

# **Translational Research Studies in Exercise-related Muscle Disorders**

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I, Renata S. Scalco, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature

Date

*To all patients with rare neuromuscular diseases, who have inspired this work*

# 1. Abstract

Translational research is the process that transfers knowledge from basic sciences to the clinical setting. This PhD thesis translates knowledge gained from animal model research in hypokalaemic periodic paralysis (HypoPP) and McArdle disease to humans affected by these conditions to identify new treatment options for both diseases. The efficacy of two compounds, sodium valproate and bumetanide, were assessed for the first time in humans with McArdle disease and HypoPP, respectively. For HypoPP, the role of the McManis test as an outcome measure was explored in a randomised, double-blind, placebo-controlled phase II clinical trial with a cross-over design. For McArdle disease, several outcome measures were explored in an open-label proof-of-concept phase II study.

2 mg bumetanide was not effective to abort a focal attack of weakness in an immobilised hand in the majority of the trial participants with HypoPP, but data presented here supports further studies of bumetanide in this population of patients. Extending the isometric exercise period to 10 minutes increased the sensitivity of the McManis test, and frequent compound muscle action potential (CMAP) amplitude assessments were shown to be useful in assessing both efficacy and safety.

20 mg/kg/day sodium valproate was ineffective in stimulating the expression of the brain glycogen phosphorylase enzyme in skeletal muscle of people with McArdle disease. Based on these results, further research into VPA as a treatment for McArdle disease is discouraged. The combination of several outcome measures contributed to data interpretation and should be considered in future studies exploring treatment efficacy in McArdle disease.

The results of this research should contribute to future clinical trials in the field of exercise-related muscle disorders and provide valuable insights for translational research.

## 2. Impact Statement

Currently, there are few pharmacological treatments for rare neuromuscular diseases. This research comprised two investigator-initiated phase II clinical trials developed to address this limitation for two such conditions: hypokalaemic periodic paralysis (HypoPP) and McArdle disease.

The first clinical trial provided Class 1 evidence that 2 mg bumetanide was not effective to abort a focal attack of weakness in an immobilised hand in the majority of the trial participants with HypoPP. However, data presented here show that a beneficial effect cannot be entirely excluded, supporting further studies of bumetanide in this population of patients. This study also provides safety data for 2 mg bumetanide in normokalaemic HypoPP patients for the first time. In addition, this clinical trial illustrates the role of the McManis test as an outcome measure in clinical trials for HypoPP. Extending the isometric exercise period to 10 minutes increased the sensitivity of this diagnostic tool in the assessed participants, and further studies should be performed to confirm the finding in a larger cohort of patients. Frequent compound muscle action potential (CMAP) amplitude assessments were shown to be useful in assessing safety.

The second clinical trial showed that oral treatment with sodium valproate (valproic acid, VPA) was ineffective in stimulating the expression of the brain glycogen phosphorylase enzyme in skeletal muscle of people with McArdle disease. Based on these results, further research into VPA as a treatment for McArdle disease is discouraged. This project also includes data relating to the safety of VPA treatment for people affected by McArdle disease. Based on findings in this study, it is reasonable to say that McArdle disease should not be considered a formal contraindication for VPA use in patients with a strong clinical indication for its prescription, such as epilepsy. In terms of study assessments, this research shows that the combination of several outcome measures contributed to data interpretation and should be considered in future studies exploring treatment efficacy in McArdle disease.

The combination of highly specialised clinical services, an academic institution, an international registry and a patients' association generated a valuable partnership for the promotion of clinical research. This was one of the most important insights gained in this research. Such partnerships may be key to overcoming the lack of treatment options for patients with very rare diseases.

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## 6. List of Abbreviations

12MWT: 12-minute walk test

ACRT: Association for Clinical Research Training

ACZ: acetazolamide

ADM: abductor digiti minimi

AE: adverse event

AGSD-UK: Association for Glycogen Storage Disease UK

AMI: amiloride

ATP: adenosine triphosphate

AUC: area under the curve

A&E: accident and emergency

BMI: body mass index

BP: bodily pain

B-P: treatment sequence bumetanide-placebo

*CACNA1S*: gene – calcium voltage-gated channel subunit alpha1 S

CF: consent form

CI: confidence interval

CK: creatine kinase

Cl: chlorine

CMAP: compound muscle action potential

CRF: case report form

DH: dominant hand

ECG: electrocardiogram

EMA: European Medicines Agency

FBC: full blood count

FDA: Food and Drug Administration

GH: general health

HDACI: histone deacetylase inhibitor

HR: heart rate

HyperPP: hyperkalaemic periodic paralysis

HypoPP: hypokalaemic periodic paralysis

HypoPP1: hypokalaemic periodic paralysis due to *CACNA1S* mutations

HypoPP2: hypokalaemic periodic paralysis due to *SCN4A* mutations

HSS: Highly Specialist Services

IMP: investigational medicinal product

K: potassium

KI: knock-in

LFT: liver function tests

MCS: mental component score

MH: mental health

MHRA: Medicines and Healthcare Products Regulatory Agency

MRC: Medical Research Council

Na: sodium

NDH: non-dominant hand

NGS: next-generation sequencing

NHNN: National Hospital for Neurology and Neurosurgery

NHS: National Health Service

NKCC: Na-K-2Cl co-transporter

NRES: National Research Ethics Service

PAS: periodic acid-Schiff

*PYGM*: muscle glycogen phosphorylase gene

PCS: physical component score

PF: physical functioning

PIS: participant information sheet

P-B: treatment sequence placebo-bumetanide

RE: role – emotional

RM: rhabdomyolysis

REC: Research Ethics Committee

RPE: rating of perceived exertion

RP: role – physical

RPP: rate of perceived pain

R&D: research and development

SAE: severe adverse event

SCN4A: gene – sodium voltage-gated channel alpha subunit 4

SD: standard deviation

SF: social functioning

SF-36: Short Form 36

SPC: summary of product characteristics

SPL: spironolactone

TR: translational research

UCL: University College London

UCLH: University College London Hospitals

UK: United Kingdom

URTI: upper respiratory tract infection

USA: United States of America

U&E: urea and electrolytes

V: vitality

VBG: venous blood gas

VO<sub>2</sub>peak: peak cardiorespiratory conditioning or peak oxygen uptake

VPA: sodium valproate

V1: visit 1

V2: visit 2

V3: visit 3

V4: visit 4

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*Eu senti saudades todos os dias!*

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Mr Paul Bassett devised the statistical analysis plan; Dr Federico Ricciardi and Dr Aidan O'Keeffe performed the statistical analysis; Dr Jasper Morrow revised the study design, grant application, the study protocol, analysis of data and the final manuscript draft; Ms Iwona Skorupinska and Dr Andreea Manole processed blood samples; Dr Emma Mathews advertised the trial at the channelopathy clinic and revised the scientific manuscript; Muscular Dystrophy UK, advertised the trial; Clinical Research and Development Committee Research Funding from the UCLH Charities – Fast Track Grants, funded the trial.

### **Sodium Valproate Clinical Trial**

Dr Ros Quinlivan supervised all the activities I have been involved in, designed the study and wrote the study protocol / PIS / CF.

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## 9. Contribution

I was the main researcher responsible for conducting all stages of both trials, from screening to completion. I was responsible for all trial assessments, including recruitment, performing the exercise assessments, evaluating adverse events and reporting safety data. I have collected all trial data, interpreted the study findings and have written the scientific manuscripts for both studies. In both studies I applied for ethics approvals and wrote Research Ethics Committee (REC) reports. I presented the study development at all trial Steering Committee meetings. In both trials I was responsible for assessing drug compliance.

In the bumetanide trial, I also contributed to the study design and outcome measure selection. I wrote the clinical trial protocol and all related documents, including the participant information sheet, consent form, study diaries and case report form. I submitted the study for ethics committee evaluation. I also completed the grant application form.

In the sodium valproate trial, I also worked as a trial coordinator. My responsibilities related to trial management, ensuring the study was conducted as per the Good Clinical Practice guidelines. Related activities included organising and updating the Trial Master File, direct interaction with the Joint Research Office, providing annual reports, training the study team, among others. I also analysed muscle biopsies.

## 10. Introduction

Developing a new drug is a lengthy and costly process, which constrains the development of new pharmacological treatments for rare conditions – a category that includes exercise-related muscle disorders. Consequently, a large proportion of people with rare neuromuscular conditions have no treatment option to satisfactorily improve their symptoms and reduce progression of the underlying disease. The combination of research projects and efforts to overcome shortcomings like these is known as translational research.

### Introduction to Translational Research

Translational research (TR) is the process of transferring knowledge from basic sciences to the clinical setting. TR provides evidence-based results that may directly change ongoing clinical practice.

An example of TR is the discovery of a new pharmacological treatment for a specific disease. It generally involves basic scientific study that explores the pathophysiology of a condition. This knowledge is later used to identify potential therapeutic targets to address the disease mechanism. In parallel, new molecules that may act in the discovered targets are developed. The molecules that have the best performance in pre-clinical studies are later assessed in humans. The safety profile and different drug doses are generally explored in healthy humans while efficacy evidence is studied in affected patients. Different clinical trial phases collect the evidence required for a market authorisation. Once the new drug is commercialised, further studies are performed to analyse the real impact of the new treatment in the real world. Once the medicine's effectiveness is confirmed, the new therapeutic drug can be standardised as a treatment option for a specific condition. TR is a complex but important process that aims to improve human health (Zoellner et al., 2015, Sung et al., 2003).

Several authors have suggested classification systems for stages of TR (Zoellner et al., 2015). A simplified model is to categorise TR according to major blocks related to the application of scientific results: from the laboratory findings/bench side to clinical research/bed side (T1 translation) and from clinical studies to the practice setting and communities (T2 translation) (Sung et al., 2003). In other words, the first stage of TR translates knowledge from laboratory studies to patient-oriented research, and the second promotes the implementation of best practice to the general public (Rubio et al.,

2010). Other authors consider basic scientific research, such as pre-clinical, *in vitro* and animal model research, as T1; early patient-oriented research, like phase I and II clinical trials and proof-of-concept studies, as T2; and late-stage clinical research, which involves studies assessing effectiveness and implementation research, as T3 (Zoellner et al., 2015). With the increasing use of the term ‘translational research’ at conferences and in training settings and academic environments, the Evaluation Committee of the Association for Clinical Research Training (ACRT) has clarified the situation by providing the following definitions to be used in the educational framework: T1 translates the findings from basic science projects to patient-oriented research, aiming for an improvement in knowledge and/or standards of care; T2 translates knowledge from patient-centred research to population-based research, aiming for improvements in community outcomes and public health by implementing best practices; T3 comprises interaction between basic science research and population-based research (Rubio et al., 2010). However, translational research is a continuous and multi-directional cycle as new knowledge can function as feedback to previous research (Figure 1). In this thesis, the ACRT definitions are used.

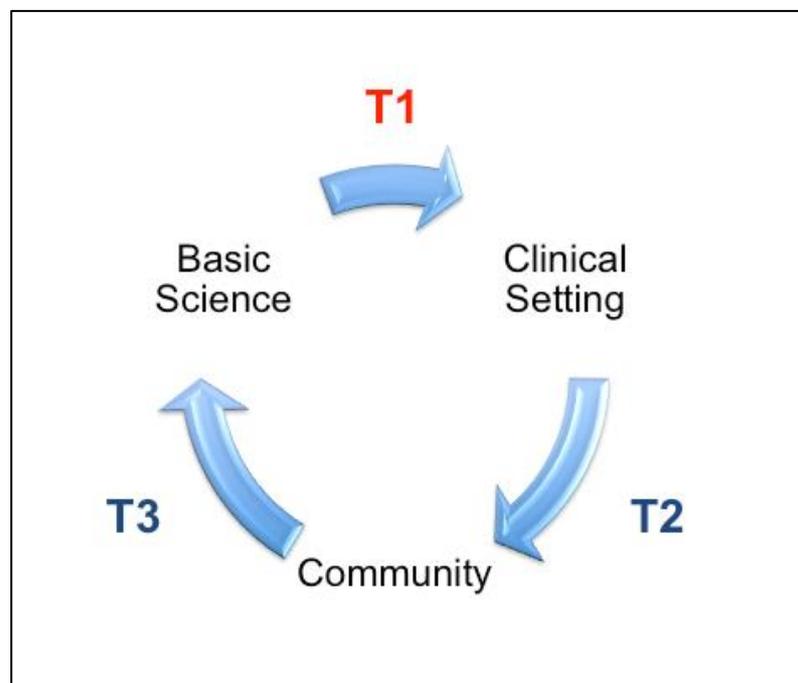


Figure 1. Translational research definitions

*In red: the translational research area explored in this thesis.*

Developing new molecules up to commercialisation is complex, lengthy and expensive. It includes but is not limited to the following steps:

- Understanding the pathophysiology of the disease
- Discovering new compounds
- Developing pre-clinical studies
- Performing early-stage clinical trials in humans to assess pharmacokinetics, safety, tolerability, dose ranging and first evidence of efficacy
- Developing further trials and pivotal studies to collect further evidence on safety, tolerability, efficacy and effectiveness
- Application for marketing authorisation
- Performing post-marketing surveillance studies to further confirm effectiveness and long-term safety

The cost of developing a new drug has been estimated to be almost £2 billion (Mullard, 2014). This figure is based on the costs 10 companies spent on drug discovery and expenses related to negative trials exploring compounds that failed the drug development process. Unfortunately, the majority of clinical trials fail to bring a new molecule to commercialisation (Ashburn and Thor, 2004). In 2014, the probability of a drug reaching a patient-oriented research level (phase I study) to be approved by the Food and Drug Administration (FDA) was estimated to be 11.7% (Van Norman, 2016). Some conditions are particularly challenging. For example Alzheimer's disease, for which the drug development failure rate was estimated to be 99.6% in the period 2002–2012 (Cummings et al., 2014).

The time taken for TR results to benefit the general population is also an obstacle. It can take approximately 12 years for a new drug to be approved by the FDA – from pre-clinical research to commercialisation (Van Norman, 2016), and additional years for the implementation of scientific innovations in general clinical practice (Trochim et al., 2011). In conclusion, it may take several years – and a few billion – for treatment guidelines to be updated.

## **Translational Research in Rare Diseases**

Developing new treatment options for rare conditions is particularly complex. Various factors contribute to this, including uncertainties related to the pathology of rare diseases, lack of knowledge on natural history, and the lack of standardised measures to assess the outcomes of clinical trials (Conwit et al., 2011).

Rare diseases, by definition, have extremely low incidence and prevalence. From a clinical trial perspective, low prevalence negatively affects recruitment rates. This is particularly challenging in single-centre studies, as eligible participants may be distributed across a wide geographic region (Forrest et al., 2011, Conwit et al., 2011). In such cases, randomised controlled trials involving larger samples are less likely to meet the recruitment target, and may be underpowered for the primary outcome analysis (Augustine et al., 2013, Conwit et al., 2011, Mitsumoto et al., 2009). In terms of trial design, the use of a placebo arm may not be feasible if a condition restricts life expectancy, such as neuromuscular conditions manifesting at early stages of child development (Augustine et al., 2013, Mitsumoto et al., 2009).

A further challenge for TR in rare diseases is the possibility that safety data will be incomplete at the time an application is made for commercialisation of a new drug. Clinical trials designed to assess safety as a primary outcome may fail to achieve the recruitment target when rare conditions are studied. Only a small proportion of people with a very rare disease will be exposed to the investigational medical product during the research phase, reducing the likelihood of rare adverse events during the pre-market stage. In such cases, post-approval surveillance studies and phase IV clinical trials may be developed to collect further evidence on safety (Zhang et al., 2016).

Another problem is the shortage of reliable outcome measures to assess functional benefits of new therapeutic medicines. This is particularly challenging in the field of exercise-related muscle disorders, as such conditions are frequently very rare, correct diagnosis is frequently delayed by several years and the disease progression is generally slow (Conwit et al., 2011, Michelson, 2018, Scalco et al., 2017c, Reason, 2016). The absence of internationally recognised standards of care may contribute to inter-subject clinical variability, resulting in further difficulties in identifying a homogeneous cohort of patients for clinical trials; this may compromise study recruitment and/or internal validity (Slack and Draugalis, 2001). In rare disorders with a chronic presentation and slow progression, uncertainties related to unidentified clinically relevant primary endpoints to assess drug efficacy in reasonable time are common, further affecting data analysis (Augustine et al., 2013). All these factors combined make it difficult to design and validate clinically relevant primary endpoints for pivotal trials, thereby hampering new drug discovery (Conwit et al., 2011).

The European Union countries have combined their efforts to improve the care provided to people with rare diseases (Taruscio et al., 2014). These efforts include the creation of Centres of Expertise, European Reference Networks and The European Union Committee of Experts on Rare Diseases (Taruscio et al., 2014). Specialised services for rare conditions may provide the perfect setting for TR development as they combine clinical expertise, larger cohorts of patients and standardised care; such centres also offer an easy access to diagnostic investigation (Reason et al., 2018, Quinlivan et al., 2014b). International registries for rare diseases may also support TR, especially in post-market surveillance studies requiring the monitoring of larger cohorts of patients (Jonker et al., 2018). In addition, international registries may provide valuable information on epidemiology data, natural history and phenotypic variability; contributing to the development of outcome measures and study planning (Evangelista et al., 2016, Mul et al., 2017). Registry findings contribute to the identification of key milestones in disease progression, which are crucial for the selection of treatment/exposure duration (Augustine et al., 2013). The registries may also provide a valuable source for the investigation of new therapeutic targets (Ask and Kolb, 2015).

The regulatory bodies may request the development of patient registries following drug approvals for further data collection. However, a recent study showed that several post-approval registries were incomplete and/or had poor enrolment rates (Jonker et al., 2018). It illustrated that further efforts are still needed not only to develop registries but also to improve their maintenance.

As a result of the above-mentioned difficulties in researching rare diseases, many trials have negative results. A negative trial illustrates clinical research in which the primary outcome data analysis does not exclude a null hypothesis ( $P \geq 0.05$ ), frequently confirming a lack of efficacy of a tested medicine (Singh et al., 2008). However, in rare diseases, a negative trial may not necessarily reflect a lack of drug efficacy (type II error) but might result from the use of an inadequate study design, underpowered trial, lack of a placebo arm, high inter-subject variability and/or short duration of exposure to the assessed compound (Augustine et al., 2013, Fletcher, 2008, Pocock and Stone, 2016). As a result, it may be hard to provide strong evidence of efficacy and safety required for a new drug approval in the field of rare conditions.

## **Regulatory Bodies – Orphan Drug Designation**

Drug development for orphan diseases has little commercial investment (Shah, 2006). The predicted difficulties in researching new treatments combined with the knowledge that just a small proportion of people will benefit from such therapies may discourage financial investments in the field of rare diseases such as, for example, exercise-related muscle disorders. As a result, there are far fewer treatments for rare conditions than for conditions with higher prevalence rates, for instance, hypertension and cardiac diseases.

To overcome these difficulties, regulatory bodies have created incentives to encourage the expansion of research in rare conditions (EuropeanMedicinesAgency, 2018, Attwood et al., 2018). The introduction of the Orphan Drug Act (1983) in the USA, followed by similar regulations in the European Union (2000) and other countries, aimed to expand pharmaceutical research in the field of rare diseases (Shah, 2006). In Europe, orphan drug designation is applied to compounds developed for debilitating or life-threatening conditions affecting fewer than five in 10,000 people for which there is no adequate treatment available (Joppi et al., 2013, Shah, 2006). Incentives offered by the European Medicines Agency (EMA) for research into orphan drugs include protocol assistance, fee reduction, 10-year market exclusivity, incentives for small enterprises and for research, and centralised authorisation procedures (EuropeanMedicinesAgency, 2017). The incentives are largely financial as orphan drug status does not seem to affect the length of the drug development process (EuropeanMedicinesAgency, 2018). Nevertheless, compounds for neurological diseases with orphan drug designation were reported to require fewer pivotal studies, smaller sample sizes and fewer randomised trials for market authorisation, illustrating that alternative designs may provide the evidence needed for regulatory approvals in the field of rare diseases (Mitsumoto et al., 2009).

In the last three decades, the number of companies researching new treatment options for rare conditions greatly increased, which is very promising (ClinicalTrials.gov, 2000, Attwood et al., 2018). Since the introduction of the Orphan Drug Act in 1983, orphan drugs have represented more than 40% of the approved pharmacological treatments in the USA (Attwood et al., 2018). In relation to neuromuscular diseases, the recent approval of the first pharmaceutical treatment for spinal muscular atrophy was a great achievement for patients and their family members (Claborn et al., 2018). Such

improvements might confirm that the incentives offered by regulatory bodies have positively contributed to the changes seen in translational research in the field of rare diseases.

## **Drug Repurposing Studies**

Discovering new molecules is complex and expensive. An alternative option is exploring new uses for existing molecules prescribed as treatment options for other diseases. Drug repurposing studies aim to expand the indications for already approved drugs. They simplify drug development research, as studies assessing safety in humans have already been performed (Pantziarka et al., 2018, Ashburn and Thor, 2004). Well-established safety profiles contribute to protocol development because expected adverse events, FDA pregnancy categorisation and contraindications may be known by the time a repurposing study is developed. Such studies are a cheaper option not only because they bypass early stages of drug development, but also because the cost of studying drugs with expired patents may be lower.

One negative aspect of repositioning studies relates to financial discouragements (Pantziarka et al., 2018). Redirecting generic medicines involves the development of novel pivotal studies to confirm the drug efficacy for a new indication (Ashburn and Thor, 2004). The financial return from generic drug sales may not satisfactorily counterbalance the cost of novel research to extend the drug indications (Pantziarka et al., 2018). Investing in off-patent drugs is likely to support competitors' sales, which may not be the most attractive option for private companies. Another limitation relates to market authorisation: prior to repurposing research, contracts should be organised when private companies hold patents for licensed drugs (Ashburn and Thor, 2004).

Academic institutions may perform off-patent drug trials, as such institutions in general have more freedom when it comes to protocol development. Also, achievements in academia are not measured purely by financial profit, but by the number of publications in high-impact journals, success in obtaining scholarship and grants, international recognition and internal promotion (Oprea et al., 2011). A great advantage of performing repurposing studies in academic environments is the easy access to Centres of Excellence and/or facilities in teaching hospitals (Oprea et al., 2011, Conwit et al., 2011). International support for academic-centred organisations regarding funding and protocol development may also be advantageous for drug discovery (Oprea et al., 2011, Conwit et al., 2011).

Because of the lower cost, known safety profile in humans and faster execution, drug repurposing studies performed in an academic environment may be of particular interest in the field of rare diseases, specifically exercise-related muscle disorders.

### **Scope of Study**

Several difficulties discourage the development of new pharmacological treatments for rare conditions. Thus, a large proportion of affected patients have no treatment option to satisfactorily improve their symptoms and reduce the progression of the underlying disease. This research was developed to address this limitation while exploring the challenges related to developing translational research studies in rare exercise-related muscle disorders. It translates knowledge gained from animal model research in Hypokalaemic Periodic Paralysis and McArdle disease to humans affected by these conditions to identify new treatment options for both diseases, making findings from basic science useful for practical applications (T1 translation). Drug-repurposing studies were developed in an academic environment as investigator-initiated studies. The efficacy of two compounds, sodium valproate and bumetanide, were assessed for the first time in humans with McArdle disease and Hypokalaemic Periodic Paralysis, respectively.

