2. Amendments

For any amendment 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.

9.3 Peer review

The study has been peer reviewed by the Funder, patient organisation and 2 individual experts in the field from other institutions including : Dr. Claudia Nuñez a radiologist expert in Pompe disease from Hospital Sant Pau in Barcelona, Spain; Dr. Giorgio Tasca, a neurologist with a large experience in neuromuscular diseases from Ospedale A. Gemelli in Rome, Italy and 2 UK patients organization including the Pompe Support Network and the Association for Glycogen Storage Disease (AGSD).

9.4 Patient & Public Involvement

Patients have and will be involved in the research process relating to this study. Patients are involved in the assessment of the acceptability of the research and the design of the research by being involved in the protocol review. Patients with Pompe disease, who meet eligibility criteria, will be invited to participate in the study. Patient organisations will be involved in the dissemination of findings as the study results will be presented at the patient organisation's annual conference.

9.5 Protocol compliance

Protocol deviations, non-compliances, or breaches are departures from the approved protocol will be document and reported as per NJRO SOPs.

9.6 Data protection and patient confidentiality

Study data will be handled in the strictest confidence in compliance with the Data Protection Act 2018 and UK GDPR. Chief Investigator/Principal Investigator and members of study team will comply with the requirements of the data protection regulations and laws with regards to the collection, storage, processing and disclosure of personal information. All data gathered on study subjects will be given a code rather than a participant's name or date of birth being used, and no data will leave the site that contains any of the participants' personal details. Clinical data from the study will be collected

electronically using REDCap system. MRI scan data will be anonymized and stored on University network storage with access control limited to those with study involvement. Data will be stored on password protected computers and paper source documents will be contained with secure storage units with restricted access Data from the project will be stored securely, in compliance with the relevant regulations and laws for 5 years.

9.7 Indemnity

The sponsor will provide indemnity in the event that trial participants suffer negligent harm due to the management of the trial. Indemnity will be provided under the NHS indemnity arrangements for clinical negligence claims in the NHS.

Newcastle University as the protocol authors will provide indemnity in the event that trial participants suffer negligent harm due to the design of the trial.

The study site will provide indemnity in the event that trial participants suffer negligent harm due to the conduct of the trial at their site under the NHS indemnity arrangements for clinical negligence claims in the NHS. NHS Organisations must ensure that site staff without substantive NHS contracts hold honorary contracts to ensure they can access patients and are covered under the NHS indemnity arrangements.

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

9.8 Access to the final study dataset

The data will be the property of the Chief Investigator and Co-Investigator(s) and access to the final study dataset will be limited to those individuals.

The dataset might be used for future research and this will be undertaken only with the consent of the study participants. All patient documentation for this study, including participant information sheets and consent documentation, will reflect the future use of these data in research.

9.9. Archiving

Study documentation will be archived in line with all relevant legal and statutory requirements. Archiving will be carried out according to NJRO SOP.

10 DISSEMINIATION POLICY

10.1 Dissemination policy

The study will be registered in the public database Clinicaltrials.gov.

The data arising from the study will be owned by Chief Investigator and Co-Investigator(s).

On completion of the study, the data will be analysed and tabulated and a Final Study Report prepared.

The full study report can be accessed by request addressed to the study Chief Investigator.

The publication and dissemination of the results of the study will be the responsibility of the Sponsor. However, in order to ensure that the Funder is able to make comments and suggestions, where pertinent, the data, literature abstracts concerning the study, reports of the study, proposed

manuscripts concerning the study and any materials for public dissemination concerning the study will be submitted to the Funder for review prior to submission for publication and/or public dissemination.

The Sponsor will ensure, that the Funder's support for the study is acknowledged in any publication by the Sponsor and/or the Chief Investigator/Principal Investigator concerning the study.

Study participants will be informed about the results of our project. A report summarising the main results of the study once the final analysis is completed will be sent to the participants that have consented to be contacted to receive the report.

10.2 Authorship eligibility guidelines and any intended use of professional writers

Authors of the final report or of any scientific publication (poster, presentation in conferences, papers) will be the team of professionals that have actively participated in the:

1. Design and setup of the study
2. Identification and recruitment of patients for the study
3. Performance of the clinical assessment of the patients, including clinical interview and muscle function tests
4. Analysis of the muscle MR data obtained with the study
5. Analysis of the study data

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12. APPENDICIES

12.1 Appendix 1 – Schedule of Procedures

Procedures	Visits	
	Screening/ Baseline	Visit 1 (year1) *)
Informed consent	X	
Demographics	X	X
Medical history	X	X
AE and SAEs review	X	X
Muscle strength assessment (Medical Research Council Scale (MRC) and Hand Held Dynamometry for specific pelvic, paraspinal and thighs muscles)	X	X
Muscle performance (100 meters walk/run test and the Timed Up&Go test)	X	X
Collection of patient reported outcome measures (Rasch-built Pompe-specific activity (R-Pact) scale)	X	X
Blood collection	X	X
MRI scan	X	X

* Only patients with Pompe disease will attend Visit 1. Controls will not attend this visit.

12.2 Appendix 2- Final R-PAct questionnaire

	Are you able to:	No (0)	Yes, but with some difficulty (1)	Yes, withouth difficullty (2)
1.	Comb your hair			
2.	Eat (swallow, chew)?			
3.	Pull on a pair of trousers (without closures)?			
4.	Prepare a meal?			
5.	Take a shower?			
6.	Reach for and grasp an object above your head?			
7.	Step over a threshold or negotiate obstacles in your path?			
8.	Turn over in bed?			
9.	Walk on an uneven surface?			
10.	Stand up from a seated position?			
11.	Walk more than 1km?			
12.	Walk up and down a complete set of stairs?			
13.	Bend over to pick something up off the ground and then stand up again?			
14.	Walk at a rapid rate?			
15.	Garden or carry out tasks in and around your yard?			
16.	Practice a sport?			
17.	Bend at the knee (squat) and then stand up again?			
18.	Run (for example to catch a train)?			