The results of this research should not only add to future research performed in the field of exercise-related muscle disorders, but also provide valuable insights to TR performed in the wider field of rare conditions.

## 11. Translational Research in Periodic Paralysis

### Introduction to HypoPP

Hypokalaemic periodic paralysis (HypoPP; ORPHA#681) is a rare autosomal dominant neuromuscular disease characterised by recurrent attacks of reversible flaccid paralysis often in association with low levels of serum potassium (Vicart et al., 2002). HypoPP is caused by mutations in genes encoding skeletal muscle ion channels. Pathogenic mutations have been described in the S4 segments of the voltage-gated skeletal muscle calcium channel gene *CACNA1S* (HypoPP1 OMIM#170400) and the voltage-gated skeletal muscle sodium channel gene *SCN4A* (HypoPP2 OMIM#613345).

Anyone can experience paralysis under extreme low levels of serum potassium, whether they have an underlying muscle disease or not. Intracellular and extracellular potassium currents influence the resting potential of the sarcolemma. Outward voltage-gated potassium channels ( $K_v$ ) and inward-rectifying potassium channels ( $K_{ir}$ ) are activated by depolarisation and by reaching the resting membrane potential, respectively. Rectifying channel conductance ( $K_{ir}$ ) is essential for setting the normal resting potential as it has a hyperpolarising role (around -85 mV (Jurkat-Rott et al., 2009)).  $K_{ir}$  has reduced activity when serum potassium levels are low (Geukes Foppen et al., 2002). During hypokalaemia, the muscle fibre membrane hyperpolarises (more negative than -90 mV) as a result of an imbalance between inward and outward ion currents. Muscle cells may have two membrane potentials under equal conditions when exposed to hypokalaemia: hyperpolarised and depolarised. This phenomenon is called bistable behaviour (Geukes Foppen et al., 2002). However, with further reduction of extracellular potassium levels, the membrane of most muscle fibres paradoxically depolarises, particularly because  $K_{ir}$  current is suppressed at low levels of extracellular potassium. Resting potential depolarisation inactivates the voltage-gated sodium channels. Sodium channel activation is central in action potential generation; thus, the inactivation of these channels reduces skeletal muscle fibre excitability, which is clinically manifested as flaccid paralysis – an acute onset of weakness (Burge and Hanna, 2012, Fialho et al., 2018).

In HypoPP, patients experience such symptoms under more physiological levels of serum potassium, such as 3.5 mmol/L, as the inward current is abnormally increased. The mutations weaken the ionic

bonds within the channels, causing the formation of a gating pore current that allows a leak of cations across the membrane, particularly when exposed to low levels of potassium. This aberrant inward current through the gating pore is, in turn, thought to cause susceptibility to sustained depolarisation of the resting potential of the sarcolemma (Burge and Hanna, 2012).

During a HypoPP acute attack of weakness, the extracellular potassium ions shift to the intrasarcolemmal compartment as a result of the abnormal ion channel function and the suppressed  $K_{ir}$ . This is clinically represented by flaccid paralysis in association with low serum potassium levels, resulting in a self-sustained diminished potassium current and depolarisation because local reduction of potassium levels triggers  $K_{ir}$  suppression in local muscle fibres, perpetuating the attack to the adjacent fibres.

### **Prevalence**

Few studies have analysed epidemiological data on the condition. The minimum prevalence of genetically confirmed HypoPP patients in England was estimated to be 1.3 per million people (Horga et al., 2013). This number is an underestimate as only those affected people referred to the National Health Service (NHS) highly specialised service for channelopathies were included in the study analysis. A higher estimated prevalence has recently been reported in the Netherlands (5.3 per million), where four genetic diagnostic centres were reviewed (Stunnenberg et al., 2018); however, this prevalence may also be an underestimate, as the diagnostic investigation of the included patients did not include complete sequencing of the *CACNA1S* gene.

### **Clinical Features**

Recurrent attacks of weakness are the main clinical feature of this condition. Affected people experience focal or generalised transitory weakness, which may last from several minutes to days (Vicart et al., 2002). The disease phenotype is similar in both currently identified forms: HypoPP1 and HypoPP2. The onset of symptoms may vary from early childhood to adulthood, but most patients are symptomatic from the second decade of life (Vicart et al., 1993a, Miller et al., 2004). This acute onset of weakness increases in frequency during teenage years and early adulthood, but appears to reduce with aging (Vicart et al., 1993a).

Acute symptoms are elicited by common everyday activities. Certain triggers like carbohydrate intake (hyperglycaemia – hyperosmolarity) activate the Na-K-2Cl co-transporter (NKCC) resulting in a

depolarising effect in the resting potential of the sarcolemma (see 'Novel Frontiers of Therapy in HypoPP: Bumetanide'); other triggers activate the Na<sup>+</sup>/K<sup>+</sup>-ATPase resulting in mild reductions in extracellular potassium content. Common triggers are carbohydrate-rich meals and the combination of exertion and rest following exercise (Venance et al., 2006). Further triggers that may induce or exacerbate an attack include strenuous exercise, a sodium-rich diet, acute illness and specific medications (Miller et al., 2004, Statland et al., 2018, Venance et al., 2006). Ambient temperature reduction may also induce sarcolemma depolarisation due to K<sub>r</sub> suppression (Geukes Foppen et al., 2002).

Acute onset of muscle weakness typically occurs during the night or in the early morning, and patients may have frequent attacks that interfere with daily activities and work. During severe attacks, hospitalisation for intravenous potassium treatment may be required and if frequent and/or requiring intensive therapy unit admission, treatment may cause a significant economic burden (Links et al., 1994a, Levitt, 2008). Muscle strength returns to normal after an attack, however, as the disease progresses, fixed muscle weakness develops in approximately 28% of affected individuals (Vicart et al., 2002, Sternberg et al., 2001), which can be disabling in later life (Links et al., 1994a).

### **Diagnosis**

Three channelopathies with overlapping phenotype manifest with periodic paralysis: Andersen-Tawil syndrome, hyperkalaemic periodic paralysis (HyperPP) and HypoPP. Diagnostic investigation in patients presenting with periodic paralysis includes taking a detailed medical history, physical examination, laboratory investigation, genetic and neurophysiology testing. A secondary cause of hypokalaemia and/or other neuromuscular disease presenting with transitory weakness (for example, myasthenia gravis) should be excluded.

A history of recurrent paralysis, positive family history and the identification of common triggers should raise the suspicion of an underlying genetic channelopathy. HypoPP diagnosis is confirmed by genetic testing. Most patients have HypoPP1, with only a small proportion of patients having mutations in *SCN4A* (Horga et al., 2013, Statland et al., 2018). HypoPP is inherited in an autosomal dominant manner with a variable penetrance, as female patients tend to be less symptomatic (Fialho et al., 2018, Vicart et al., 1993a, Miller et al., 2004, Ke et al., 2013).

## *Neurophysiology*

Neurophysiology may be used as a diagnostic tool in periodic weakness investigation. The McManis test is a safe, reliable and reproducible investigation to diagnose periodic paralysis (Tan et al., 2011, McManis et al., 1986, Fournier et al., 2004). It involves a standardised procedure to evoke and assess localised disease symptoms in people with HypoPP. This procedure is known to be positive in approximately 80% of patients with HypoPP1 (Fournier et al., 2004). The long exercise test first described by McManis and his colleagues in 1986 applies a standardised regime of isometric exercise to an intrinsic hand muscle followed by rest (McManis et al., 1986). In patients with periodic paralysis this often induces a focal attack of weakness. The compound muscle action potential (CMAP) represents the summation of all muscle fibre action potentials evoked following motor nerve stimulation and recorded via surface electrodes during the McManis test. During an attack, the depolarised muscle fibre membranes are electrically unexcitable, which is reflected in reduced CMAP amplitude. The severity of the weakness can be quantified indirectly by recording the decrement of the CMAP amplitude. A CMAP amplitude decrement of 40% or more from the maximum response obtained during or within 50 minutes following the isometric exercise is considered abnormal, and is used as a diagnostic cut-off point for channelopathies (McManis et al., 1986, Kuntzer et al., 2000). The change in CMAP is a quantitative measurement that not only shows the impaired ion channel function, but also reflects the patients' focal weakness.

Systematic use of the McManis test in the diagnostic work-up of skeletal muscle channelopathies, together with the short exercise protocols and advances in genetic testing, has replaced the previous clinical practice of inducing generalised attacks of weakness by exposing patients to a range of known provocative factors (for example, oral glucose load and/or insulin administration).

### **Exercise-related Disease**

Exertion is the commonest reported trigger for acute symptoms (Miller et al., 2004), and patients are advised to avoid high-intensity unaccustomed physical activity (Fialho et al., 2018). Paradoxically, gentle physical activity may improve patients' symptoms.

Potassium loss from the intracellular compartment during exercise has been reported in healthy people (Lindinger and Sjogaard, 1991). During exercise, the plasma potassium concentration increases following muscle contraction as a result of several factors that share a common final

pathway: skeletal muscle cell leakage (Medbo and Sejersted, 1990, Hopkins, 2006). It has been reported that the level of serum potassium may even double during exhausting running (Medbo and Sejersted, 1990). The excess is cleared from the extracellular compartment once the activity is interrupted; however, a rebound effect reduces the serum potassium concentration to below its resting levels (Medbo and Sejersted, 1990). In addition, several mechanisms activate Na<sup>+</sup>/K<sup>+</sup> ATPase during exertion, which has a depolarising effect in muscle fibres related to extracellular potassium reduction.

The exact differences between HypoPP and the physiology of high-intensity exercise in healthy individuals are not known, and multi-factorial mechanisms may contribute to the generalised weakness seen in HypoPP.

### **Management**

Treatment of HypoPP is suboptimal in many patients. Conservative treatment involves dietary changes and avoidance of triggering factors but this alone is rarely enough to prevent attacks. Behavioural strategies including gentle physical activity at the beginning of acute weakness and avoidance of dehydration/hyperglycaemia (hyperosmolar states) are also recommended (Statland et al., 2018).

The majority of drugs available are used for attack prevention and include, most commonly, carbonic anhydrase inhibitors used prophylactically. Acetazolamide may reduce the frequency of attacks in some patients, but the exact mechanism of action remains unclear (Venance et al., 2006, Matthews and Hanna, 2010). Some reports suggest that acetazolamide may even exacerbate symptoms in patients with HypoPP2 (Vicart et al., 2002). Dichlorphenamide has been shown in a clinical trial (Class I evidence) to be a reasonable therapy to reduce attack frequency and severity (Sansone et al., 2016); it is five times more potent than acetazolamide, but is more commonly associated with side effects, such as drowsiness and confusion (Tawil et al., 2000). Other agents that reduce urinary potassium loss are also prescribed; for example, spironolactone and triamterene (Vicart et al., 2002).

Potassium chloride supplementation is the only treatment option for these patients during an acute attack of weakness (Links et al., 1994b, Levitt, 2008, Vicart et al., 2002), even though there are no randomised controlled clinical trials to assess its efficacy. Oral potassium chloride is often used to abort mild and moderate symptoms (Alkaabi et al., 2010, Vicart et al., 2002), but inpatient admission

for intravenous potassium may be required for severe symptoms (for example, respiratory dysfunction) and/or severe hypokalaemia with associated generalised weakness (Vicart et al., 1993b). During an acute attack, potassium from serum is shifted to the intracellular compartment, resulting in hypokalaemia with no real potassium loss. After recovery from an attack, the potassium is shifted back and normokalaemia is restored. Hence, potassium supplementation may result in hyperkalaemia and associated risks.

#### *Opportunities for Translational Research*

There have been very few randomised controlled trials that demonstrate medication efficacy in HypoPP (Sansone et al., 2008, Sansone et al., 2016). Although the evidence remains poor, there is clinical suspicion that active treatment to reduce the frequency and/or severity of attacks may improve long-term disease prognosis, in particular the development of fixed weakness, which is a chronic myopathy (Cavel-Greant et al., 2012, Jurkat-Rott et al., 2009). Consequently, it is important to develop more-effective abortive treatments for acute attacks of weakness in HypoPP. Moreover, finding more effective agents to reduce HypoPP symptoms will improve quality of life and prevent hospital admissions in these patients. It is well acknowledged that alternative therapies are needed (Matthews et al., 2011, Statland et al., 2018).

## **Novel Frontiers of Therapy in HypoPP: Bumetanide**

### **Pre-clinical Research**

With respect to treatment, experimental data from the use of bumetanide in mouse models of HypoPP has provided convincing evidence that it can both prevent and abort paralytic attacks. Bumetanide is a potent loop diuretic of the sulfanyl category, which has a rapid onset and short duration of action (Asbury et al., 1972, Ward and Heel, 1984).

At low concentrations, bumetanide is a specific inhibitor of NKCC (Greger and Wangemann, 1987). Skeletal muscle has a high resting membrane conductance to chloride ions. This is mostly related to the skeletal muscle voltage-gated chloride channel CLC1, which plays an important role in repolarisation of the resting potential of the sarcolemma. The NKCC normally allows influx of potassium, sodium and two chloride ions with no net current. Intracellular chloride accumulation above its equilibrium potential has a depolarising effect in myocytes due to the passive efflux of

chloride ions (Geukes Foppen et al., 2002). Therefore, normal function of the NKCC has an important depolarising effect on sarcolemma as it increases the intracellular chloride ion concentration (Geukes Foppen et al., 2002). It has been demonstrated that if sarcoplasmic chloride ion concentration cannot be increased by the NKCC, then paradoxical depolarisation at the sarcolemma resting potential in low potassium states is less likely to occur and therefore an attack would be less likely (Geukes Foppen et al., 2002).

Hypertonic medium influences chloride ion transport by stimulating the NKCC, shifting the extracellular potassium 'switch-off' value to higher levels (Geukes Foppen et al., 2002). In other words, muscle fibres depolarise under higher potassium levels when exposed to a hyperosmolar environment.

Early *in vitro* studies in wild mouse muscle provided evidence that the resting potential of muscle fibres exposed to bumetanide effectively hyperpolarised in hypertonic and hypokalaemic media, preventing the paradoxical depolarisation by reducing both the 'switch-off' and 'switch-on' values of extracellular potassium (Geukes Foppen et al., 2002). Bumetanide exposure also prevented depolarisation at low temperatures (Geukes Foppen et al., 2002).

Further studies of HypoPP in the Nav1.4-R669H (Wu et al., 2013b) and CaV1.1-R528H knock-in (KI) mouse models (Wu et al., 2013a) provided convincing evidence that bumetanide was very effective at preventing the loss of muscle force that occurred at low potassium levels as well as inducing recovery of force during an established attack. *In vitro* experiments with continuous exposure to bumetanide following a hypokalaemic challenge showed an abortive treatment efficacy (Wu et al., 2013b, Wu et al., 2013a). An *in vivo* study showed the efficacy of a single intravenous dose of 0.08mg/kg bumetanide in preventing an acute attack following continuous intravenous infusion of glucose with insulin (Wu et al., 2013a). Wu et al (2013) demonstrated, as hypothesised, that the protection from loss of force was dependent on chloride ions and illustrated that bumetanide was more effective than acetazolamide (the current prophylactic treatment) in preventing loss of force in low potassium (Wu et al., 2013b, Wu et al., 2013a).

### **Clinical Research**

To date there have been no clinical trials in patients with HypoPP using bumetanide, but its safety in humans has been analysed by several studies of this drug in other conditions. Bumetanide is a drug

already approved by the MHRA for use as a diuretic. Bumetanide has been extensively used in both healthy and critically ill humans and full-term and preterm infants to treat fluid volume overload due to cardiac and/or pulmonary disease (Wargo and Banta, 2009). It has well-established pharmacokinetic and pharmacodynamic properties in adult humans and has relatively few side effects (Staley, 2006, Kahle and Staley, 2008). It is about 40 times more potent than furosemide, with the exception of its effects on urinary potassium excretion, where its potency is lower (Ward and Heel, 1984). Following oral administration of 2 mg, peak activity is reached between one and two hours. It is eliminated rapidly in humans, with a half-life of between one and 1.5 hours (Tata et al., 1993).

## **Outcome Measures in Clinical Trials**

### **Opportunities for Translational Research**

It is difficult to analyse the efficacy of an abortive treatment strategy in people with HypoPP as there is no outcome measure standardised to reliably assess treatment benefits. Inducing generalised attacks of weakness in people with HypoPP, for instance by inducing hypokalaemia with intravenous administration of glucose and insulin, has many disadvantages. These include the difficulties of quantifying the attack severity and duration in the context of variable distribution of muscle involvement and variable response to triggering factors. There are also significant associated safety risks, such as cardiac complications due to severe hypokalaemia.

Requesting patients to perform effort against resistance during the Medical Research Council (MRC) muscle scale assessment, especially if repeatedly stimulated (for example, every few minutes), could potentially induce attack recovery related to the gentle physical activity benefits. Thus, this scale may not be an accurate outcome measure to assess therapeutic efficacy for acute symptoms in HypoPP. Thus, there is a need to develop highly sensitive, reliable and clinically relevant outcome measures for HypoPP.

I hypothesise the McManis test may be a reliable outcome measure to be used in clinical trials as 1) it reproduces an exercise-induced attack of weakness (validity); 2) symptoms may be triggered by exercise with concomitant normal serum potassium levels (ensures safety); 3) a small hand muscle is analysed, which facilitates study assessments while avoiding a generalised attack of weakness (feasibility); 4) CMAP decrement is an objective measure of the skeletal muscle depolarisation, which

could be used to indicate therapeutic effects as it is sensitive to changes (responsiveness); 5) there are norms available for comparison as the cut-off point for an ongoing attack (40% decrement) has already been standardised (interpretability); and 6) it is easily reproducible and has internal consistency (reliability).

Katz *et al* (1999) reported improvements in a modified version of the long exercise test following treatment with tocainide in a patient with Andersen-Tawil syndrome, which was consistent with the patient's documented therapeutic response (Katz et al., 1999), suggesting that this test might also be useful in determining therapy effectiveness; however, no clinical trial has assessed its role as an outcome measure to date ([clinicaltrials.gov](http://clinicaltrials.gov)).

## 12. Translational Research in McArdle Disease

### Introduction to McArdle Disease

McArdle disease (Glycogen Storage Disease Type V – GSDV; ORPHA#368; OMIM#232600) is a rare inherited metabolic disorder of skeletal muscle. The condition is caused by homozygous or compound heterozygous mutations in the muscle glycogen phosphorylase gene (*PYGM*) located at chromosome 11q13. Many pathogenic mutations have been identified in *PYGM*, and most are population specific (Bruno et al., 2006, Andreu et al., 2007). The most common mutation in Northern European and North American patients is a nonsense mutation at p.Arg50X (p.R50X) in exon 1, which has been previously referred to as p.R49X (Bartram et al., 1993, Quinlivan et al., 2010, Santalla et al., 2017). Other frequent mutations in this population are p.Gly205Ser (p.G205S) (Quinlivan et al., 2010) and p.Trp798Arg (p.W798R) in the Spanish population (Andreu et al., 2007, Santalla et al., 2017).

Affected people are unable to use skeletal muscle glycogen as an energy source due to a congenital lack of the enzyme muscle glycogen phosphorylase (myophosphorylase), which is essential for glycogen metabolism (Figure 2) (McArdle, 1951, Mommaerts et al., 1959, Schmid and Mahler, 1959, Andreu et al., 2007). The inability to metabolise glycogen into glucose 1-phosphate in this tissue results in severe impairment of physical activity that is dependent on glycolysis. Consequently, people with this condition will experience an acute skeletal muscle energy crisis when performing tasks relying on anaerobic metabolism and in the early stages of any physical activity. Affected people present with exercise-related symptoms within minutes of initiating physical activity as further detailed in 'Clinical Features'.

- Available in: printed thesis

Figure 2. Impaired skeletal muscle glycogen metabolism in McArdle disease. Reproduced from (Lucia et al., 2008)

### Exercise-related Disease

Adenosine triphosphate (ATP) breakdown and energy production is essential for skeletal muscle contraction (Baker et al., 2010). During physical activity, skeletal muscle metabolic demand increases, and three different mechanisms contribute to ATP resynthesis: phosphagen system, glycolytic system and mitochondrial respiration (Baker et al., 2010).

The glycolytic system is compromised in people with McArdle disease, while the other two metabolic pathways are unaffected. Understanding the normal function of each metabolic pathway facilitates the understanding of the McArdle disease clinical phenotype and management strategies. Figure 3 illustrates these metabolic systems according to exercise duration and in relation to McArdle disease pathophysiology.

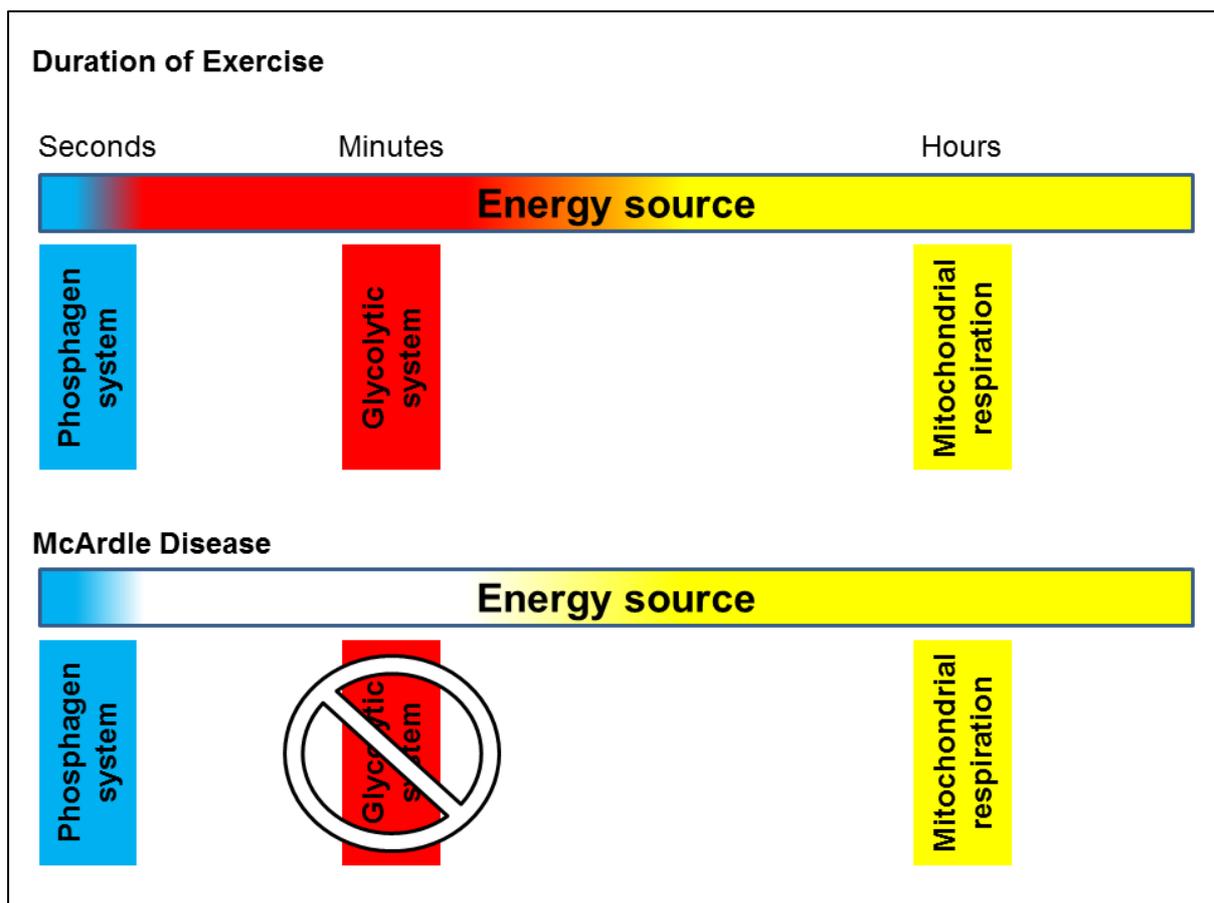


Figure 3. Skeletal muscle energy sources in relation to exercise duration and McArdle disease

### *Phosphagen System*

The phosphagen pathway (ATP-phosphocreatine) provides immediate ATP for a short period of up to 10 seconds. It is the simplest and fastest system for ATP production, but limited phosphocreatine stores restricts its availability (Draper and Marshall, 2013). Activities involving rapid and high-intensity movements, including explosive efforts such as 100m sprint, rely on this metabolic pathway (Baker et al., 2010, Draper and Marshall, 2013).

The phosphagen pathway is unaffected in McArdle disease, thus patients may use this energetic option to perform high-intensity physical activity safely for a very short time if needed; for example, carrying heavy luggage for a few seconds (Wakelin, 2013, Santalla et al., 2014a, Garcia-Benitez et al., 2013). Patients are advised to rest for at least 30 seconds and up to three minutes between periods of short-burst activity to restore phosphocreatine stores (Garcia-Benitez et al., 2013, Santalla et al., 2014a).

### *Glycolytic System*

The glycolytic system provides ATP rapidly via glycolysis (Baker et al., 2010, Draper and Marshall, 2013). This metabolic pathway involves a series of steps catabolising stored muscle glycogen and blood glucose. In this pathway, ATP is resynthesised and pyruvate is produced as a final metabolite. Pyruvate may be converted into lactate (anaerobic), or be catabolised by the mitochondria as part of the carbohydrate oxidation pathway (aerobic) (Draper and Marshall, 2013). Hence, pyruvate conversion to lactate and the consequent increase in levels of lactate correlates with exertion levels and is a physiological response to exercise (Goodwin et al., 2007, Draper and Marshall, 2013).

The glycolytic system supersedes the phosphagen system in activities involving maximum effort lasting for 10–90 seconds, or for a sprint finish, as in a rowing race (Draper and Marshall, 2013). Also, muscle glycogen storage closely correlates with endurance capacity, especially for moderate- to high-intensity exercise or if the exertion is performed for a prolonged period (Lars et al., 1967).

The glycolytic system is compromised in people with McArdle disease: the absent glycogen myophosphorylase enzyme compromises the metabolism of the stored glycogen in skeletal muscle (Santalla et al., 2014b).

However, glucose metabolism in skeletal muscle is unaffected in McArdle disease – it also produces pyruvate, which is used in mitochondrial respiration. Hence, high pre-exercise liver glycogen storage and pre-workout glucose supplementation may optimise exercise performance in this patient population (Santalla et al., 2014b, Haller and Vissing, 2002). The small amount of pyruvate produced via glucose breakdown may also be converted into lactate. For this reason, a blunt increase in serum lactate during exercise may be seen in affected people (Hogrel et al., 2015, Haller and Vissing, 2002). Plasma lactate may increase during exercise with IV glucose supplementation, confirming an unaffected glucose breakdown system in skeletal muscles (Haller and Vissing, 2002).

### *Mitochondrial Respiration*

Mitochondrial respiration, an aerobic process, provides ATP for a prolonged period. Mitochondrial energy sources include fatty acid oxidation, carbohydrate oxidation or amino acid oxidation (Baker et al., 2010). Activities involving aerobic exercise, including long-duration activities such as walking, rely on this metabolic pathway. This is the primary source of energy in resting skeletal muscle and cardiac muscle (Berg et al., 2002).

This metabolic pathway is generally not affected in people with McArdle disease. However, affected people have reduced carbohydrate oxidation as pyruvate production from the glycolic system is greatly reduced (Santalla et al., 2014b, Haller and Vissing, 2002).

### **Prevalence**

Epidemiologic data on the condition are limited to a few small studies (Santalla et al., 2017). The largest series of McArdle patients reported to date (n = 333) showed a disease prevalence of ~1/139,543 in individuals in Spain (Santalla et al., 2017). This number may be an underestimate, as the correct genetic diagnosis often takes several years (Scalco et al., 2017c, Reason, 2016, Scalco et al., 2014b, Santalla et al., 2017).

### **Clinical Features**

The three most common clinical features of McArdle disease are exercise intolerance, the presence of the 'second wind' phenomenon and raised serum creatine kinase (CK) levels (Santalla et al., 2017). Other clinical features are muscle hypertrophy, which is present in ~24% of the UK patients (Quinlivan et al., 2010), and fixed muscle weakness. Muscle weakness is seen in 16–21% of patients; it predominantly affects shoulder girdle and axial (paraspinal) muscles of older patients (Nogales-

Gadea et al., 2007, Santalla et al., 2017, Quinlivan et al., 2010, Quinlivan et al., 2017). Symptom severity may vary (Santalla et al., 2017). Factors associated with more severe symptoms are sedentariness and ageing (Santalla et al., 2017, Lucia et al., 2013, Munguia-Izquierdo et al., 2015).

### *Exercise Intolerance*

In affected people, exercise intolerance is seen from early childhood (Scalco et al., 2017c, Santalla et al., 2017, Quinlivan et al., 2010). It is commonly described as muscle fatigue, muscle discomfort and muscle pain starting within minutes of initiating any kind of physical activity, particularly activities dependent on muscle energy from glycogenolysis (Quinlivan et al., 2010). Symptoms are more severe with more strenuous exercise, such as climbing stairs or walking up hills, and isometric contraction (heavy lifting or squatting) (Nogales-Gadea et al., 2007).

If the activity is continued at the same or greater intensity despite the onset of symptoms and fatigue, symptoms are likely to become more intense and severe muscle contracture may occur. McArdle-related muscle contracture is usually described as severe muscle cramp with prolonged muscle spasm/rigidity; but unlike cramp, they are electrically silent. Contractures may lead to acute muscle damage and rhabdomyolysis (RM), a clinical syndrome characterised by acute skeletal muscle breakdown that results in the release of intracellular muscle constituents into the circulatory system (Chatzizisis et al., 2008). There is no clear diagnostic consensus for RM. Raised serum CK levels (often >10 times the upper limit of normal) in association with RM-related symptoms are frequently considered diagnostic criteria (Scalco et al., 2016d, Zutt et al., 2014). RM symptoms vary and may include flu-like symptoms, muscle soreness, muscle swelling, severe myalgia and dark urine (myoglobinuria).

People with McArdle disease may report frequent episodes of myoglobinuria and RM, particularly before the diagnosis is confirmed.

Affected people may report muscle contractures and RM following routine daily activities that are not necessarily exercise related, as long as the activities are rapid isotonic or prolonged isometric in nature. These include emotionally charged situations such as anger, excitement and pain (Brady et al., 2014a). RM can also occur following prolonged healthcare procedures where patients are required to hold certain positions, such as dental procedures, as prolonged isometric muscle activity

can elicit muscle contractures in McArdle patients (Scalco et al., 2016b). Sexual intercourse may also trigger RM in this patient population (Quinlivan et al., 2010, McCormick et al., 2013).

### *The Second Wind Phenomenon*

During the first few minutes after initiating physical activity, affected people have exercise-related muscle symptoms in association with tachycardia. During this symptomatic phase, in order to prevent muscle damage, patients have to slow down frequently and occasionally stop to have brief rests while warming up (Scalco et al., 2014a, Santalla et al., 2014b). Once in the *second wind*, they are able to continue the physical activity without experiencing McArdle-related symptoms and without causing muscle harm.

The *second wind* occurs after about 8–10 minutes of physical activity and is characterised by an easing of symptoms and an associated decrease in heart rate (HR) leading to an improvement in exercise tolerance. The *second wind* occurs as a consequence of increased availability of alternative fuel substrates, namely free fatty acids metabolised through oxidative phosphorylation and glucose supplied from the liver due to increased blood flow in muscle tissue (Haller et al., 1985, Braakhekke et al., 1986, Bartram et al., 1995).

Although the phenomenon is generally considered to be the hallmark of McArdle disease, around 22% of affected people do not recognise the phenomenon before the diagnostic confirmation (Quinlivan et al., 2010). When assessed by functional testing, however, the phenomenon is present in almost 100% of patients (Quinlivan et al., 2010, Santalla et al., 2017). Functional testing in McArdle disease is further discussed later in this chapter in 'Outcome Measures in Clinical Trials'.

### *Raised Creatine Kinase*

Baseline CK levels are almost always elevated, often higher than 2,000 IU/L (Quinlivan et al., 2010, Reason, 2016).

### **Comorbidities**

Comorbidities of McArdle disease include not only muscle damage due to recurrent RM, but also distressing childhood experiences, depression, anxiety and the adoption of a sedentary lifestyle (Reason, 2016, Scalco et al., 2017c). Sedentariness can lead to more muscle weakness, physical

deconditioning, poor bone health, obesity and related complications (Reason, 2016, Rodriguez-Gomez et al., 2018, Quinlivan et al., 2010).

Severe RM may result in acute kidney dysfunction, compartment syndrome and disseminated intravascular coagulopathy, which are life-threatening complications, highlighting the clinical significance of preventing RM (Zhao et al., 2017, Zutt et al., 2014, McCormick et al., 2013). In the UK, 11% of people with McArdle disease have experienced acute kidney failure earlier in life (Quinlivan et al., 2010). RM-related acute kidney failure may be the main complication prompting the diagnostic investigation of an underlying metabolic myopathy (Fikri-Benbrahim et al., 2013, Zhao et al., 2017, Costa et al., 2013). Chronic kidney failure is not commonly seen in this patient population (Santalla et al., 2017).

### **Diagnosis**

In the UK, there is a delay of approximately 33 years from presenting with symptoms until the correct diagnosis is confirmed (Scalco et al., 2017c). The delay is frequently related to misdiagnosis, as 90% of patients receive an incorrect explanation for their symptoms earlier in life (Scalco et al., 2017c, Reason, 2016, Scalco et al., 2014a, Quinlivan et al., 2010). These numbers improved after the development of new diagnostic techniques, the creation of a highly specialised service for McArdle disease and related disorders and the active support of patients' associations such as the Association for Glycogen Storage Disease UK (AGSD-UK, 1986, Quinlivan, 2012, Scalco et al., 2017c). Diagnostic delay has also been reported in the largest published McArdle cohort (Santalla et al., 2017).

McArdle disease diagnosis is confirmed by the identification of homozygous or compound heterozygous mutations in *PYGM* and/or by typical findings in a skeletal muscle biopsy.

Other screening/diagnostic tools such as objective assessments of the *second wind* and the non-ischaeamic forearm exercise test are discussed later in this chapter in 'Outcome Measures in Clinical Trials'.

### *Genetic Testing*

Currently, genetic testing is considered the first-line investigation for McArdle disease (Quinlivan et al., 2017). Screening for p.Arg50X and p.Gly205Ser is frequently the first diagnostic assessment in

the UK as most British patients carry these mutations (Quinlivan et al., 2017, Quinlivan et al., 2010, Santalla et al., 2017, Godfrey and Quinlivan, 2016). Whole *PYGM* sequencing is indicated for cases presenting with a typical McArdle disease phenotype where the genetic confirmation is not fully elucidated by the diagnostic screening of the most common pathogenic variants.

For cases presenting with atypical clinical features, including people with a milder phenotype, next generation sequencing (NGS) may be a useful screening tool to assess all genes related to glycogen storage disorders (NHS\_England). NGS may also confirm or exclude mutations in other genes associated with recurrent RM (Scalco et al., 2015b). The NGS technique is a cheaper diagnostic option and is less invasive than skeletal muscle biopsy.

### *Muscle Biopsy*

Typical McArdle disease findings in skeletal muscle biopsy are well established (Figure 4). The main features are subsarcolemmal vacuoles, glycogen accumulation and absence (or virtual absence in rare cases) of phosphorylase enzymatic activity (Goebel et al., 2013, Lucia et al., 2008). Reduced enzymatic activity may be demonstrated by histochemistry (phosphorylase stain) and/or muscle biochemical enzyme analysis, contributing to the diagnosis of the condition in centres where genetic evaluation is not available. A skeletal muscle sample from an unaffected person may be used as an external control, preventing false positive results related to a faded phosphorylase stain (Sato, 2002). Increased glycogen content is demonstrated by periodic acid-Schiff (PAS) staining. Biochemical analysis may demonstrate increased glycogen content and reduced enzymatic activity.

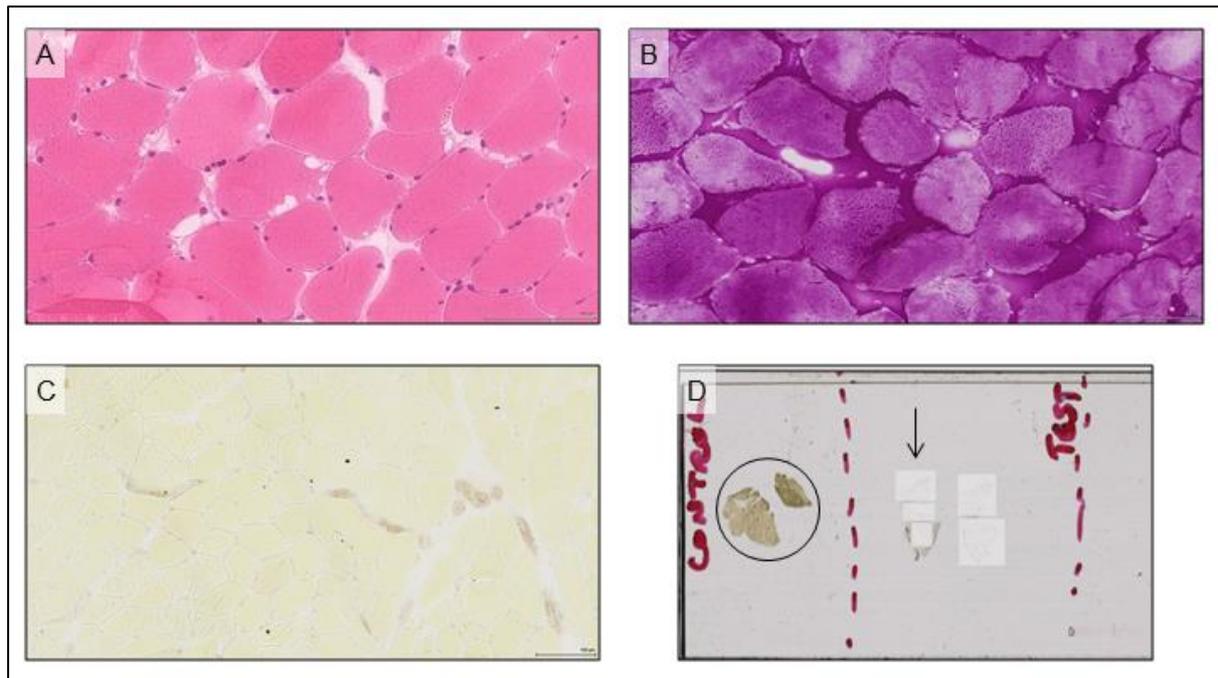


Figure 4. Biopsy abnormalities in McArdle disease

A) Vacuolar myopathy (haematoxylin and eosin); B) Glycogen accumulation (periodic acid-Schiff (PAS)); C) Absence of enzymatic staining in skeletal muscle fibres (phosphorylase); D) The use of a non-affected control (circle) for the evaluation of phosphorylase stain in people with suspected McArdle disease (arrow). Positive phosphorylase activity is seen in smooth muscle fibres of blood vessels as an internal control (C) and in the external control (D).

## Management

Once the diagnosis is made, patients are counselled about the disease mechanism. They are taught how to exercise in a safe way in order to prevent recurrent RM.

Currently there is no satisfactory pharmacological treatment for McArdle disease (Quinlivan et al., 2014a), but regular aerobic training programmes can help by conditioning skeletal muscle for improved fatty acid oxidation and  $VO_2$  peak (Haller et al., 2006, Mate-Munoz et al., 2007, Santalla et al., 2017). Prevention of sedentariness improves patients' wellbeing and prevents comorbidities (Rodriguez-Gomez et al., 2018, Munguia-Izquierdo et al., 2015).

Resistance training may be performed safely if specialised support is provided on an individual basis. Specific protocols designed for McArdle disease are used: the *second wind* is reached prior to workout following an aerobic warm-up, appropriate exertion/rest windows related to the skeletal muscle energetic pathway are respected, and glucose supplementation is used prior to exertion and

after contraindications for exercising are carefully reviewed by a specialist doctor (Quinlivan et al., 2017, Santalla et al., 2014a, Pietrusz et al., 2018).

Even though scientific evidence supports the use of regular exercise as a treatment option for people with McArdle disease, exercise can be a major barrier for this patient population because of painful symptoms and fear of muscle damage (Lucia et al., 2013). Thus, many patients are sedentary, poorly conditioned and so significantly impaired.

#### *Opportunities for Translational Research*

To date, no randomised controlled trial registered in ClinicalTrials.gov demonstrates a pharmacological benefit for McArdle disease (Quinlivan et al., 2014a, ClinicalTrials.gov, 2000). An ongoing clinical trial is assessing the benefits of triheptanoin oil (ClinicalTrials.gov, 2000).

### **Novel Frontiers of Therapy in McArdle disease: Sodium Valproate**

Mammals have three isozymes of glycogen phosphorylase named in association with the tissue in which they predominate: muscle, liver and brain (Crerar et al., 1995). These isozymes have a high degree of amino acid sequence homology, particularly the brain and the muscle forms (Bartram et al., 1995). Each isozyme is encoded by a different gene located in different chromosomes (Newgard et al., 1989). The muscle isozyme encoded by the *PYGM* is the predominant form found in mature fibres of skeletal muscle. This isozyme is absent in people with McArdle disease (Crerar et al., 1995). The brain isozyme encoded by the *PYGB* is found in the brain, heart and foetal tissue (Sato et al., 1977). It is thought to play an important role in periods of anoxia or hypoglycaemia (Crerar et al., 1995). The brain isozyme is also expressed in regenerating skeletal muscle fibres following muscle damage (DiMauro et al., 1977, Martinuzzi et al., 1999). A possible explanation for this finding could be related to the methylation of the CpG islands in the brain isozyme promoters, which would reduce/suppress its expression in mature skeletal muscle fibres. This could explain the post-natal downregulation of this enzyme in foetal tissues.

Surprisingly, studies performed on human cell cultures derived from skeletal muscle of people with McArdle disease expressed both the brain (foetal) and the liver isozymes but not the muscle isozyme (Sato et al., 1977). For this reason, human cultured muscle fibres fail as *an in vitro* model: the different isozymes prevent glycogen accumulation, the hallmark of McArdle disease.

The human *in vitro* study findings combined with the normal physiological response to muscle damage (muscle regeneration) illustrate that skeletal muscle fibres from affected McArdle humans are able to express other isozymes of the missing enzyme under special circumstances (Sato et al., 1977). Thus, finding a therapeutic agent to activate the expression of the foetal/brain or the liver isozymes for a prolonged time in mature skeletal muscle fibres in the absence of muscle damage may be a potential therapeutic strategy for the condition.

### **Pre-clinical Research**

Experiments using notexin injections to produce damage to skeletal muscle fibres in a sheep animal model of McArdle disease have shown widespread expression of the foetal/brain and the liver isoforms in skeletal muscle (Howell et al., 2014). Further studies in the sheep model showed that both injected and enteral administration of sodium valproate (valproic acid, VPA) increased the number of phosphorylase-positive skeletal muscle fibres, suggesting activation of different isoforms of the missing enzyme in this tissue (Howell et al., 2015), but glycogen storage content and the specific isozyme type of the expressed phosphorylase were not analysed. Similar results were obtained in an *in vitro* KI mouse model homozygous for the p.R50X mutation. Following VPA exposure, myotubes expressed the brain isozyme in association with a dose-dependent decrease in glycogen accumulation (de Luna et al., 2015).

VPA belongs to a group of drugs known as histone deacetylase inhibitors (HDACIs). HDACIs increase the accessibility of demethylase enzyme to DNA (de Luna et al., 2015), regulating gene expression by activating the expression of methylated genes (Brodie and Brandes, 2014, Ghodke-Puranik et al., 2013). Based on animal model research, VPA could be considered as a potential gene modulator treatment for people with McArdle disease, allowing the expression of the brain isozyme with a potential functional benefit.

There has been growing interest in VPA function as an HDACI (Natasha et al., 2008, Duenas-Gonzalez et al., 2014, Huber et al., 2011, Ghodke-Puranik et al., 2013). However, it is not possible to compare its efficacy as the conditions studied have different pathophysiologies.

To date there has been no clinical trial of VPA in humans with McArdle disease (clinicaltrials.gov).

## **Clinical Research**

VPA safety in humans has already been proven. It is licensed for epilepsy and bipolar disorders in the UK. VPA has well-established pharmacokinetic and pharmacodynamic properties in adult humans (Ghodke-Puranik et al., 2013). It blocks sodium ion channels and inhibits enzymes that deactivate the neurotransmitter gamma-aminobutyric acid (GABA). Because of its mechanisms of action, VPA has efficacy in partial and generalised seizures (Glauser et al., 2013).

More recently, growing evidence has proven the teratogenic effect of VPA if used by women during pregnancy (Guveli et al., 2017, Gotlib et al., 2017). Currently, women of childbearing potential are advised to use other treatments when possible. If VPA is indicated, an effective method of birth control while using VPA is required (Wise, 2017).

## **Outcome Measures in Clinical Trials**

### **Muscle Biopsy**

Muscle biopsy use as an outcome measure in clinical trials in neuromuscular diseases is well reported (clinicaltrials.gov). Its use in McArdle disease may objectively confirm the presence of an alternative isozyme, and for this reason it was the main outcome measure that demonstrated the VPA benefits in the McArdle sheep animal model study (Howell et al., 2015).

VPA efficacy can be confirmed by the demonstration of phosphorylase enzyme expression in skeletal muscle fibres following treatment. Further studies in fresh muscle samples can also confirm the type of isozyme expressed, quantify the enzyme activity and enable analysis of glycogen content.

### **Cycle Ergometer Test**

Use of the cycle ergometer test as an outcome measure in clinical trials is well established (ClinicalTrials.gov, 2000, Quinlivan et al., 2015). The test is described in detail in 'Cycle Ergometer Test' (Methods section). Exercise endurance on the cycle ergometer can be analysed by assessing changes in the following parameters: rating of perceived exertion (RPE), submaximal oxygen consumption, maximum HR, maximum workload and  $VO_2$  peak (also peak cardiorespiratory conditioning or peak oxygen uptake).

VO<sub>2</sub>peak is an indicator of human health. When the cycle test is used to determine VO<sub>2</sub>peak, the workload is increased continuously (incremental test). VO<sub>2</sub>peak is the product of peak cardiac output and peak O<sub>2</sub> uptake by the working skeletal muscles. Assessment of VO<sub>2</sub>peak in a large cohort of patients with McArdle disease confirms that it is safe to perform an incremental test to maximal exercise intensity in this population (Munguia-Izquierdo et al., 2015, Santalla et al., 2017). The cycle test, when performed with a constant workload, is used as a screening/diagnostic tool in McArdle disease as it documents the *second wind* phenomenon (Haller et al., 2006, Quinlivan et al., 2015).

## **Opportunities for Translational Research**

### *12-minute Walk Test*

The 12-minute walk test (12MWT) is a simple and non-invasive self-paced test used in the UK clinic for McArdle disease as a diagnostic tool to demonstrate the presence of the *second wind* phenomenon (Buckley et al., 2014, Scalco et al., 2014a, Quinlivan et al., 2010). During this test, patients are requested to walk as far as they can for 12 minutes. The HR, total walked distance and muscle symptoms are recorded every minute. Symptoms are assessed by the Borg Rate of Perceived Pain (RPP) scale (Borg, 1970). The 12MWT documents the pre *second wind* characteristic increase in HR and muscle symptoms followed by the decrease in both clinical findings once *second wind* is reached. The six-minute walk test, classically used as an outcome measure in neuromuscular diseases (clinicaltrials.gov), is too short to document the improvement in clinical findings seen in McArdle patients during the *second wind* phenomenon as this occurs after walking for more than six minutes. For this reason, a prolonged test has more clinical utility when assessing this patient population.

To date, the role of the 12MWT as an outcome measure for clinical trials assessing drug efficacy in McArdle disease has never been studied (clinicaltrials.gov).

### *Forearm Exercise Test*

The non-ischaemic forearm exercise test consists of repetitive maximal handgrip contractions followed by hand/arm rest and blood sampling (Kazemi-Esfarjani et al., 2002). Ammonia and lactate levels are regularly measured following exercise. People with McArdle disease have a suboptimal rise (<3 fold) in serum lactate following exercise as a consequence of an impaired glycolytic system. This test, used to screen people with suspected McArdle disease (Scalco et al., 2015b, Kazemi-Esfarjani

et al., 2002, Hogrel et al., 2015), has a sensitivity and specificity of 100% and 99.7%, respectively, for discriminating McArdle disease patients from healthy controls (Hogrel et al., 2015).

The functional benefits of an alternative isozyme may be demonstrated, theoretically, by documenting a lactate rise during the forearm exercise test. To date, the non-ischaemic forearm exercise test has never been used as an outcome measure in clinical trials assessing drug efficacy in McArdle disease registered at ClinicalTrials.gov (clinicaltrials.gov).

### *Short Form 36*

The Short Form 36 (SF-36) health survey questionnaire consists of two component T-scores: mental component score (MCS) and physical component score (PCS); and eight health domain scales: physical functioning (PF), role – physical (RP), bodily pain (BP), general health (GH), vitality (V), social functioning (SF), role – emotional (RE) and mental health (MH). Questionnaire data is scored on a scale of zero to 100. Fifty is considered the mean score standardised for the general population. Higher scores represent better function in health domains, while lower scores represent the opposite.

The use of SF-36 in clinical trials is well established (clinicaltrials.gov), and its internal consistency and normative data are well described (Jenkinson et al., 1993).

Because the SF-36 has never been used in registered clinical trials for McArdle disease, it is not known whether it is a reliable tool for the assessment of the health status of people with the condition when exposed to a new pharmacological treatment (clinicaltrials.gov). A correlation between cardiorespiratory fitness and quality of life has however been reported in a large cohort of patients with McArdle disease (Munguia-Izquierdo et al., 2015). In addition, affected people had lower SF-36 scores when compared with healthy individuals (Munguia-Izquierdo et al., 2015). It is possible that the SF-36 questionnaire may consistently assess improvement in patients' quality of life in clinical trials for McArdle disease, especially if investigational medicinal products increase participants' fitness levels.

## 13. Research Questions

In this PhD, the main research challenge was how to translate knowledge from animal model studies to humans with rare exercise-related muscle disorders (T1 translation). To address this challenge, two main focuses were explored:

### Developing New Treatment Options for Rare Diseases

- To assess whether the inhibition of the NKCC by 2 mg bumetanide can abort a focal attack of weakness in people with HypoPP
- To assess whether the inhibition of the NKCC by 2 mg bumetanide can reduce the severity of a focal attack of weakness in people with HypoPP
- To assess whether 2 mg bumetanide is safe in people with HypoPP
- To assess whether 20 mg/kg/day sodium valproate can stimulate the expression of brain and/or liver glycogen phosphorylase enzyme in skeletal muscle of people with McArdle disease
- To assess whether treatment with 20 mg/kg/day VPA can improve the VO<sub>2</sub>peak of people with McArdle disease
- To assess whether treatment with 20 mg/kg/day VPA is safe in people with McArdle disease

### Developing New Outcome Measures for Rare Diseases

- To assess whether the abductor digiti minimi CMAP measured by the long exercise test (McManis test) is an objective outcome measure to assess the efficacy of drug therapies in HypoPP patients
- To assess whether the 12MWT is an objective outcome measure to assess the efficacy of drug therapies in McArdle patients
- To assess the feasibility of using several outcome measures in clinical trials for McArdle disease

# 14. Randomised Controlled Trial of Bumetanide in Hypokalaemic Periodic Paralysis

## Methods

### Aims and Hypothesis

The aim of this project was to assess a new treatment option for people with HypoPP and to investigate a new outcome measure to be used in clinical trials in this patient population. The initial hypothesis was that bumetanide would reduce both the severity and the duration of an attack of weakness in people with HypoPP based on previously published mouse model studies (Wu et al., 2013a, Wu et al., 2013b). Treatment benefit was assessed by neurophysiology assessments.

All participants were expected to show a reduction in CMAP amplitudes after isometric exercise. A CMAP reduction illustrates an ongoing acute attack of weakness as it demonstrates a significant proportion of skeletal muscle fibres are depolarised: further supramaximal stimulations in the ulnar nerve do not recruit additional muscle fibres. Once the CMAP amplitude recovers to normal levels, it confirms the acute attack is resolved as the skeletal muscle fibres are repolarised: supramaximal stimulation activates muscle action potentials.

As a primary outcome measure, CMAP amplitudes expressed as a percentage of the peak CMAP during or after exercise measured at 60 minutes following treatment intake were compared. The main aim of the primary outcome was to assess whether treatment intake reduced the severity of an attack of weakness one hour following treatment intake.

The outcome of four hours of McManis protocol was not known, as the test had never been performed for such a prolonged time. The initial hypothesis was that all participants would eventually recover, even the ones who received placebo, as they would have undergone the test under normokalaemia. The initial hypothesis was that the improvement would be faster in the bumetanide group compared to that in the placebo group. For this reason, a secondary outcome measure analysed the duration of an attack of weakness. This was assessed by comparing the exact time it took for CMAP amplitude to return to normal value following treatment intake, representing a repolarised muscle. The analysis of this outcome would provide evidence that bumetanide may facilitate the repolarisation of skeletal

muscle fibres, supporting the use of bumetanide to rescue an attack of weakness in this patient population.

Early and late efficacy were analysed by assessing all CMAP amplitudes from zero to 120 minutes and from 121 minutes to 240 minutes, respectively, following treatment intake.

As this was the first time bumetanide has been used in people with HypoPP, safety was analysed as a secondary outcome measure. It was initially hypothesised that 2 mg bumetanide would be safe as a single dose. As a potent diuretic drug, expected adverse events include hypotension and hypokalaemia due to potassium loss in urine. For this reason, repeated assessments of vital signs and serum potassium levels were made and analysed. Reported adverse events were also collected.

The McManis test was hypothesised to be a valuable tool to objectively document attack recovery in HypoPP. This was assessed by analysis of serial CMAP recordings.

### **Study Protocol and Ethics Statement**

This protocol was registered in ClinicalTrials.gov (NCT02582476).

This study and related documents were reviewed and approved by the University College London Hospital (UCLH) Trust (R&D), by the research ethics committee (NRES Committee London - Westminster) and by the MHRA. Ethics approval documentation (REC reference 14/LO/0774) is provided in Available in: printed thesis

Appendix 1. Written informed consent was obtained from all participants prior to study assessments. Copies of the final study participant information sheet (PIS) and study consent form (CF) are provided in Appendix 2 and Appendix 3, respectively. The study was performed under the ethical guidelines issued by the University College London (UCL) for clinical trials. All investigators involved in this study regularly attended Good Clinical Practice courses in the UK.

### **Study Design**

A randomised controlled trial was developed to provide the strongest scientific evidence (Class 1) of treatment benefit with less bias. This was a randomised, double-blind, placebo-controlled, single-centre, phase II clinical trial with a cross-over design assessing patients who fulfilled the study inclusion and exclusion criteria.

Because HypoPP is rare and in the light of the inclusion and exclusion criteria, it was expected that it would be difficult to recruit participants for this trial. To address this, a cross-over design was chosen, ensuring all participants would receive both treatments on two different occasions. To avoid a carry-over effect, a washout period of at least two weeks was selected. As the drug has a half-life of one to one and a half hours, it was predicted that there should be no drug in the system after seven and a half hours post drug intake.

After recruitment, each participant undertook two assessment visits following an identical protocol at two to eight weeks apart as illustrated in Figure 5. At the end of the study assessments, participants were discharged home.

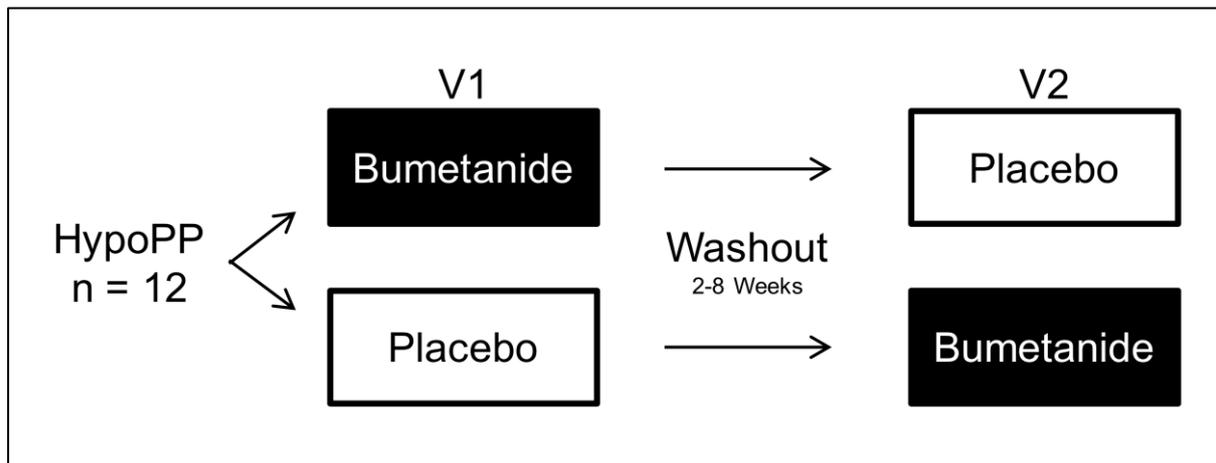


Figure 5. The schematic diagram of overall trial design

*HypoPP: Hypokalaemic periodic paralysis; V1: visit 1, V2: visit 2. Washout period lasted for 2–8 weeks.*

#### *Randomisation*

Each participant received one capsule containing either placebo or 2 mg bumetanide on two different occasions. The treatment order was randomly assigned: participants started with either bumetanide or placebo in a 1:1 allocation. A computer, based on pseudo-random numbers, generated the randomisation list. The study pharmacist held the randomisation list until the end of the study. Blocked randomisation was used to ensure a balance to the treatment orders throughout the study.

### *Blinding*

All trial personnel and study participants and their family members were blinded to treatment assignment for the duration of the trial. Over-encapsulated bumetanide tablets and manufactured placebo capsules looking identical to the over-encapsulated bumetanide were provided. Both active drug and placebo were bottled and labelled in a blinded fashion.

The study code was unblinded on one occasion for a valid medical reason: an unexpected pregnancy in one trial participant, which was diagnosed following visit 1 (V1) procedures as described in the Results section. The University College London (UCL) Joint Research Office approved the unblinding of the selected participant.

### **Participant Selection**

Inclusion and exclusion criteria were selected based on drug interaction with bumetanide, known complications related to bumetanide use (for example, potassium loss in urine, increase micturition), and difficulties in performing hand exercise, which would be a McManis protocol requirement. A list containing all exclusion criteria is given in Table 1.

Table 1. Inclusion and exclusion criteria

**Inclusion Criteria:**

- At least 18 years of age
- Diagnosis of genetically confirmed HypoPP
- Clinical symptoms or signs of active symptomatic disease (at least one attack of weakness in the last 12 months)
- Practising an acceptable method of birth control as described in the participant information sheet

**Exclusion Criteria:**

- Inability or unwillingness to provide informed consent
- People older than 64 years of age
- Other conditions causing hand weakness that could interfere with study measurements (for example, due to a stroke, trauma or arthritis)
- Patients with a history of cardiac disease, renal failure or hepatic disease
- Pregnant or breast-feeding
- Patients with a history of diabetes, porphyria, symptomatic hypotension, prostatic hypertrophy or difficulty with micturition, or allergy to sulfonamides or thiazides
- Patients taking lithium, digoxin, nephrotoxic or ototoxic drugs
- Patients known to be allergic to bumetanide or its excipients
- Patients with a history of inadequately treated Addison's disease
- Patients who had participated in another interventional trial in the previous one month

*HypoPP: hypokalaemic periodic paralysis.*

Potential participants were identified from a database of all patients with HypoPP at the National Hospital for Neurology and Neurosurgery (NHNN), London, UK. Potential participants were contacted by invitation letter, phone and/or in person during a routine clinic appointment at the NHNN. The trial was advertised via the MRC Centre for Neuromuscular Diseases website ([ucl.ac.uk/cnmd](http://ucl.ac.uk/cnmd)) and the Muscular Dystrophy UK website ([muscular dystrophyuk.org](http://muscular dystrophyuk.org)). New patients were also informed at the highly specialised clinic for channelopathy and during neurophysiology appointments at the NHNN. The trial was presented annually at the Channel Patients' day as poster sessions and oral sessions.

All participants received the study PIS prior to the screening visit. They were given time to read and discuss the study with their family members. The screening visit was booked at least 24 hours after PIS distribution. Interested participants attended a screening visit to determine study eligibility. After consenting, a physical examination was conducted and the following were assessed: medical history, vital signs (blood pressure and pulse), blood sampling for laboratory evaluation, electrocardiogram (ECG) and urine pregnancy test for all female participants. Laboratory assessments included kidney function, liver function, magnesium, chloride, bicarbonate, glucose and CK. This information was collected to determine whether participants were eligible for the study.

### **Study Visits Assessments**

To avoid inter-rater variability, all trial-related procedures were performed by the same investigator (me) and closely supervised by the PhD co-supervisor.

Standardised baseline assessments were performed in all study visits. These were performed to ensure the disease had not changed or progressed between study visits, which would have required rescreening and/or exclusion from the trial as a cross-over design was used. Baseline assessments were also performed to ensure safety, as visits would have been postponed in cases of ongoing symptoms or hypokalaemia prior to drug intake. A list of all baseline assessments is presented in Table 2.

Table 2. Baseline assessments performed on visit 1, visit 2 and extra visits

**Baseline Assessments**

- Medical history
- Physical examination (general, skin, cardiac, pulmonary, chest, abdomen, extremities, neurological, other as required)
- Vital signs (blood pressure and pulse)
- Contraceptive compliance
- MRC muscle scale
- Instant potassium levels\*
- Serum potassium levels\*\*
- ADM CMAP amplitudes (both limbs)

*\* measured by venous blood gas analyser; \*\* measured by the NHNN laboratory; MRC: Medical Research Council; ADM: abductor digiti minimi; CMAP: compound muscle action potential.*

### *Potassium Evaluation*

As a potent loop diuretic, an expected adverse event of bumetanide intake is hypokalaemia due to increased potassium loss in urine. Such an adverse event could in theory cause more harm than good in patients with HypoPP, as low levels of serum potassium can trigger acute weakness.

For this reason, baseline potassium levels were measured using two different techniques. Instant potassium levels were measured using a venous blood gas (VBG) analyser. The results were used to assess safety and guide prompt treatment (for example, to detect hypokalaemia at an early stage and provide quick rescue treatment). Further blood samples were sent to the NHNN laboratory for independent analysis of serum potassium levels. These results were used to assess bumetanide safety by confirming whether or not bumetanide caused severe potassium depletion. These results were analysed as part of a secondary outcome measure (safety).

### Definitions

To facilitate reading, in this thesis the term 'instant potassium levels' is used to represent the VBG results and 'serum potassium levels' to represent the NHNN laboratory results. The following definitions were used in the adverse event (AE) classification: a) hypokalaemia:  $K^+ < 3.5$  mmol/L; b) severe hypokalaemia:  $K^+ < 2.5$  mmol/L.

Instant potassium levels were measured at:

- Baseline procedures, performed prior to the McManis protocol, and were used as a criterion for postponing a study visit – as described in 'Criteria for Postponing a Study Visit'
- 30, 60, 90, 120 and 180 minutes post treatment intake, and were used for immediate assessment of possible hypokalaemia
- Any time, as extra potassium assessments were performed whenever required – for example, to confirm a previous assessment result, to assess participants presenting with muscle symptoms, etc.

Serum potassium levels were measured at:

- The beginning of study visits, prior to the McManis protocol
- The end of study visits, at participants' discharge

### *Localised Attack of Weakness*

This PhD project was designed to test an abortive treatment strategy as it has more utility in terms of clinical practice. In this scenario, participants receive treatment during an ongoing acute attack of weakness. To ensure safety, a localised attack instead of a generalised one was triggered. The focal attack was triggered in all participants at all study visits. A small hand muscle was chosen to facilitate neurophysiology assessments.

Following baseline assessments and procedures, the localised attack of weakness was induced by isometric exercise of the abductor digiti minimi (ADM) in the non-dominant hand, as routinely performed during the McManis protocol. The technique is described below. The non-dominant hand was selected so that participants would be able to use the dominant hand for accessing toilet facilities, using mobile devices or any other requirements.

#### Protocol for the Long Exercise Test (McManis Protocol)

Room thermometers and air conditioning were used to keep the study room temperature constant at 24 °C. Neurophysiology assessments and CMAP amplitude recordings were performed using skin electrodes attached to a VikingQuest Portable system (Neurodiagnostic System Version 11.1.0; Viasys Healthcare Neurocare Group, Judex A/S).

Electrodes and Supramaximal Stimulation: Self-adhesive electrodes were applied to the skin of both hands (Figure 6). The active electrode was placed on the hypothenar eminence overlying ADM, the reference electrode on the proximal fifth digit, and the ground electrode on the dorsum of the hand. Supramaximal stimulation was performed by a bipolar stimulator placed 1–3 cm proximal to the distal wrist crease, as standardised for ulnar motor studies (Wertsch et al., 2000, Misra and Kalita, 2006).

Five ADM CMAP measurements were collected, one every 30 seconds, to obtain stable baseline amplitudes.

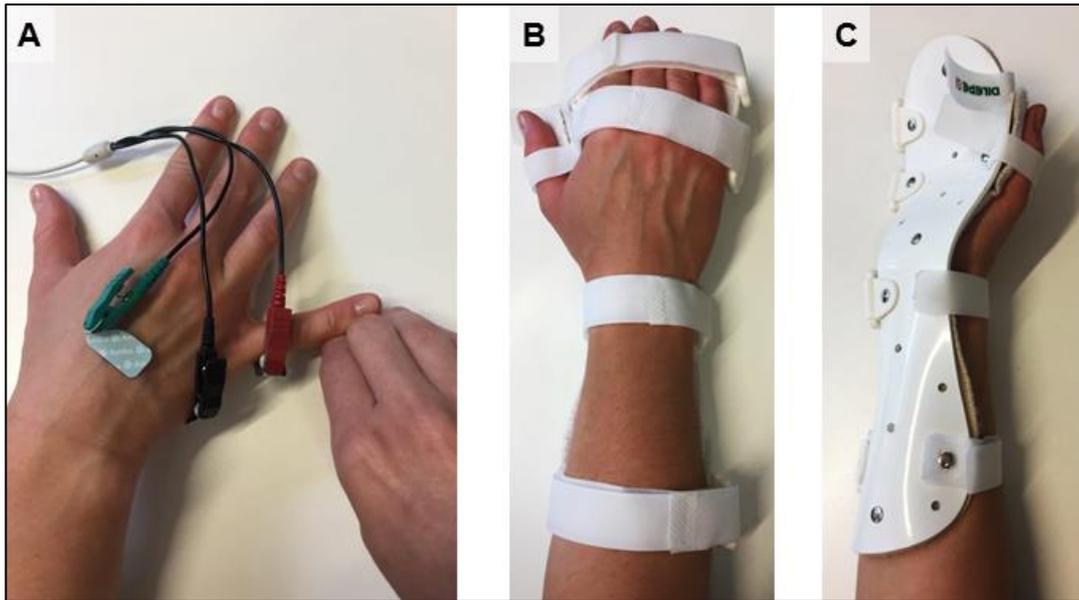


Figure 6. Electrode placement and custom-made splint for limb immobilisation

*In the non-dominant hand, CMAP amplitude was recorded every minute following the exercise until treatment intake, every two minutes for the first hour after treatment and every 5–10 minutes for the next three hours. A) Active electrode (black), reference (red) and ground electrode (green); B and C) Custom-made splint for limb immobilisation.*

Hand exercise: The long exercise test first described by McManis et al. (1986) applies a standardised regime of exercise followed by rest to an intrinsic hand muscle (McManis et al., 1986). Following baseline CMAP assessments, participants were instructed to contract the ADM isometrically against resistance as strongly as possible for five minutes with brief rest periods of three to four seconds every 15 seconds. During the five-minute exercise, the CMAP amplitude was recorded every minute. Following the ADM isometric exercise, participants were then instructed to relax the assessed hand completely for the following four to five hours. CMAPs were recorded every minute until the study medication was given. Following ingestion of treatment, CMAP amplitudes were recorded every two minutes for the first hour after treatment, and every five to 10 minutes for the next three hours.

Hand immobilisation: Tested hands and forearms were immobilised using a custom-made splint in order to prevent inadvertent ADM activation while allowing a degree of mobilisation to prevent induction of major attacks of paralysis and to allow access to bathroom facilities.

Splints for left and right limbs were available.

Figure 6 shows the electrode placement and a custom-made splint.

*Opposite limb:* Each study visit lasted for approximately six hours. As participants would be seated for a long time, there was a risk of generalised attack of weakness being triggered by persistent inactivity. All participants were encouraged to gently walk in the trial room to avoid prolonged immobilisation of lower limb muscles and core muscles. CMAP amplitudes were also evaluated in the dominant (non-immobilised) limb. This was performed as a safety procedure to identify the development of a generalised attack of weakness at an early stage. This would have been confirmed by documenting a progressive decrease in CMAP amplitudes in the dominant hand. Generalised attacks would increase the risk of unplanned hospital admissions for general support and, possibly, for respiratory support. For this reason, the data collected in the dominant hand was used to assess the need for prompt rescue treatment with potassium supplementation. Dominant-hand CMAP amplitudes were assessed before the long exercise test as part of the baseline procedures, and at every hour following exercise. This was repeated if needed until the visit was ended.

#### Extended McManis

If a participant did not show 40% CMAP decrement within 50 minutes after exercise, the exercise was repeated once using the same protocol, thereby extending the exercise period to 10 minutes. If this again failed to induce a focal ADM attack of weakness in V1, the participant was excluded from the study.

#### Attack Duration

CMAP amplitudes were compared to the peak CMAP amplitude, which was the highest value recorded during or after exercise. The following cut-off points were used:

- Attack onset: 40% CMAP amplitude decrement (60% of peak CMAP amplitude)
- Attack recovery: 35% CMAP amplitude decrement (65% of peak CMAP amplitude)

Figure 7 is a visual representation of the cut-off points used in a long exercise test.

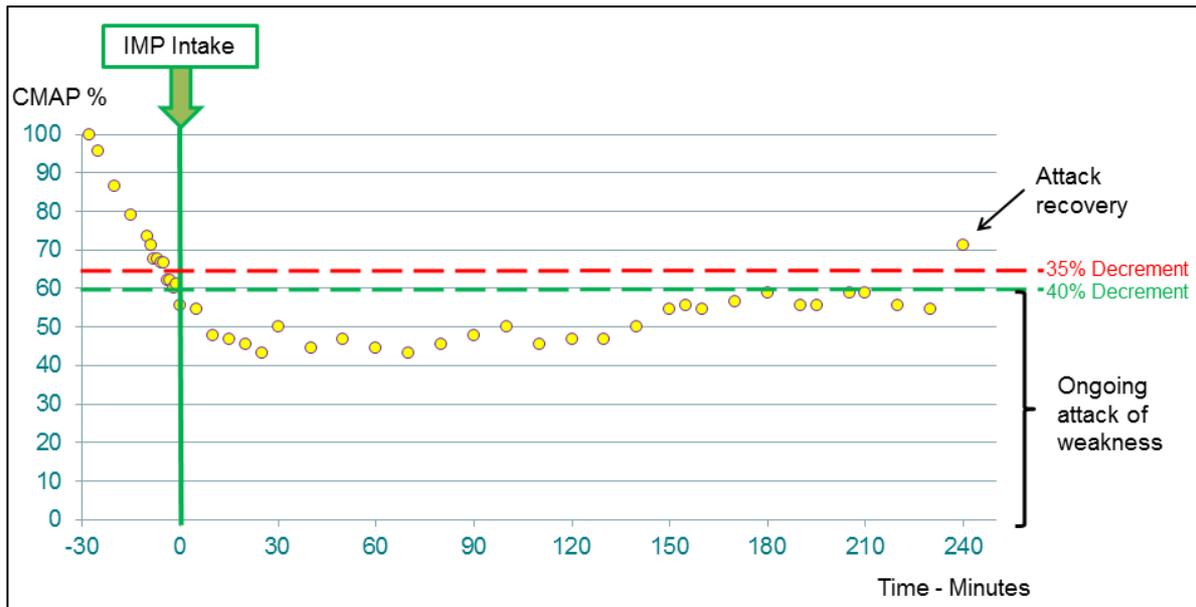


Figure 7. Visual representation of the cut-off points used in this trial during a long exercise test performed by one patient with HypoPP

CMAP: compound muscle action potential; IMP: investigational medicinal product (in this clinical trial, placebo or bumetanide).

### Study Diaries

It is well known that environmental factors influence serum potassium levels and, consequently, HypoPP symptoms – as previously reviewed in ‘Introduction to HypoPP’. To reduce the impact of non-treatment-related factors in both study visits, and to improve the comparison between treatment groups, a diary was developed to reduce the variation of environmental factors prior to both study visits. All participants had to complete a food and activity diary 24 hours prior to V1. In this diary, participants recorded their dietary intake, all physical activities performed and details on resting periods. They were encouraged to follow the same dietary intake protocol and similar physical activity pattern during the 24 hours prior to visit 2 (V2). Participants were given the food and activity diary during their screening visit.

Following each study visit (1, 2 or extra), participants also received a home diary in which they were advised to record any AE that occurred in the seven days following each study visit. Participants received two home diaries in total.

## **Treatment Administration**

The assigned treatment was taken by mouth at the onset of a focal attack, defined as 40% decrement in ADM CMAP amplitude, equivalent to 60% CMAP amplitude from peak amplitude, as illustrated in Figure 7. To ensure 100% compliance, the same investigator (me) administered all treatment tablets.

### *Bumetanide Dosage*

As a proof-of-concept study, a 2 mg dose was chosen. The aim was to assess efficacy in a clinically relevant outcome. Further information on study dose selection is reviewed in 'Discussion – Bumetanide Dosage'.

### *Previous Treatments*

Participants who had been prescribed oral potassium and potassium-sparing diuretics before entering the study were allowed to continue their treatment throughout the trial to avoid worsening of symptoms related to treatment interruption.

Participants who had been taking carbonic anhydrase inhibitor (including acetazolamide and dichlorphenamide) were asked to stop these medications for a minimum of 72 hours before each study visit so as not to interfere with the long exercise test protocol. Participants were allowed to restart treatment immediately after each study visit.

Participants were also required to withhold anti-inflammatory medications for at least 72 hours prior to assessment visits due to potential drug interaction with bumetanide.

## **Data Collection**

Clinical and paraclinical data from the patients were abstracted into a standardised Case Report Form (CRF) reproduced in Appendix 4. The collected data plus any other relevant information were recorded in participants' source folders, participants' medical notes and participants' CRFs. Data quality checks were regularly performed by the study monitor, who represented the UCL Joint Research Office / Study Sponsor. All data was recorded in a specifically designed database.

## **Safety Procedures**

Even though not expected with a single 2 mg dose, there were risks of severe hypotension due to increased micturition and severe reduction in potassium levels following bumetanide intake. Allergic reaction to bumetanide or placebo pill components was also not expected, but the risk of such an

unexpected adverse event was also considered. The following protocols were followed during all study visits to ensure safety in a rare event of a clinical emergency:

- Participants were seen as a day case at the MRC Centre for Neuromuscular Diseases at the NHNN, where medical facilities were available in case of severe adverse events (SAEs) related to drug use
- A medical doctor (me) closely supervised all participants during all study visits
- All medical doctors involved in this trial were trained in basic life support at UCLH
- An emergency/resuscitation trolley was available in the clinical trial room for immediate advanced cardiac life support, and a resuscitation team was nearby for prompt assistance if needed
- A trial nurse was present during the study visits for further support if needed
- Participants were encouraged to drink plenty of water during the course of all trial visits to prevent dehydration and associated hypotension

The following safety procedures were also carried out during all study visits:

- All female participants performed a urine pregnancy test before any study procedure
- All participants were asked about contraceptive-method compliance

Safety of bumetanide was assessed by physical examination, AE recordings, changes in vital signs and serum potassium levels.

#### *Vital Signs and Potassium Levels*

Vital signs were measured prior to the McManis test and at 30, 60, 90, 120 and 180 minutes following treatment intake. Potassium levels were collected as previously described in 'Potassium Evaluation'.

#### *Generalised Weakness*

In order to identify generalised weakness, muscle power was evaluated using MRC muscle scale for muscle strength at the beginning and end of each study visit. This study assessment was used to assess the need for postponing a study visit and/or to delay a visit discharge in case of ongoing generalised weakness. To avoid inter-rater variability, the same investigator (me) performed the MRC muscle scale assessment for all study participants at all study visits.

#### Definitions

- Minor attack of weakness: HypoPP-related weakness, participant fully ambulant
- Major attack of weakness: HypoPP symptoms affecting ability to walk

- Atypical attack of weakness: not characteristic of typical HypoPP symptoms

#### *Criteria for Postponing a Study Visit*

The criteria for postponing a study visit were a) not fully recovered from a recent attack the day before each visit; b) an ongoing acute attack of weakness at the time of the study visit assessments; c) pre-treatment instant potassium level lower than 3.5 mmol/L; d) medical concern raised by medical history and physical examination on the visit day; e) if the extended McManis failed to induce an attack. In such cases, V1 or V2 were postponed and an extra visit was booked.

#### *Hospital Admission*

Hospital admission was considered an SAE. In this protocol, the following hospital admission criteria were used: a) generalised and typical HypoPP attack of weakness during the study assessments; and b) persistent hypokalaemia beyond three hours of completing the observation period.

#### *Rescue Medication*

As explained earlier, bumetanide may cause potassium depletion in urine. Variation in serum potassium levels was expected; however, the significance of this was not entirely clear. The clinical impact of bumetanide-related hypokalaemia in people with HypoPP has never been explored. Even with all safety procedures in place, it was known that participants could require urgent treatment for acute potassium loss. However, it was not known whether potassium supplementation could influence the CMAP amplitude assessments. To standardise treatment approach, specific procedures were defined prior to commencement of the clinical trial.

In HypoPP, the use of potassium supplementation is standardised (Statland et al., 2018). For this reason, oral tablets of potassium were used as a rescue treatment. These were available in the trial room for instant intake. Rescue treatment comprised two Sando-K effervescent tablets.

In the event of an emergency, such as cardiac arrhythmia due to severe hypokalaemia, intravenous potassium was available for administration. This would have been used to manage severe HypoPP symptoms affecting respiratory muscles, severe hypokalaemia as previously defined in 'Potassium Evaluation', and prolonged attack of weakness if hospital admission were required and as medically indicated.

### *Adverse Events*

AEs were regularly evaluated at each study visit and following phone call evaluations performed seven days after each study visit. Participants were advised to record any AE occurring within one week of treatment in a diary at home as described in 'Study Diaries'.

### **Telephone Consultation**

All participants were called a day prior to each study visit to ensure they were fit to attend the trial appointment. During this telephone consultation, the criteria for postponing study visits were evaluated, such as assessing ongoing HypoPP symptoms for AE review. Prior to V1, participants were reminded to complete their food and activity diary; prior to V2, they were reminded to follow the diet and activity pattern recorded in their food and activity diary.

A telephone consultation was also performed seven days following each study visit for AEs review.

### **Guessing Treatment Assignment**

In this trial, no participant-reported outcome measure was used. For this reason, during the final follow-up telephone consultation, participants were asked whether they could guess the treatment they had been assigned. This was performed to better comprehend the impact of AEs when participants guessed correctly based on AE perception. It was also used to further understand bumetanide efficacy when participants guessed correctly based on symptom improvement. Table 3 summarises all study procedures previously described in this protocol.

Table 3. Flowchart of study assessments

	Screening	Treatment Phase								
	Week -8 to -1	72h	24h	Week 0	Week 1	Washout Week 2-8	72h	24h	Week 3-9	Week 4-10
			Call		Call			Call		Call
Visit #				1					2	
Informed Consent	X									
Medical History / Physical Examination	X			X					X	
Acute Attack of Weakness Assessment	X		X	X	X			X	X	X
Pregnancy Test	X			X					X	
Vital Signs	X			X					X	
ECG	X									
Eligibility Determination	X			X					X	
Cannulation				X					X	
Blood Sample	X			X					X	
Neurophysiology (McManis Test)				X					X	
MRC Muscle Scale	X			X					X	
Randomisation				X						
Food and Activity Diary			X					X		
IMP Administration				X					X	
Adverse Events Review	X		X	X	X			X	X	X
Patient Reported Symptoms	X		X	X	X			X	X	X
Concomitant Medication Review	X		X	X	X			X	X	X
Titrate Drug Down (previous treatments)*		X					X			
Restart Previous Treatments**				X					X	
Home Diary (AE)				X	X				X	X
Guessing Treatment Assignment					X					X

Call: telephone consultation; 72h: 72 hours prior to a study visit. \*72 hours prior to each study visit: titrate drug down (acetazolamide, non-steroidal anti-inflammatory drugs); \*\*Restart (previous treatments): on the same day of each study visit, immediately after the visit discharge. If needed, extra visits were booked following the same pattern of visit 1 or visit 2. ECG: electrocardiogram; MRC: Medical Research Council; IMP: investigational medicinal product; AE: adverse event.

## Data Analysis

The sample size was calculated based on the primary outcome of the CMAP amplitude one hour after treatment administration. After one hour, the CMAP amplitude was expected to fall to a mean of 40% in the control period. A difference in CMAP amplitude of 15% between treatments was regarded as clinically important, so the sample size calculation was based on identifying this difference. Based on clinical experience, it was expected that the standard deviation of the difference in CMAP amplitude between the two groups would be 15%. Using a 5% significance level and 90% power it was calculated that 11 participants were required, so 12 participants were selected to facilitate randomisation in each study arm (even number). In the event of a drop-out, a replacement subject could be recruited.

Baseline covariates (i.e., age, weight and height) were analysed descriptively.

### *Primary Outcome*

- The primary outcome measure was the focal attack severity one hour after treatment administration. This was measured as CMAP amplitude expressed as a percent of peak CMAP amplitude

The primary outcome was measured on a continuous scale. To allow for a cross-over design of the study, a multi-level regression model was used to estimate treatment effect. A two-level model was used, with measurement from individual time periods nested within patients. Terms were included in the model for time period (first or second treatment administration) and also treatment group. Carry-over from the first to the second period was examined by fitting the treatment by time interaction. In both cases, point estimates, 95% confidence intervals and p-values for the parameter associated with treatment assignment were obtained. A p-value equal to 0.05 or lower would have been indicative of statistical significance. All the above-mentioned analyses were performed on an intention-to-treat basis and no adjustments for missing data were made.

### *Secondary Outcomes*

This study was mainly developed to assess bumetanide efficacy, and for this reason interpretation of the following analyses reflected that these were secondary outcome measures, and the study design was not powered to assess these independently.

The secondary outcomes used in this trial were:

- The effect of treatment on focal attack duration was measured as the time between treatment administration until CMAP returned to 65% of peak CMAP
- The effect of treatment on severity of a focal attack within the first two hours was measured as CMAP amplitude (% compared to peak) area under the curve (AUC) from treatment administration until two hours post-treatment
- The late effect of treatment on severity of a focal attack was measured as CMAP amplitude (% compared to peak) AUC from treatment administration during the third and the fourth hours post-treatment
- Safety of bumetanide was assessed by physical examination, AE recordings, changes in vital signs and serum potassium levels

A multi-linear regression model was used to assess the CMAP-related secondary outcomes. Attack duration was summarised by descriptive statistics.

Safety of bumetanide was summarised using descriptive statistics. The following findings were included in the safety analysis:

- AEs for both placebo and bumetanide according to study visit and telephone consultation
- Potassium levels, according to treatment intake and time
- Vital signs (blood pressure and pulse – mean values), and pulse variation according to treatment intake and time

To evaluate mild serum potassium reduction, a multi-level regression model for potassium levels following treatment intake, controlling for their baseline values, has been fitted to the data.

## Results

I contacted and invited 37 people to take part in this clinical trial. Eleven participants agreed to attend a screening visit. The first participant was enrolled in February 2015. In May 2017 the study was terminated due to expired funding and slow recruitment.

One person failed the screening procedures as she did not have a genetically confirmed disease at the screening visit appointment; she was later diagnosed with Andersen-Tawil Syndrome.

A total of 10 participants were randomised: five participants received placebo at V1 and bumetanide at V2, treatment sequence placebo-bumetanide (P-B), and five participants were allocated to the treatment sequence bumetanide-placebo (B-P) as illustrated in Figure 8. Participant 002 had V2 cancelled/postponed as his baseline serum potassium level measured by VBG was 3.3 mmol/L; he attended an extra visit to complete trial procedures. Participant 006, randomised to sequence B-P, was excluded from this study during the washout period between the first and the second study visits due to pregnancy.

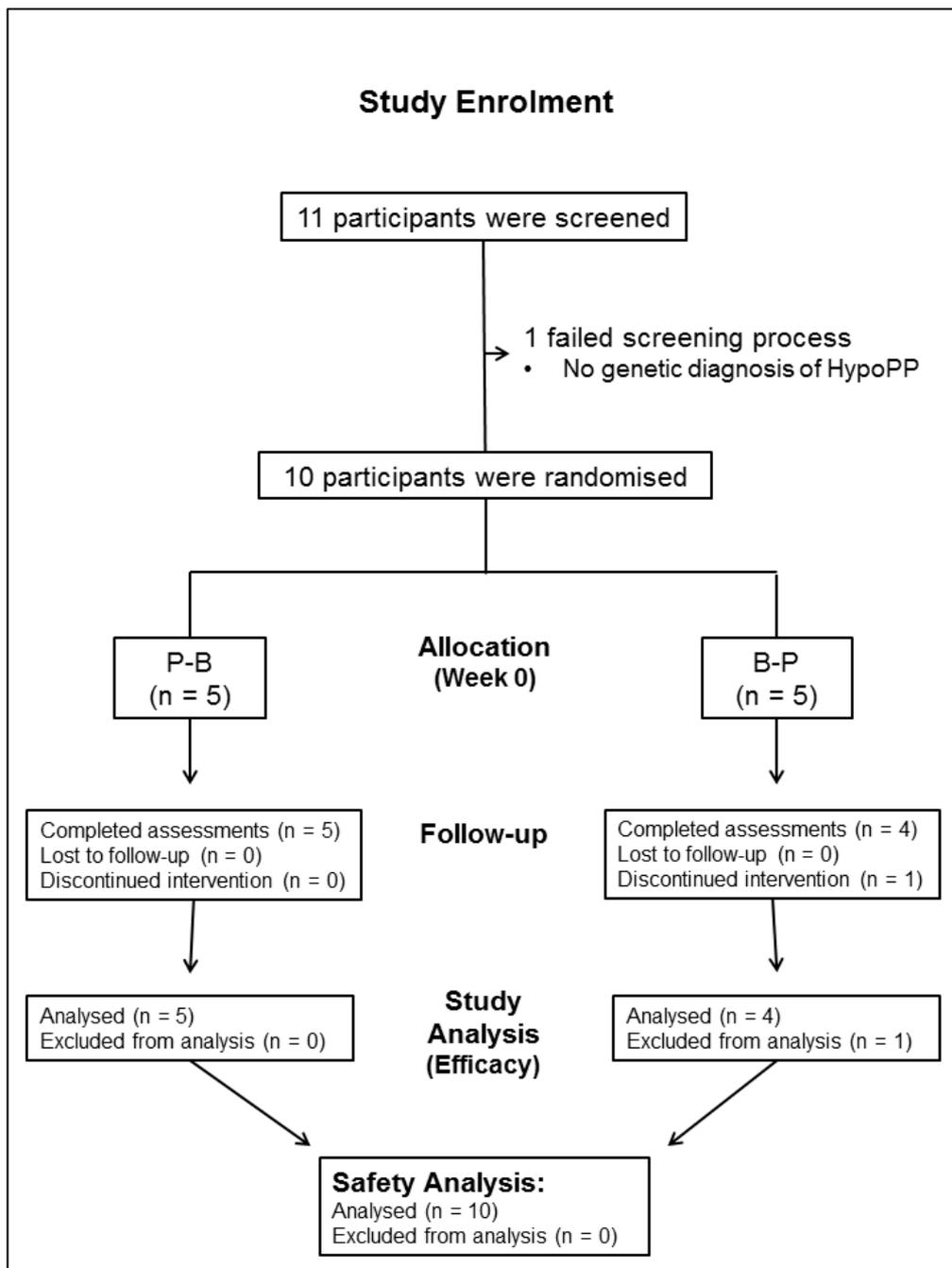


Figure 8. Flow diagram showing enrolment and outcome analysis

*P-B: treatment sequence placebo-bumetanide (female n = 2; male n = 3); B-P: treatment sequence bumetanide-placebo (female n = 1; male n = 4). Screening failure: not meeting inclusion criteria. Efficacy data was analysed for 9 participants as one was excluded because of an unexpected pregnancy. Safety data was analysed for all participants. HypoPP: hypokalaemic periodic paralysis.*

All participants had mutations in *CACNA1S* – HypoPP1: p.R1239H (n = 6), p.R528H (n = 3), and p.R498H (n = 1). Serum potassium levels measured at the screening visit by the NHNN laboratory were within normal limits. Continuous baseline variables are summarised in Table 4 and Table 5.

Table 4. Baseline findings for recruited participants

<b>Study Number</b>	<b>Gender</b>	<b>Previous HypoPP Treatment</b>	<b>Screening K<sup>+</sup> (mmol/L)</b>	<b>Treatment Allocation</b>
001	Male	ACZ, oral potassium	4.1	P-B
002	Male	None	4.4	B-P
003	Female	ACZ, SPL, oral potassium	4.4	P-B
004	Male	ACZ, K	4.3	P-B
005	Male	ACZ, AMI, SPL, oral potassium	3.7	B-P
006	Female	AMI, oral potassium	4.3	B-P
007	Male	ACZ, oral potassium	4.2	B-P
008	Male	ACZ, oral potassium	4.4	P-B
009	Male	ACZ, oral potassium	4.1	B-P
010	Female	ACZ, oral potassium	4.0	P-B

*Screening K<sup>+</sup>: serum potassium levels measured at the screening visit; ACZ: acetazolamide; SPL: spironolactone; AMI: amiloride; Treatment allocation: P-B: treatment sequence placebo-bumetanide, B-P: treatment sequence bumetanide-placebo.*

Table 5. Descriptive statistics for continuous baseline variables, by treatment order

	<b>Median</b>	<b>Min</b>	<b>Max</b>	<b>n</b>
<b>Treatment Order P-B</b>				
<b>Weight</b> (kg)	77	50	93	5
<b>Height</b> (cm)	169	161	175	5
<b>Age</b> (years)	47	28	55	5
<b>Treatment Order B-P</b>				
<b>Weight</b> (kg)	73	66	82	5
<b>Height</b> (cm)	180	168	186	5
<b>Age</b> (years)	38	18	54	5
<b>Study Sample</b>				
<b>Age</b> (years)	45	18	55	10

*Min: minimum; Max: maximum. P-B: treatment sequence placebo-bumetanide; B-P: treatment sequence bumetanide-placebo.*

Three trial participants (007, 008 and 009) continued acetazolamide as previously prescribed for the duration of the trial (protocol deviation). The participants were more severely affected, thus treatment was maintained prior to both visits to prevent the occurrence of severe attacks of weakness. They stopped acetazolamide treatment 12 hours prior to each study visit.

### **Guessing Treatment Assignment**

Of the eight participants who guessed their treatment assignment correctly, as shown in Table 6, three considered 'symptom improvement' the main reason to support their choice. Only one based his choice on adverse event perception (increased micturition). Participant 007 felt weaker on V2 and thought this could have been an adverse event of bumetanide intake; in fact, 007 received bumetanide on the day he felt stronger and received placebo on V2. Participant 001 did not notice significant differences between V1 and V2. Participants 002 and 004 did not give any particular reason for their choices. Participant 008 described "having a feeling" he had received bumetanide on the correct date, but did not know how to describe it.

Table 6. Guessing treatment assignment

Participant Number	Guessing Treatment Assignment	Reason for Participant's Choice
001	Did not answer	Did not notice differences between the two visits
002	Correct	No reason
003	Correct	Symptom improvement
004	Incorrect	No reason
005	Correct	Symptom improvement
006	-	-
007	Incorrect	Weakness was perceived as a bumetanide AE
008	Correct	No reason
009	Correct	Bumetanide-related adverse event (increased micturition)
010	Correct	Symptom improvement

*No reason: participants gave no specific explanation for their choice; AE: adverse event.*

### Neurophysiology: Extended McManis

In five out of 19 visits (26.3%) participants did not show 40% CMAP decrement within 50 minutes following five minutes of isometric ADM exercise as standardised by the McManis protocol. All these participants presented a 40% decrement following 10 minutes of isometric ADM exercise (extended protocol) as illustrated in Table 7. Following the standard and the extended McManis protocol, 40% CMAP decrement was seen after a median of 30.5 (12–49) and 28 (18–44) minutes, respectively.

Table 7. Neurophysiology assessments according to study participants

Study Participant	001		002		003		004		005		006	007		008		009		010	
Study Visit	V1	V2	V1	V3	V1	V2	V1	V2	V1	V2	V1	V1	V2	V1	V2	V1	V2	V1	V2
Exercise (min)	5	5	5	5+10	5+10	5	5+10	5	5	5	5	5	5	5+10	5+10	5	5	5	5
<b>Non-dominant Hand</b>																			
NDH Baseline CMAP	4.4	5.4	8.5	7.7	8.2	8.5	8.0	8.2	6.0	5.2	8.6	4.3	5.2	4.2	4.4	6.2	5.0	11.9	10.8
NDH Max CMAP	6.0	6.5	9.0	8.2	9.3	9.0	8.4	9.6	7.5	7.4	9.3	5.3	8.5	7.0	6.0	6.5	6.3	12.1	11.3
Time 40% Decrement (min)	22	28	44	18	24	38	44	33	35	27	20	20	12	28	37	34	41	22	49
Time 35% Decrement (min)	NK	NK	NK	NK	NK	240	NK	NK	NK	NK	NK	26	NK	NK	NK	NK	NK	NK	NK
NDH CMAP 1h	2.0	2.2	3.0	3.5	1.8	4.0	4.0	3.1	2.8	2.7	4.5	3.5	2.9	1.8	1.8	2.4	1.6	6.0	4.9
NDH CMAP 2h	1.8	2.0	3.9	3.2	1.8	4.2	3.9	3.2	2.4	2.4	3.2	2.3	2.4	1.5	1.0	2.5	1.4	NK	5.1
NDH CMAP 4h	1.4	2.6	4.2	3.1	3.9	6.4	3.9	4.4	2.7	3.0	4.3	2.8	1.7	1.0	0.9	3.3	2.3	4.9	6.4
<b>Dominant Hand</b>																			
DH Basal CMAP	7.3	9.8	7.7	8.3	8.4	8.2	8.3	9.3	8.3	7.5	7.2	4.5	5.9	3.4	5.4	4.6	4.6	13.6	12.6
DH CMAP 1h	7.5	8.6	6.7	7.8	7.5	7.5	7.2	7.6	9.0	6.5	7.7	4.6	5.8	4.5	6.5	4.6	4.3	15.0	10.9

DH CMAP 2h	7.8	9.1	7.2	8.2	7.5	8.0	7.5	8.5	7.8	6.2	8.6	6.1	4.8	4.0	6.2	4.1	4.3	14.3	13.4
DH CMAP 3h	8.2	10.3	6.2	8.0	7.4	8.3	7.8	10.4	9.8	7.7	7.6	6.3	6.3	4.0	6.4	4.6	5.2	13.2	12.1
DH CMAP 4h	7.7	11.2	6.6	8.3	7.0	9.1	7.8	10.0	10.5	6.1	9.3	6.4	6.8	5.2	5.6	5.6	5.5	12.2	12.4

*V1: visit 1; V2: visit 2; V3: extra visit – visit 3; NDH: non-dominant hand; DH: dominant hand; CMAP: compound muscle action potential. Max: maximum; Time 40% Decrement (min): Time in minutes when there was a 40% decrement in ADM CMAP amplitude from the peak response; Time 35% Decrement (min): Time in minutes when 35% decrement (or less) was reached in ADM CMAP amplitude from the peak response; NK: not known. 002 attended a third visit (V3) as V2 was cancelled/postponed due to low instant potassium levels at baseline assessments; 006 attended V1 only.*

## Primary Outcome Analysis

The primary outcome was the CMAP amplitude relative to the peak value one hour after the administration of treatment. Histogram and Normal quantile-quantile plot (Figure 9) show that it was reasonable to assume the primary outcome was normally distributed.

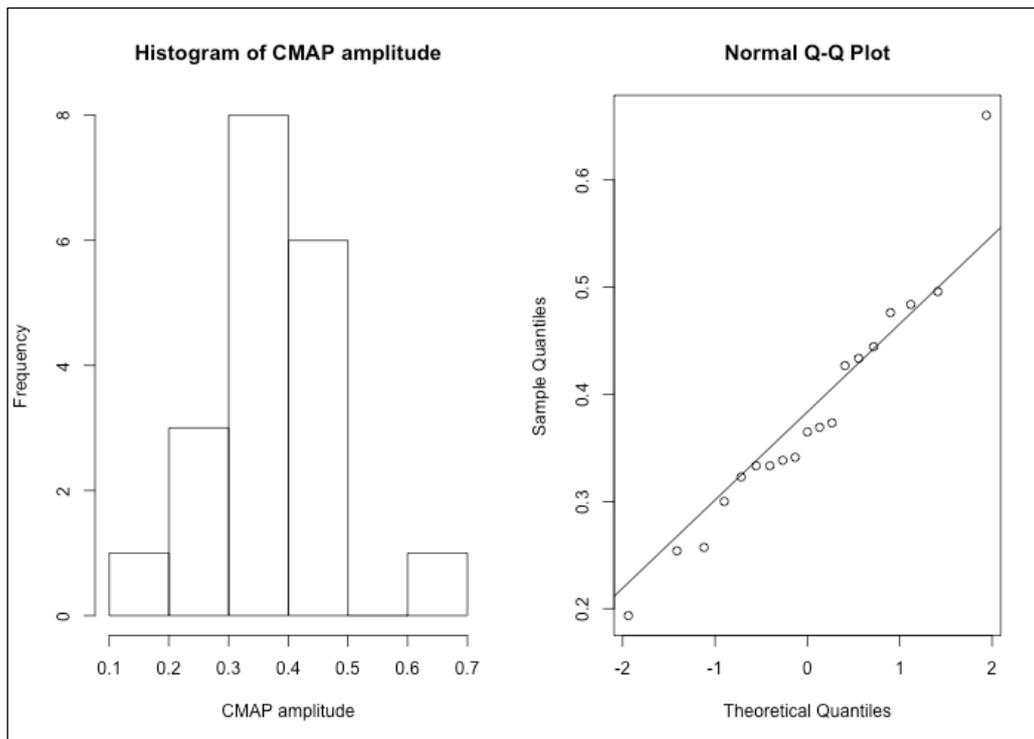


Figure 9. Normality checks for the primary outcome

*Figure generated by Mr Federico Ricciardi.*

### *Random Effects Model*

The mean CMAP percentages of peak amplitudes for bumetanide and placebo groups were 40.6% and 34.9%, respectively. A random effects model was fitted to the data to estimate the effect of the treatment on primary outcome, adjusting for time period and treatment group. Table 8 shows the estimated effect difference for CMAP amplitude relative to peak of being treated with bumetanide compared to placebo. The small effect estimate and large p-value implied that there was insufficient evidence to support any difference in CMAP amplitude between the treatment groups.

Table 8. Estimated difference for CMAP amplitude in bumetanide group compared to placebo group

Effect Estimate	95% Confidence Interval	P-value
0.059	(-0.057; 0.175)	0.27

*Model Checking for Primary Outcome*

The normality of the estimated error terms was analysed using normal quantile-quantile plots (Figure 10), while the homoscedasticity of the estimated error terms was assessed using a scatter plot of the residual (Figure 11). Both graphs are reassuring about model assumptions, and no data transformation was required. No outliers for primary outcome have been identified.

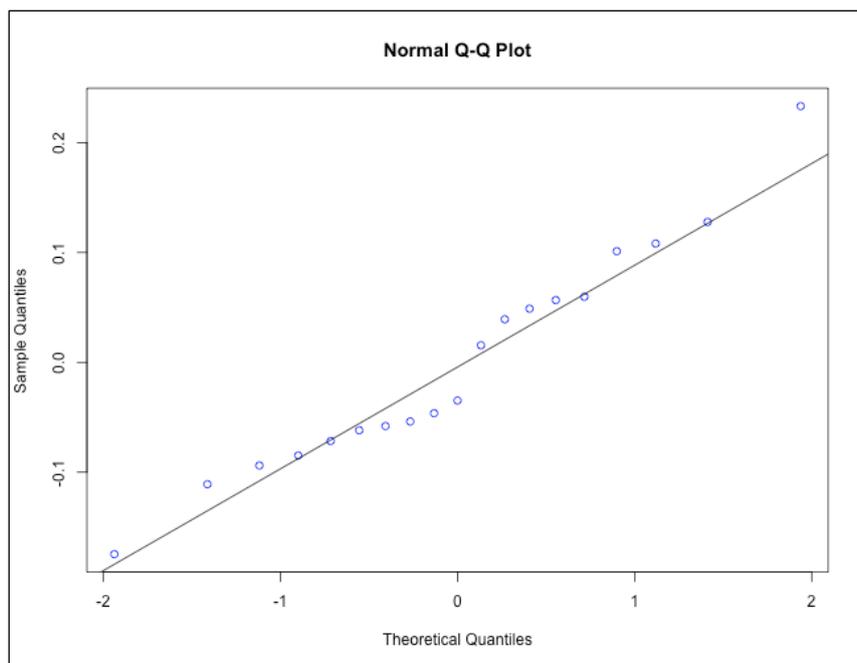


Figure 10. Normal QQ plot of residuals

*Figure generated by Mr Federico Ricciardi.*

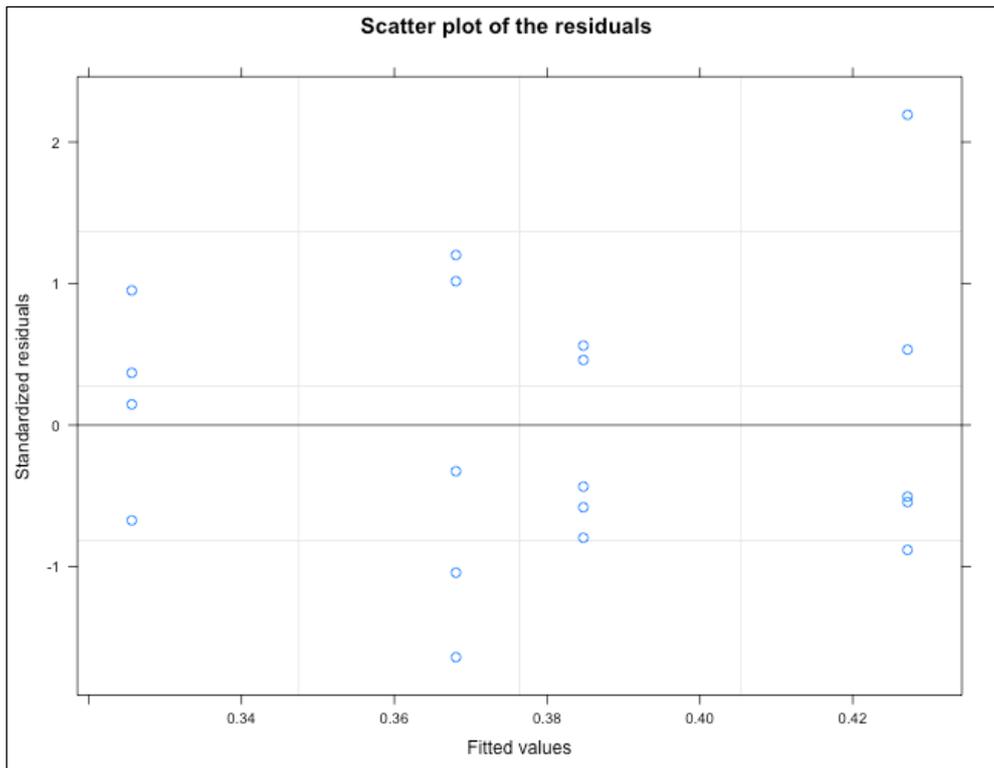


Figure 11. Scatter plot of the residuals

Figure generated by Mr Federico Ricciardi.

### Carry-over Effect

The carry-over effect from the first to the second period was examined by fitting a second model adding the treatment by time interaction. The coefficient for this effect was equal to -0.072 and it proved to be non-significant (p-value = 0.5), with 95% confidence interval -0.315 to 0.172 (Table 9).

Table 9. Estimated carry-over effect, with associated 95% confidence interval and P-value for a test of the null hypothesis that the true effect is zero

Effect Estimate	95% Confidence Interval	P-value
-0.072	(-0.315; 0.172)	0.5

### Secondary Outcomes

#### Attack Duration

The effect of treatment on focal attack duration was measured as the time in minutes between treatment administration and CMAP returning to 65% of peak CMAP amplitude. None of the

participants recovered following placebo intake, hence it was not possible to analyse the outcome. When given bumetanide, two participants recovered at 26 and 240 minutes, respectively.

#### *Initial and Late Treatment Effects*

The early effect of treatment on severity of a focal attack was measured as CMAP percentages of peak amplitude AUC from treatment administration until two hours post-treatment. Late effect was measured as CMAP percentages of peak amplitude AUC from treatment administration during the third and the fourth hours post-treatment. Initial and late treatment effects were analysed using the same regression model used for analysing primary outcome.

As both p-values exceed the threshold level of 0.05, these effects were not statistically significant.

The mean CMAP percentage of peak amplitudes for early and late treatment following bumetanide intake were 41% and 40%, respectively. For the placebo group, early and late treatment were 37% and 31%, respectively.

The estimated effect differences for the mean CMAP percentage of peak amplitudes in the bumetanide group compared to the placebo group according to early and late efficacy were 4.3% (p-value: 0.3) for early efficacy, and 8.5% (p-value: 0.1) for late efficacy.

#### Post-hoc Analysis – CMAP Changes Following Treatment Administration

Mean CMAP percentages of peak following treatment administration until McManis protocol completion (240 minutes) is illustrated in Figure 12. Figure 13 shows the longitudinal profiles of CMAP amplitude according to study participant and treatment for 240 minutes after treatment administration during the McManis test.

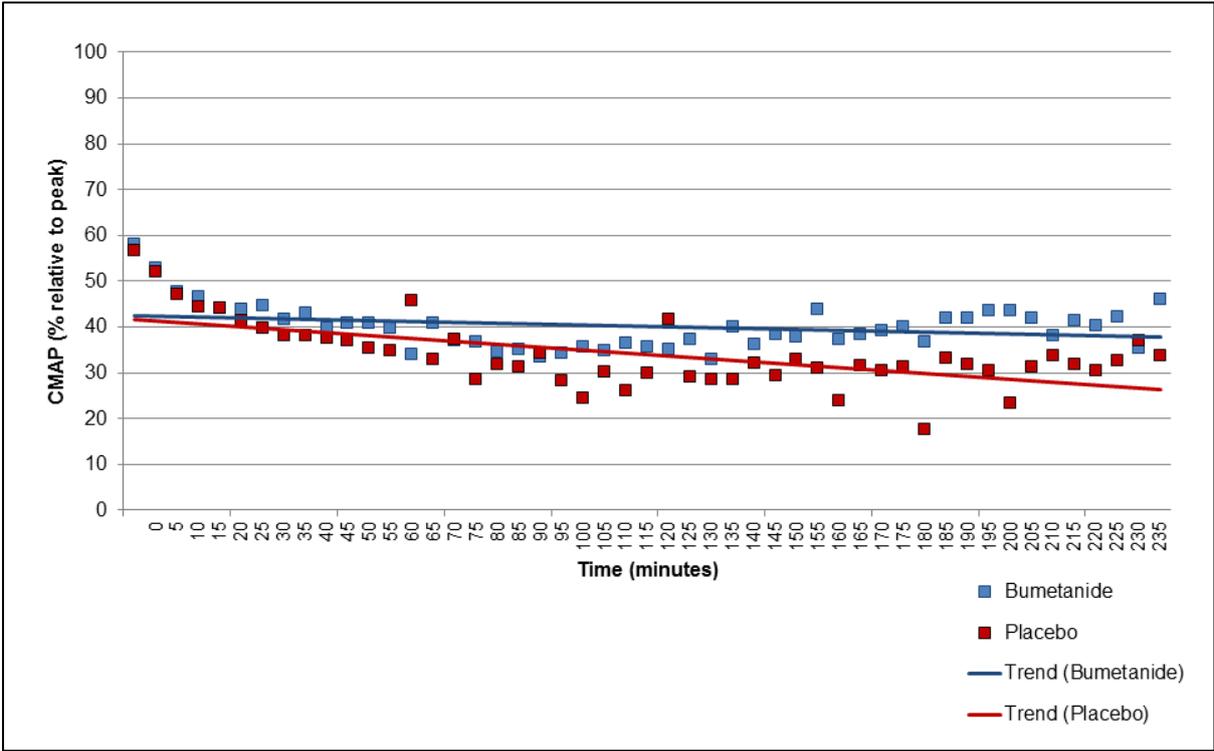


Figure 12. Average CMAP percentage of peak amplitudes following treatment administration

CMAP: compound muscle action potential. Outliers after the first hour are explained by missing data points as measurements were not performed for the same time points for every individual.

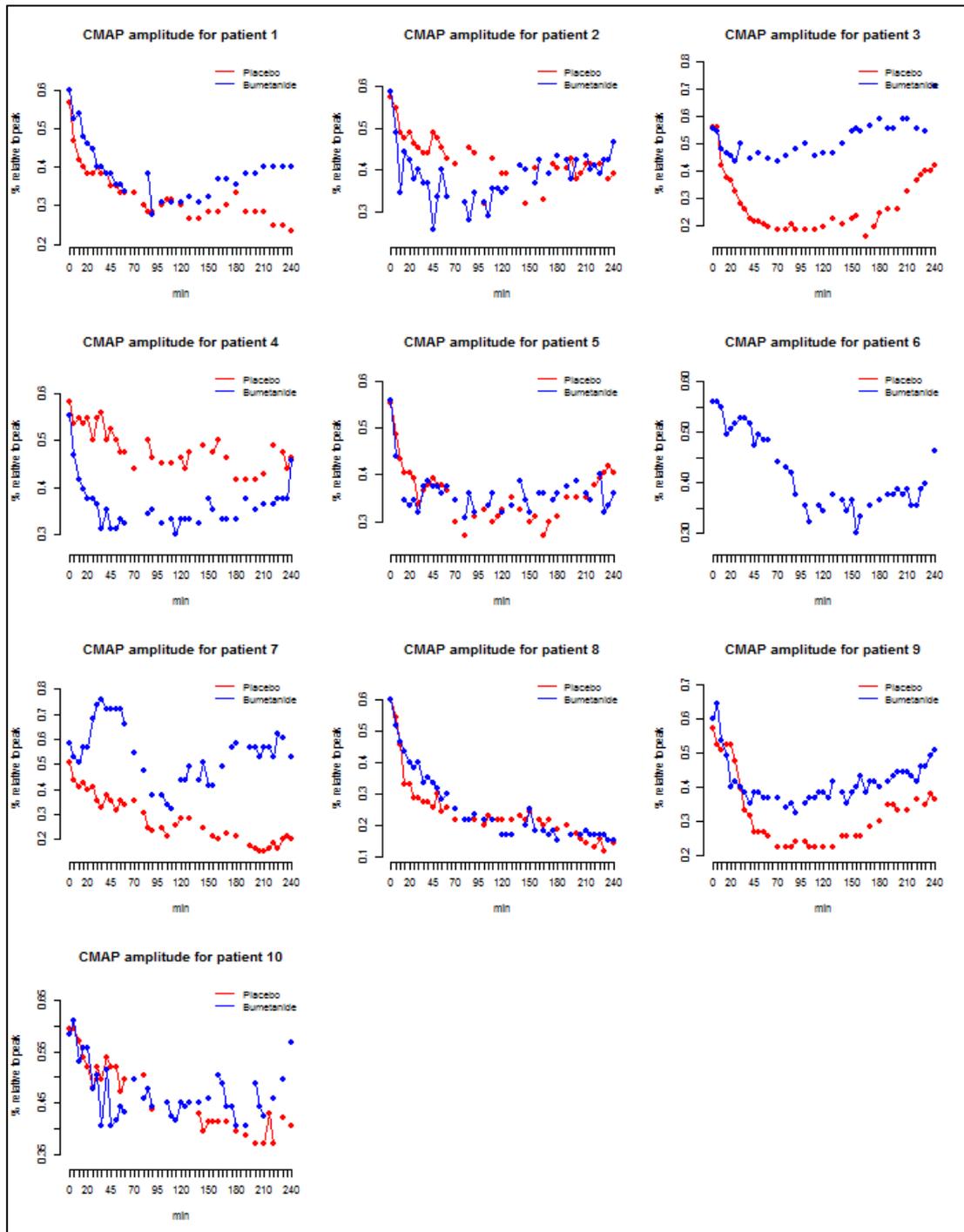


Figure 13. CMAP amplitude during the McManis test, by study participant and treatment  
*In blue: bumetanide visits; in red: placebo visits. CMAP: compound muscle action potential.*

### *Safety of Treatment*

Safety of bumetanide was assessed by physical examination, AE recordings, changes in vital signs and serum potassium levels.

### Adverse Events

No study participant had a severe adverse event. Frequencies of AEs recorded during study visits and telephone consultations are shown in Table 10 according to treatment.

Table 10. Adverse events recorded during study visits and telephone consultations

<b>Adverse Events</b>	<b>Placebo (n)</b>	<b>Bumetanide (n)</b>
<b>Adverse Events Prior to Study Assessments</b>		
Minor attack of weakness (legs)	0	1
<b>Adverse Events During Study Visits</b>		
Weakness in exercised hand *	6	6
Atypical attack of weakness	0	1
Burning pain (hand) while exercising	0	1
Hypokalaemia	2	3
Asymptomatic bradycardia	0	1
Focal arm rash (reaction to cannula plaster)	1	0
Haematoma after cannula removal	1	0
Cold symptoms	1	1
Increased micturition	0	6
Headache	1	1
Tingling	2	1
<b>Adverse Events within One Week of Study Visit</b>		
Tingling	0	1
Stomach pain	0	1
Minor HypoPP attack	6	3
Major HypoPP attack	1	1
Weakness in exercised hand	0	1
Stiffness	1	0
Cold symptoms	1	1
Muscle soreness	1	0
Increased micturition	0	1
Headache	1	0
<b>Total</b>	<b>25</b>	<b>31</b>

*Because some participants reported more than one adverse event, the total number of AEs exceeded the number of trial participants. Atypical attack of weakness: not characteristic of typical HypoPP symptoms. \* at visit end.*

## Potassium Levels

None of the participants had symptomatic hypokalaemia during this clinical trial. Rescue treatment with oral potassium was given to four participants in five occasions for the following reasons:

- 003: acute symptoms of weakness  
The study participant reported “generalised weakness”. She was walking in the trials room and her symptoms started suddenly when she sat down. Extra CMAP measurements were assessed in her dominant hand. These were within normal limits and did not correlate with reported arm/hand weakness. There was no respiratory discomfort in association with the reported generalised symptoms. She was given two tablets of oral potassium as a treatment option for generalised weakness, even though her serum potassium levels were within normal limits (extra instant potassium assessment prior to oral potassium intake: 4.1 mmol/L). Her symptoms completely improved within 24 minutes of rescue treatment intake. The atypical symptoms were discussed with the study team, the trial steering committee and the multi-disciplinary channelopathy team responsible for her care. Her symptoms were considered to be an attack of functional weakness not related to periodic paralysis. This was based on the atypical presentation, absence of a CMAP decrement on the dominant hand, normal serum potassium levels, sudden onset and quick resolution. Her vital signs were stable and she made a full recovery. Oral potassium was given at 225 minutes following bumetanide intake. The presentation was not considered a serious adverse event as discussed and agreed at the steering committee meeting.
- 006: asymptomatic hypokalaemia  
An instant potassium level measured by VBG machine at 180 minutes following bumetanide intake was abnormal ( $K^+$ : 3.0 mmol/L).
- 008: asymptomatic hypokalaemia  
An instant potassium level measured by VBG machine at 120 minutes following bumetanide intake was abnormal ( $K^+$ : 3.1 mmol/L).
- 010: asymptomatic hypokalaemia  
This participant received rescue treatment twice in the same visit following placebo intake. She presented with asymptomatic hypokalaemia ( $K^+$ : 3.0 mmol/L) measured by VBG machine at 30 minutes following placebo intake. This improved with rescue treatment, but later she presented a second episode of asymptomatic hypokalaemia ( $K^+$ : 3.2 mmol/L) measured by VBG machine at 120 minutes following placebo intake. Each hypokalaemic episode was recorded as an AE ( $n = 2$ ). In total, she received four tablets of oral potassium during the same study visit.

Serum potassium levels measured at the NHNN laboratory at the beginning of visit assessments (pre exercise) and at the end of study visits are shown in Table 11. One participant had a serum

potassium level of 3.3 mmol/L at visit discharge following at least 240 minutes bumetanide intake; this was recorded as an AE once the laboratory report had been reviewed, but no rescue treatment was provided as the laboratory report was available for review after a few hours following participant discharge.

Table 11. Descriptive statistics for serum potassium levels

		Mean	SD	Min	Max	n
<b>Placebo</b>						
<b>Serum Potassium Levels</b> (mmol/L)	Before exercise	4.2	0.28	3.8	4.6	7
	At discharge	4.3	0.3	4.1	4.8	6
<b>Bumetanide</b>						
<b>Serum Potassium Levels</b> (mmol/L)	Before exercise	4.4	0.19	4	4.6	8
	At discharge	3.9	0.38	3.3	4.4	9

Post-hoc Analysis – Serum Potassium (Laboratory Analysis)

There was a mild potassium reduction during the bumetanide visits as compared to the placebo visits (coeff: -0.238); this difference was not statistically significant ( $p = 0.18$ ; 95% CI = [-0.675 ; 0.198]). However, the trial was not powered for such an outcome.

Post-hoc Analysis – Instant Potassium Levels

Figure 14 illustrates the mean instant potassium values analysed by VBG according to treatment. Occasional high potassium levels measured by VBG were considered artefacts due to haemolysis during sample collection. For this reason, all values higher than 6.0 mmol/L were excluded from this analysis. Figure 15 shows the longitudinal profile of all instant potassium levels measured by VBG in all trial participants following IMP intake.

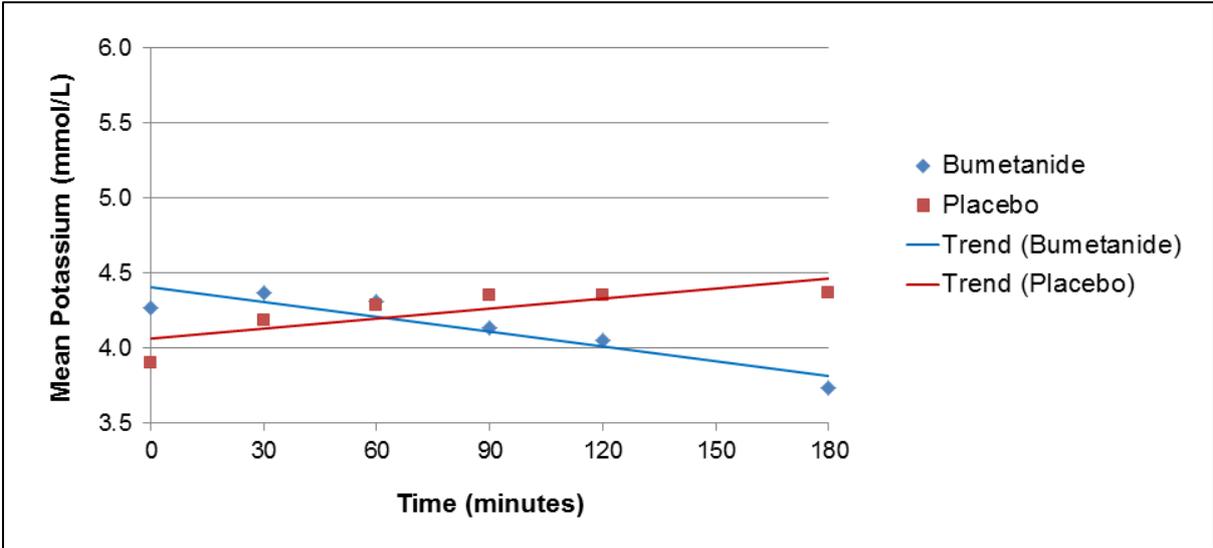


Figure 14. Mean potassium values according to treatment

*In blue: bumetanide visits; in red: placebo visits. Potassium values higher than 6.0 mmol/L were excluded from the mean analysis.*

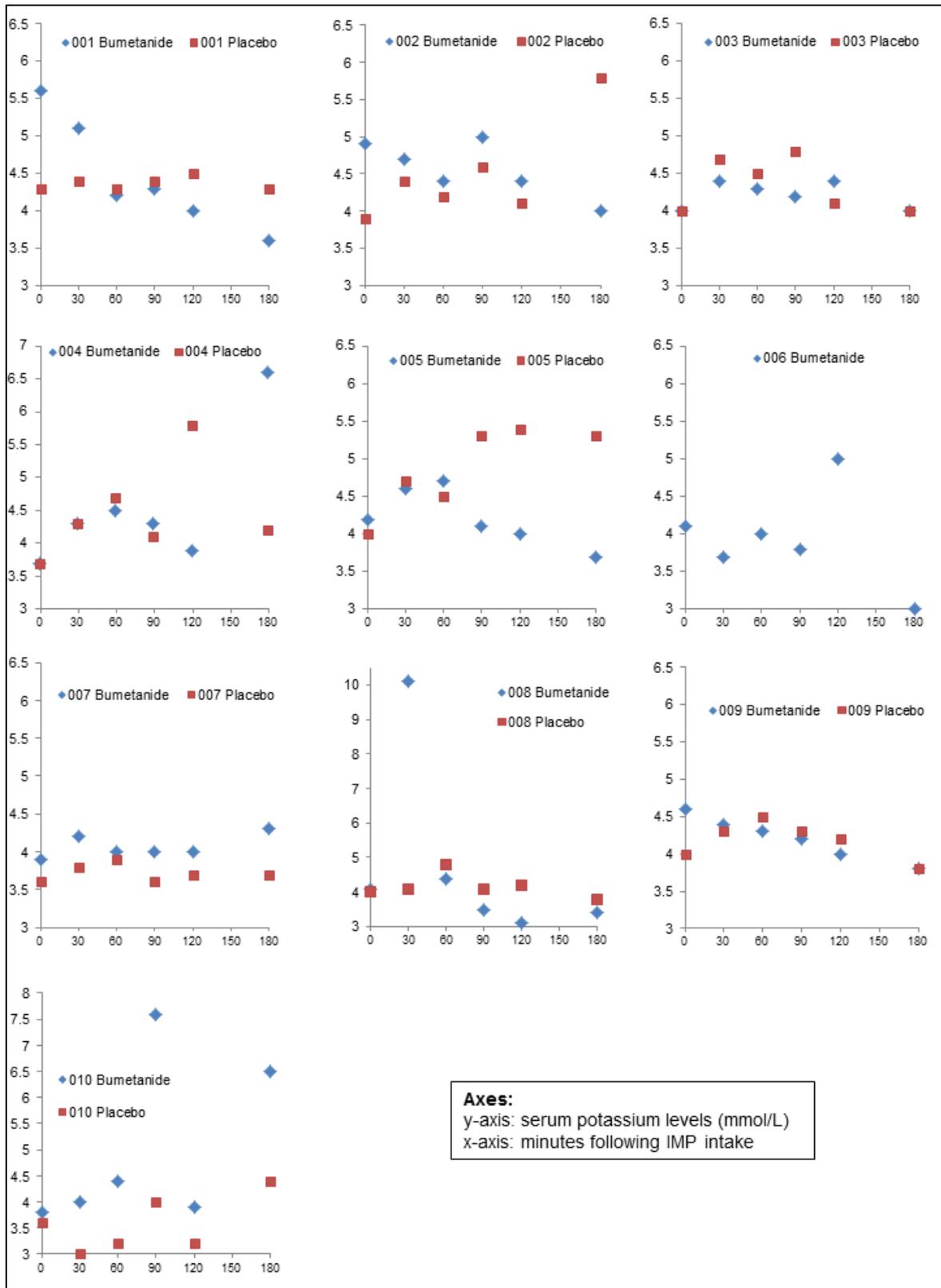


Figure 15. Instant potassium levels by study participant and treatment

Occasional high potassium levels considered artefacts due to haemolysis: 002 – placebo at 180 minutes (5.8 mmol/L), 004 – placebo at 120 minutes (5.8 mmol/L), 004 – bumetanide at 180 minutes (6.6 mmol/L), 006 – bumetanide at 120 minutes (5.0 mmol/L), 008 – bumetanide at 30 minutes (10.1 mmol/L), 010 – bumetanide at 90 minutes (7.6 mmol/L) and at 180 minutes (6.5 mmol/L). IMP: investigational medical product.

### Vital Signs

None of the participants had symptomatic hypotension. Descriptive statistics for systolic and diastolic blood pressure and pulse recorded before exercise and after the McManis test are reported in Table 12 separately, by treatment assignment. Figure 16 illustrates pulse variation according to treatment assignment. There was no symptomatic tachycardia or bradycardia.

Table 12. Descriptive statistics for blood pressure and pulse by treatment assignment

		Mean	SD	Min	Max	n
<b>Placebo</b>						
<b>Systolic Blood Pressure</b>	Before exercise	119	11.49	106	142	9
	After placebo administration	117	13.07	98	143	9
<b>Diastolic Blood Pressure</b>	Before exercise	75	5.41	67	82	9
	After placebo administration	76	7.89	63	87	9
<b>Pulse</b>	Before exercise	72	7.76	61	81	9
	After placebo administration	66	12	50	83	9
<b>Bumetanide</b>						
<b>Systolic Blood Pressure</b>	Before exercise	118	15.54	104	158	10
	After bumetanide administration	114	11.49	98	132	10
<b>Diastolic Blood Pressure</b>	Before exercise	74	6.92	66	88	10
	After bumetanide administration	77	8.9	63	93	10
<b>Pulse</b>	Before exercise	79	16.37	63	116	10
	After bumetanide administration	79	13.18	60	103	10

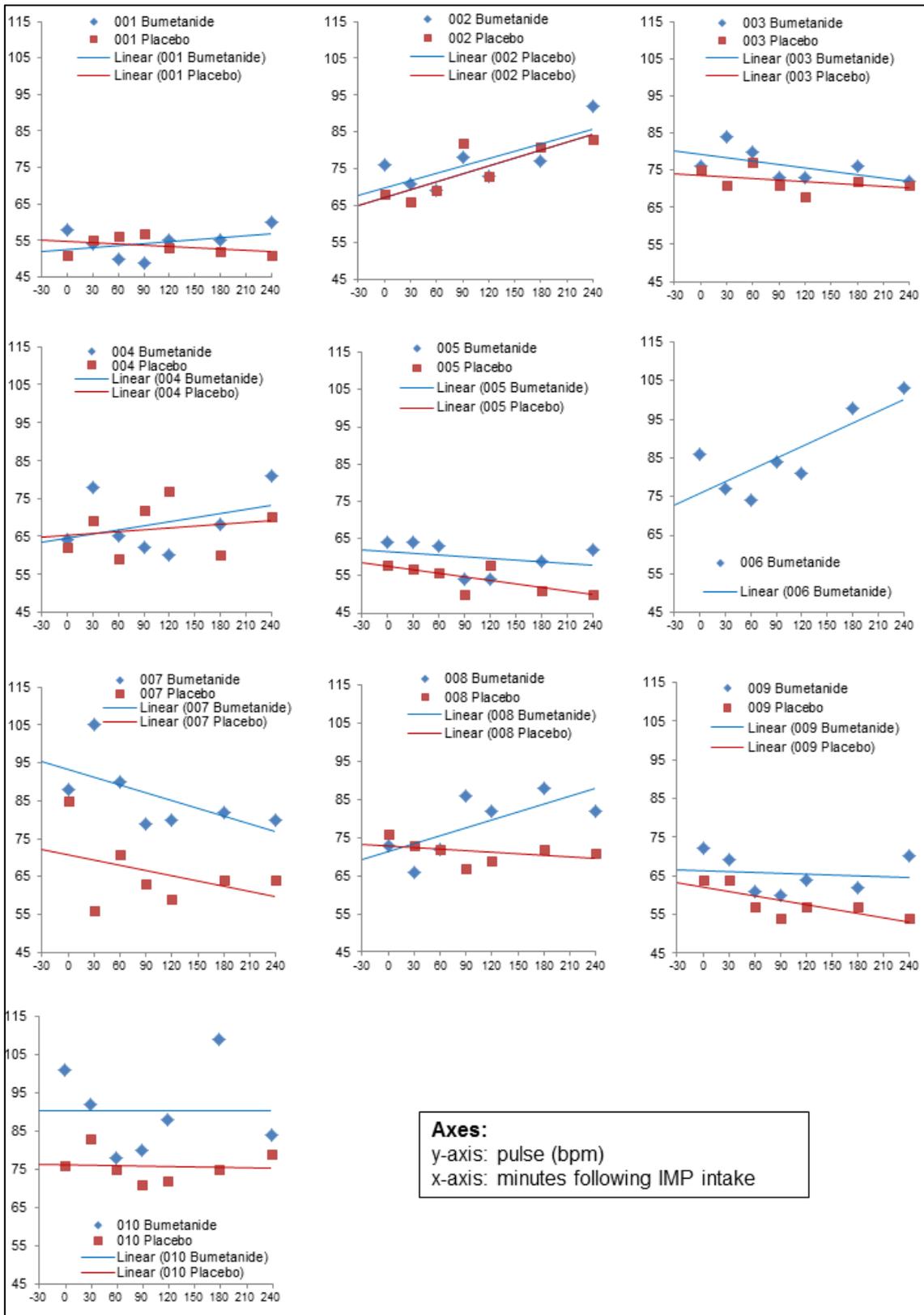


Figure 16. Pulse recorded during the McManis test, by patient and treatment  
*bpm: beats per minute; IMP: investigational medicinal product.*

### Physical Examination

MRC muscle scale scores were obtained before and after the McManis test. Table 13 illustrates the MRC scores, by treatment assignment.

Table 13. MRC muscle scale scores before and after McManis test, by treatment

	Score	Placebo				Bumetanide			
		Before McManis		After McManis		Before McManis		After McManis	
		n	%	n	%	n	%	n	%
Arm Abduction	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4-	0	0	0	0	1	10	0	0
	4	1	11.1	0	0	1	10	0	0
	4+	0	0	0	0	0	0	0	0
	5	8	88.9	9	100	8	80	10	100
Elbow Flexion	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	2	20	1	10
	4-	1	11.1	0	0	0	0	1	10
	4	1	11.1	1	11.1	2	20	0	0
	4+	1	11.1	0	0	2	20	0	0
	5	6	66.7	8	88.9	4	40	8	80
Elbow Extension	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	1	10	0	0
	4-	2	22.2	0	0	0	0	2	20
	4	1	11.1	1	11.1	2	20	1	10
	4+	1	11.1	1	11.1	2	20	1	10
	5	5	55.6	7	77.8	5	50	6	60
Wrist Extension	1	0	0	0	0	0	0	0	0
	2	0	0	1	11.1	0	0	0	0
	3	1	11.1	1	11.1	2	20	0	0
	4-	0	0	0	0	0	0	1	10
	4	0	0	2	22.2	3	30	1	10
	4+	2	22.2	2	22.2	0	0	2	20
	5	6	66.7	3	33.3	5	50	6	60

		Placebo				Bumetanide			
		Before McManis		After McManis		Before McManis		After McManis	
	Score	n	%	n	%	n	%	n	%
Wrist Flexion	1	0	0	0	0	0	0	0	0
	2	0	0	1	11.1	0	0	0	0
	3	2	22.2	1	11.1	2	20	2	20
	4-	0	0	0	0	0	0	1	10
	4	0	0	2	22.2	3	30	0	0
	4+	2	22.2	2	22.2	0	0	1	10
	5	5	55.6	3	33.3	5	50	6	60
Finger Extension	1	0	0	1	11.1	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	2	22.2	3	33.3	1	10	0	0
	4-	0	0	1	11.1	1	10	1	10
	4	2	22.2	0	0	2	20	3	30
	4+	2	22.2	1	11.1	2	20	1	10
	5	3	33.3	3	33.3	4	40	5	50
Finger Flexion	1	0	0	0	0	0	0	0	0
	2	0	0	1	11.1	0	0	0	0
	3	1	11.1	1	11.1	2	20	1	10
	4-	2	22.2	1	11.1	1	10	0	0
	4	2	22.2	1	11.1	1	10	3	30
	4+	2	22.2	2	22.2	1	10	0	0
	5	2	22.2	3	33.3	5	50	6	60
Finger Abduction	1	0	0	0	0	0	0	0	0
	2	0	0	1	11.1	0	0	0	0
	3	5	55.6	8	88.9	5	50	8	80
	4-	1	11.1	0	0	2	20	1	10
	4	2	22.2	0	0	2	20	0	0
	4+	1	11.1	0	0	1	10	1	10
	5	0	0	0	0	0	0	0	0

	Score	Placebo				Bumetanide			
		Before McManis		After McManis		Before McManis		After McManis	
		n	%	n	%	n	%	n	%
Finger Adduction	1	0	0	0	0	0	0	0	0
	2	0	0	1	11.1	0	0	0	0
	3	4	44.4	8	88.9	6	60	9	90
	4-	2	22.2	0	0	2	20	0	0
	4	2	22.2	0	0	1	10	0	0
	4+	1	11.1	0	0	1	10	0	0
	5	0	0	0	0	0	0	1	10
Hip Flexion	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	1	10	0	0
	3	3	33.3	3	33.3	3	30	3	30
	4-	2	22.2	0	0	0	0	1	10
	4	1	11.1	2	22.2	1	10	0	0
	4+	0	0	2	22.2	2	20	1	10
	5	3	33.3	2	22.2	3	30	5	50
Hip Extension	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	1	11.1	1	11.1	1	10	1	10
	4-	0	0	0	0	0	0	1	10
	4	3	33.3	2	22.2	5	50	1	10
	4+	1	11.1	2	22.2	1	10	1	10
	5	4	44.4	4	44.4	3	30	6	60
Knee Flexion	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	2	22.2	0	0	1	10	0	0
	4-	0	0	1	11.1	1	10	2	20
	4	4	44.4	1	11.1	3	30	1	10
	4+	0	0	3	33.3	1	10	1	10
	5	3	33.3	4	44.4	4	40	6	60

	Score	Placebo				Bumetanide			
		Before McManis		After McManis		Before McManis		After McManis	
		n	%	n	%	n	%	n	%
Knee Extension	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	2	22.2	2	22.2	2	20	0	0
	4-	0	0	0	0	1	10	3	30
	4	4	44.4	3	33.3	1	10	2	20
	4+	1	11.1	1	11.1	2	20	0	0
	5	2	22.2	3	33.3	4	40	5	50
Ankle Dorsiflexion	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	1	10	1	10
	4-	0	0	0	0	1	10	1	10
	4	5	55.6	1	11.1	1	10	2	20
	4+	1	11.1	4	44.4	1	10	0	0
	5	3	33.3	4	44.4	6	60	6	60
Ankle Plantarflexion	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4-	0	0	0	0	1	10	3	30
	4	5	55.6	2	22.2	2	20	1	10
	4+	1	11.1	3	33.3	1	10	0	0
	5	3	33.3	4	44.4	6	60	6	60

### Pregnancy

Participant 006, randomised to sequence B-P, was excluded from the study during the washout period between her first and the second visits due to pregnancy. The participant reported good compliance with oral contraception. Urine pregnancy tests, performed at the screening visit and visit 1, were both negative. However, it is possible that this participant was at early stages of pregnancy when she received 2 mg bumetanide.

The pregnancy was reported immediately to the study Sponsor, and the participant was unblinded for treatment assignment. The unexpected pregnancy was discussed at the trial steering committee meeting and by the multi-disciplinary channelopathy team responsible for her medical care.

Following pregnancy confirmation, she was advised by her local doctor to stop amiloride as this drug has been associated with intrauterine growth restriction (Almeida and Spinnato, 1989). Amiloride treatment discontinuation was not related to the clinical trial procedures. After treatment discontinuation her HypoPP symptoms worsened. Amiloride was restarted later in her pregnancy to treat severe HypoPP symptoms. Foetal growth monitoring was performed on a monthly basis. She had an induced vaginal delivery with episiotomy and epidural anaesthesia at 38 weeks. She did not have HypoPP symptoms during delivery. She had a healthy newborn (2.976 kg).

## **Discussion**

The innovative potential of this project comprised identifying a new treatment option for a rare disease while exploring a new outcome measure for drug development. It also explored the challenges in performing investigator-initiated studies in rare neuromuscular diseases within an academic environment. To date, this is the first randomised controlled trial assessing a treatment strategy to abort attacks of paralysis for HypoPP. It is also the first study to assess the role of the McManis test as an outcome measure for clinical trials.

In this chapter, the challenges related to protocol development and the main study findings will be discussed in two separate sections. The first section covers aspects considered while writing the trial protocol and the second discusses drug safety, efficacy and outcome measure analysis.

### **Protocol Development**

Bumetanide is a well-known diuretic drug. It is relatively safe and has few adverse events. Animal model studies provided sufficient evidence that bumetanide prevented and aborted acute attacks of weakness in KI mouse models of HypoPP, warranting further studies of this drug in humans (Wu et al., 2013a, Wu et al., 2013b). In this PhD, the knowledge gained from such animal model studies was translated to humans, assessing bumetanide efficacy in reducing the severity and duration of a focal attack of weakness in people with HypoPP. As this was the first time people with genetically confirmed HypoPP have taken bumetanide, safety of the drug in this patient population was also analysed.

#### *Choosing Treatment Strategy*

The evidence gained from previous HypoPP mouse model studies provided stronger evidence of a preventative treatment effect than an abortive treatment effect, as discussed in 'Bumetanide Dosage' (Discussion section). This project was designed to test an abortive treatment strategy because it has more utility in terms of clinical practice. Other treatment options are available for preventing acute symptoms, such as the well-established use of acetazolamide. Avoiding triggers for paralysis attacks may partially prevent symptoms, especially when they are diet related.

Important factors limiting the chronic use of bumetanide were its pharmacokinetic parameters and known adverse events (AEs). Bumetanide has a very short half-life (up to 1½ hours); thus, its use on

a daily basis may be inconvenient for patients as it would require frequent dosing. Low plasma levels during sleep periods may be an issue associated with the short half-life of the drug. As bumetanide is a potent diuretic drug, certain AEs are expected, including increased micturition, postural hypotension, muscle cramps, dehydration and hypokalaemia. Those events may be particularly distressing for people with a debilitating neuromuscular disease such as HypoPP. An example would be the difficulty in accessing toilet facilities due to HypoPP leg weakness combined with the need to urinate several times following a 2 mg dose – for example, one participant used the toilet approximately seven times during the four hours following bumetanide intake.

It is known that bumetanide AEs are more frequently reported in association with long-term drug use or multiple-dosage regimes (Stone et al., 1981, Lemonnier et al., 2017, Pressler et al., 2015). As this is the first study to assess bumetanide safety in HypoPP, a single dose was considered a safer option than multiple doses or long-term prescription.

#### *Translating Knowledge from Animal Model Studies to Humans with HypoPP: Anticipated Challenges*

##### Risk of Hypokalaemia

Because bumetanide is a potent loop diuretic, hypokalaemia is an expected AE due to increased potassium loss in urine. Such an AE could in theory cause more harm than good in patients with HypoPP, since low levels of serum potassium may trigger attacks of acute weakness.

During an acute attack of weakness in HypoPP, there is a shift of potassium into the muscle intracellular space, which produces a temporary reduction in the serum concentration, as previously described in 'Introduction to HypoPP'. Potassium loss in urine following treatment with bumetanide, combined with the temporary decrease in serum potassium concentration due to the intracellular shift related to the HypoPP pathophysiology, was of special concern because of a potential risk of cardiac arrhythmia. Severe hypokalaemia may cause sudden cardiac events, including cardiac arrest (Finsterer and Stollberger, 2014, Jauregui-Garrido and Jauregui-Lobera, 2012). In this scenario, inducing a generalised attack of weakness in people with HypoPP to assess bumetanide efficacy could potentially be unsafe and cause harm. For this reason, during this study, a focal attack of weakness was provoked in a controlled environment in people with normokalaemia. This was also the main reason why different bumetanide doses were not explored as discussed in 'Bumetanide Dosage' (Discussion section).

### Poor Recruitment

HypoPP is a rare disease and finding individuals eligible to enter the trial proved to be more challenging than expected. As a consequence, the trial did not meet its recruitment target despite efforts to advertise it nationally.

To address potential recruitment difficulties, a cross-over design was chosen, so that all participants would receive both bumetanide and placebo during the trial. This allowed the use of a smaller sample size.

In HypoPP, non-treatment-related factors may influence the attack severity, as reflected in the well-known phenotypic differences related to gender and age. In this study, each participant acted as his or her own control, thus reducing inter-subject variability and providing more reliable results (Stoney and Lee Johnson, 2012).

Prior to the study, it was not known whether findings of the McManis protocol would vary between participants. The cross-over design helped reduce possible inter-subject variability of primary and secondary outcome measures.

The condition is not expected to deteriorate over a short period of time, particularly during the wash-out period. For this reason it was felt that a cross-over study would be an appropriate trial design. Cross-over trials are valuable for assessing diseases with an 'episodic phenotype' such as HypoPP (Stoney and Lee Johnson, 2012). As bumetanide has a very short half-life, a carry-over effect was not expected, which could potentially limit the use of a cross-over design.

### Prevention of Expected Severe Adverse Events

A severe attack of weakness in someone with HypoPP may require emergency hospital admission, particularly if the respiratory muscles are affected. In such cases, intravenous potassium supplementation is used as a rescue treatment option.

For the purpose of clinical trials, any type of hospital admission is usually considered an SAE, requiring prompt safety review. Consequently, in this trial, any hospital admission would have been considered an SAE. As per trial protocol, a series of procedures ensuring safety were in place, guaranteeing an urgent report of all SAEs to the study sponsor. To prevent such occurrences, specific criteria for postponing study visits were developed prior to the start of the trial. Postponing study visits

if clinically indicated, as described in 'Criteria for Postponing a Study Visit', enabled participants to be seen on alternative dates, thereby avoiding participants' exclusion due to hypokalaemia or an ongoing acute attack of paralysis on the study visit day. Also, specific instructions on how to manage acute reductions in serum potassium levels instantly measured by blood gas analyser were standardised at the early stages of protocol development. Several instant potassium measurements and dominant-hand CMAP measurements were performed to detect hypokalaemia and the development of acute weakness, respectively. Such procedures allowed the reliable screening of known HypoPP comorbidities and bumetanide AEs on a regular basis.

In this trial, the safety measures in place may have contributed to the absence of SAEs, as no study participant was admitted to hospital due to severe hypokalaemia or severe HypoPP symptoms.

#### Precise Assessment of Efficacy

To date, there is no registered clinical trial assessing a treatment strategy to abort attacks of weakness in people with HypoPP (clinicaltrials.gov). The lack of reliable and internationally recognised outcome measures in this condition was an exceptional challenge to this project. To assess drug efficacy, it was extremely important to record exact times for the start and cessation of the attack of weakness. Finding a way to objectively grade symptom severity was also challenging.

Therefore, to identify preliminary efficacy evidence in a clinically relevant endpoint (Schmidt, 2006), an innovative proof-of-concept study was developed to detect whether oral intake of bumetanide affected HypoPP pathophysiology. A focal attack of weakness was induced in a controlled environment. To facilitate assessments, a small muscle in the hand was tested. The long exercise test (McManis protocol) was used to induce HypoPP symptoms and objectively assess the severity of the attack. The McManis protocol is internationally recognised as a diagnostic tool in HypoPP. It is easily reproducible and the Neurophysiology Department at the NHNN has many years' experience of using this technique on a regular basis.

#### Impact of External Factors

Increased micturition, a common and expected AE of diuretics, would eventually stimulate hand movement while participants were accessing toilet facilities. As gentle movement may influence symptom recovery, a hand/forearm splint was used on the assessed limb to prevent mobilisation. This

ensured reliability of the study results by neutralising this external factor, which has a direct influence on symptom worsening or improvement.

Dietary intake and physical activity pattern may either trigger or prevent attacks of weakness in HypoPP, thus influencing the severity of symptoms and potentially affecting the validity of the study. To address this challenge, a 24-hour diary was developed in an attempt to reduce the influence of such environmental factors by 'standardising' pre-visit exposure based on participants' personal dietary regime and physical activity patterns.

### **Developing New Treatment Options for Rare Diseases**

#### *Bumetanide Efficacy*

This research showed that 2 mg of bumetanide was not effective in aborting a focal attack in an immobilised hand in the majority of the trial participants. There were no statistically significant differences in CMAP amplitudes between the bumetanide and placebo groups at one hour, the primary outcome, and at early and late effect assessed by the area under the curve for nought to two hours and two to four hours following treatment intake. When planning the study protocol, it was originally assumed that all participants would eventually recover from a minor attack of weakness in a small hand muscle within four hours of study assessment. The effect of treatment on focal attack duration, one of the secondary outcome measures, was measured as the time between treatment administrations until CMAP returned to 65% of peak CMAP. It is interesting to note, however, that none of the participants recovered from an attack following placebo intake. Therefore it was not possible to carry out statistical analysis on this outcome.

#### Prolonged Immobilisation

One possible explanation for the negative trial results was the effect of prolonged immobilisation due to the splint use. Prolonged immobilisation may have prevented symptom recovery in all participants following placebo intake, illustrating the importance of gentle physical activity for symptom improvement. It is very likely that the same negative effect associated with prolonged resting prevented a more dramatic beneficial effect of bumetanide treatment.

The McManis test, when used as a diagnostic tool, evaluates CMAPs for 50 minutes following isometric hand exercise. In this trial, neurophysiologic parameters were collected for a) 50 minutes

following exercise or until 40% decrement was confirmed, and b) for four hours following treatment intake. Extra time was occasionally taken (n = 5) when participants performed the extended protocol with 10-minutes' exercise. This was the first time genetically confirmed HypoPP patients have undergone such a prolonged McManis protocol. The placebo data collected in this trial confirmed that variations in CMAP amplitudes may occur in people with HypoPP. However, no improvement of up to 65% of peak CMAP was seen within the four hours following attack onset. The placebo data provided important evidence regarding the importance of physical activity and gentle movement in symptom recovery, which could give further insights into HypoPP pathophysiology and management of symptoms. All trial participants made a full recovery from their symptoms of hand weakness by the end of the study visits or within a few minutes or hours following visit discharge, further illustrating the role of gentle movement in symptom improvement. Gentle exercise may potentially be used as a treatment option for HypoPP, and future clinical trials could address this.

In conclusion, the impact of prolonged immobilisation was underestimated and it probably prevented recovery from symptoms following both placebo and bumetanide intake. This might have biased the trial results. It is impossible to know, however, whether higher doses of bumetanide would have helped recovery even with prolonged immobilisation.

#### Small Sample Size

Another possible explanation for the negative findings was the small study sample. Even though the recruitment target was very small (n = 12), difficulty with recruitment was an issue. Ten out of 12 participants were recruited, but only nine completed both trial visits and were included in the primary outcome analysis. Factors that contributed to the failure in reaching the target sample were mainly related to the inclusion and exclusion criteria. Some patients decided against participating in this trial for the following reasons: they were too symptomatic to discontinue acetazolamide treatment for 72 hours prior to each study visit, they had not experienced attacks of weakness within the last year, they had concerns about the discomfort related to neurophysiology assessments (electric pulses), they were unable to take time off work to attend trial visits in London. Such difficulties should be discussed and considered when planning future clinical trials in patients with rare conditions such as HypoPP.

### Bumetanide Dosage

A dramatic benefit of bumetanide was reported in mouse model studies (Wu et al., 2013b, Wu et al., 2013a). *In vitro* studies assessed the benefit of bumetanide in both attack prevention and recovery. The *in vitro* experiment designed to assess an abortive treatment strategy tested recovery from an acute attack following exposure to a low potassium concentration bath, which induced loss of contractile force – hypokalaemic challenge (Wu et al., 2013a, Wu et al., 2013b). In these studies, there was a 30-minute continuous exposure to a bumetanide bath, and attack recovery was assessed by direct measurement of isometric contractile force of the soleus muscle in response to tetanic stimulation (Wu et al., 2013a). There has been just one *in vivo* study of bumetanide in the R528H<sup>m/m</sup> HypoPP mouse model (*CACNA1S*). This study tested the role of bumetanide in preventing loss of muscle excitability following a continuous intravenous infusion of glucose and insulin (Wu et al., 2013a). In this experiment, an intravenous bolus of 0.08 mg/kg bumetanide was administered as a single dose. Muscle excitability was measured every minute for two hours by neurophysiology (CMAP amplitude). To date, there has been no *in vivo* study testing an abortive treatment strategy of bumetanide in HypoPP.

The dose used in this phase II clinical trial was 2 mg. Unfortunately, higher doses may increase the number and the severity of drug-related AEs. The potential harm related to an increased bumetanide dose and related increased potassium depletion outweighed its theoretical benefits, and for this reason, and as a first HypoPP human study of bumetanide, a 2 mg dose was chosen in a proof-of-concept trial. It is possible that the 2 mg dose was too low for attack recovery in an immobilised limb.

Therefore one limitation of this study was not assessing different dosing regimens. It is impossible to predict whether higher doses would be associated with better outcomes.

### Indications of Bumetanide Benefits

The combination of prolonged immobilisation, small study sample and low bumetanide dose may possibly have contributed to the negative results reported here. Even though a negative trial, this study also provided indications that 2 mg bumetanide might benefit this patient population, supporting further clinical trials in this field:

- Two participants recovered from the attack of weakness within four hours following bumetanide intake even with prolonged immobilisation, while none recovered following placebo intake
- Half of the participants who guessed their treatment assignment correctly based their choice on symptom improvement
- One participant who guessed treatment assignment incorrectly reported worsening of symptoms following placebo intake as opposed to improvement of symptoms following bumetanide intake
- The mean CMAP amplitudes following placebo intake were overall lower than the ones following bumetanide treatment, suggesting 2 mg bumetanide may partially improve acute symptoms

The data provided by this study support future studies of bumetanide as a treatment option for people with HypoPP.

#### *Bumetanide Safety*

There were no SAEs in this trial. Two milligrams bumetanide administration in people with HypoPP with normal baseline potassium levels in association with certain safety procedures prevented symptomatic hypokalaemia or generalised attacks of weakness. The safety measures used in this project succeeded in preventing hospital admissions to manage SAEs.

Asymptomatic hypokalaemia was seen following both bumetanide and placebo intake. Only one participant received rescue treatment twice during the same visit, which was following placebo intake. There was a non-significant trend of potassium reduction following bumetanide intake; however, the study was not powered for such an outcome, and further studies to assess the finding are required.

The most common AE related to bumetanide use was increased micturition. As a diuretic drug, this was expected. Only one participant guessed treatment assignment based on drug-related AE, and in general the drug was very well tolerated. There was no symptomatic hypotension.

A common adverse event associated with trial assessments was exercised hand/upper limb weakness following splint removal. Such symptoms were mild and improved the same day.

In this study, regular CMAP amplitude evaluations in the opposite limb detected generalised attacks of weakness more reliably than the MRC muscle scale. Only one participant reported generalised weakness during the study visits. Even though her acute symptoms received low grades when

measured using the MRC muscle scale, normal CMAP amplitude assessments confirmed there was no real HypoPP attack of weakness; therefore, her weakness was most probably functional. This study showed that regular CMAP assessments were a good and reliable strategy to detect the beginning of a generalised attack of weakness in HypoPP. Future studies may further develop the use of the ADM CMAP amplitude as a safety outcome measure in HypoPP clinical trials.

In summary, 2 mg of bumetanide was shown to be safe in the study population. A mild reduction in serum potassium levels was seen in a few patients following bumetanide intake, but no associated symptoms were seen. To ensure safety, bumetanide should most probably be used in combination with oral potassium when treating acute symptoms. Further studies are required to understand the impact of such drug-related AEs in people with HypoPP during a generalised attack of weakness.

### Pregnancy

An unexpected pregnancy occurred in this trial despite the formal requirement for use of contraception. This illustrates that methods of birth control may fail, highlighting the need to address this in future studies. Requesting the use of at least two acceptable methods of birth control for the duration of a clinical trial may be a better option, but it may also increase the out-of-pocket costs for study participants.

Even though urine pregnancy tests performed at the screening visit and at the study V1 were both negative, it is possible that the participant was at the early stages of her pregnancy when she received bumetanide. The pregnancy outcome was a healthy newborn.

### **Assessing New Outcome Measures: The Long Exercise Test**

The long exercise test was well tolerated. The most common AE associated with the technique used was hand weakness due to isometric exercise and prolonged immobilisation. Mild focal weakness was seen following both bumetanide and placebo treatment. It is not known, however, whether hand weakness would also be seen following the administration of more potent drugs or higher doses of bumetanide. For this reason, it is not possible to completely exclude the use of prolonged immobilisation during the long exercise test when it is used as an outcome measure in clinical trials assessing abortive treatment strategies. The McManis test may also be useful in clinical trials assessing a preventative treatment strategy, and thus future research should evaluate this.

An advantage of this technique is its use to assess safety. CMAP amplitudes collected on the dominant hand confirmed bumetanide intake did not trigger generalised symptoms. It was also used to confirm the non-HypoPP aetiology of the atypical symptoms seen in one trial participant as described in 'Bumetanide Safety'.

Even though the technique was easily reproduced, the use of the McManis protocol required specific training. The main assessor (me) joined the Neurophysiology Department for several months to learn how to perform the technique and to practice in a systematic way. Several aspects may influence the quality of the collected data, such as room temperature, skin electrode placement, intensity of the supramaximal stimulus applied and the position where the stimulation is applied at the wrist. With so many variables affecting the data quality, inter-rater reliability should be explored. It may be advisable to ensure that the same trained researcher collects all trial data. This may limit the use of this technique in international multi-centre clinical trials.

#### *Exercise Length*

The McManis protocol included five minutes of hand exercise; it was performed 19 times in 10 trial participants during this study. In five (26.3%) of those tests the 40% decrement in CMAP amplitude did not occur within 50 minutes. It is unclear whether the use of other regular HypoPP treatments (for example, spironolactone) could have contributed to the lack of significant CMAP decrement. The degree of decrement was proportional to the extent of exercise and most patients required a high level of encouragement to sustain maximum exertion for the entire period of the exercise. Lower intensity of effort could possibly contribute to false negative results.

The repeated test with a prolonged length of isometric exercise, 10 minutes, induced 40% decrement in the CMAP amplitude in all (100%) participants who were initially resistant to the five-minute exercise protocol. It is possible that the extended McManis protocol designed in this study might increase the sensitivity of this diagnostic tool in HypoPP patients with *CACNA1S* variants; however, more studies are required to confirm this finding.

Because the study sample was so small – only five tests were extended, it is difficult to confirm whether the length of exercise (five minutes versus five + 10 minutes) could have interfered with the level of CMAP decrement following IMP intake and whether it affected related outcomes. A

suggestion to address this limitation would be to perform 10 minutes of hand exercise in all participants when the McManis test is used as an outcome measure in clinical trials.

Table 14 summarises the pros and cons of using the long exercise test as an outcome measure in clinical trials.

Table 14. Pros and cons of the long exercise test

Pros	Cons
<ul style="list-style-type: none"> <li>• Reproducible</li> <li>• Outcome measure to assess:               <ul style="list-style-type: none"> <li>○ Safety</li> <li>○ Drug efficacy:</li> <li>○ Abortive treatment strategy* Preventative treatment strategy*</li> </ul> </li> <li>• Extended McManis: Sensitivity: 100%*</li> </ul>	<ul style="list-style-type: none"> <li>• Requires training</li> <li>• Participants may require vocal stimulus / high level of encouragement during the exercise period</li> <li>• May be uncomfortable</li> <li>• Not a patient-reported outcome measure</li> <li>• Risk of false negative results</li> <li>• Several aspects may influence data quality, such as room temperature</li> </ul>

\* Further studies are needed to confirm the findings.

## Conclusions

This project illustrated several challenges related to performing an investigator initiated study of people with HypoPP within an academic environment. The lack of standardised outcome measures to assess abortive treatment strategy in HypoPP, uncertainty about prolonged McManis protocol execution and the inexistent safety data relating to use of bumetanide in this patient population were all challenges that had to be addressed at the very early stages of the protocol development. Because this was an investigator-initiated study, such challenges were addressed by a local study team as part of a PhD project.

Even though several aspects of safety and efficacy were planned ahead and succeeded in preventing SAEs, the immobilisation bias was not previously expected. This research project adds to the field of

channelopathies as it objectively illustrated the negative aspects of prolonged immobilisation/rest during four hours of McManis test.

This study provided Class 1 evidence that 2 mg bumetanide was not effective in aborting a focal attack of weakness in an immobilised hand in the majority of the trial participants. Although a negative trial, this study provides safety data for 2 mg bumetanide in normokalaemic HypoPP patients for the first time. Bumetanide was found not to be as highly effective as it was in the mouse model, but data presented here shows that a beneficial effect cannot be entirely excluded, supporting the need for further studies of this agent in this population of patients. One limitation of this study was that it did not assess different dosages, and it is impossible to predict whether higher doses would have better outcomes. In conclusion, the data provided by this study support further research of bumetanide as a treatment option for HypoPP.

The McManis protocol used as an objective outcome measure in a clinical trial for the first time was well tolerated. It succeeded in documenting a prolonged attack of weakness, and should be considered as an outcome measure in future research where such an assessment may be relevant to explore treatment efficacy or safety.

This is the first time a clinical project has documented strategies to increase the sensitivity of the McManis test. Performing 10 minutes of isometric exercise in all study participants should be considered in future clinical trials using this functional neurophysiology tool as an outcome measure. Prolonged immobilisation during the McManis protocol very likely prevented attack recovery and perhaps should be avoided in future trials designed to explore abortive treatment strategies. However, the McManis test using immobilisation could still be a useful outcome measure in studies assessing preventative treatment strategies.

## **Next Steps**

### *Future Clinical Trials*

Unfortunately, clinical trials assessing abortive treatment strategies in HypoPP still face a major limitation: the lack of standardised outcome measures. It is clear that further research should be performed to evaluate new assessment tools. This research attempted to address this by exploring the use of the McManis protocol as an outcome measure to assess safety and efficacy.

This study confirmed that HypoPP patients with confirmed *CACNA1S* variants may have a negative McManis test. Such findings emphasised that genetic investigation for people presenting with typical clinical manifestations of HypoPP should not be precluded following a normal McManis test. It is possible that people with underlying HypoPP may be underdiagnosed or even misdiagnosed, especially when taking into account the rarity of the condition and the fact that not all doctors are familiar with the disease or aware of false negative results related to neurophysiology assessments. More studies are required to confirm whether 10 minutes' exercise increases the McManis test sensitivity in a larger cohort of patients. If so, a McManis protocol with extended isometric exertion could improve the use of this neurophysiology tool as both a diagnostic test and as an outcome measure in clinical trials.

However, performing a 10-minute exertion protocol on a regular basis is time consuming and exhaustive for patients. When used as a diagnostic tool, the prolonged exertion period could be used to assess selected patients with strong clinical suspicion of an underlying channelopathy who had a normal McManis test following the five-minute exertion protocol.

For clinical trial purposes, it is interesting to consider performing only one cycle of 10-minute exertion to reduce the bias of having two groups of participants: one group exposed to a five-minute exertion period, and the other exposed to a five-minute plus a 10-minute exertion period. More importantly, false negative results could lead to overestimates of drug efficacy when assessing preventative treatment strategies using the McManis test; a ten-minute exertion period may mitigate this test limitation.

This study also provides the first evidence that bumetanide may be effective in humans with HypoPP. The small effect of 2 mg bumetanide in big muscle groups during a generalised attack of weakness could be enough to kick-start symptom improvement and speed up recovery. The combination of bumetanide, gentle movement and oral potassium supplementation may be of great benefit to patients affected by this condition. The trial results were presented and discussed at The 2017 Muscle Study Group Scientific Annual Meeting in Utah, USA (Scalco et al., 2017b). It was suggested that future research should include the development of an n-of-1 trial to assess the efficacy of bumetanide in HypoPP patients presenting with generalised attacks in a controlled environment such as an elective hospital admission. Results from such a pilot trial could guide the development of a multi-

centre randomised controlled trial in a larger cohort in an outpatient setting to assess the potential benefits of bumetanide in aborting acute HypoPP symptoms. Such a pilot study could also assess different dosing regimens and could further confirm safety data.

## **15. A phase II study of sodium valproate in McArdle Disease**

### **Methods**

#### **Aims and Hypothesis**

The aim of this project was to assess a new treatment option for people with McArdle disease and to investigate the feasibility of using a series of outcome measures in clinical trials in this patient population. The initial hypothesis was that VPA would induce the stimulation of an alternative isoform of the phosphorylase enzyme in skeletal muscle based on previous animal model studies (Howell et al., 2015, de Luna et al., 2015). The brain and/or liver glycogen phosphorylase would break down the stored glycogen and restore the glycolytic pathway, resulting in symptom improvement in this patient population. Treatment benefit was assessed using several outcome measures to evaluate the functional benefits while evidencing the presence of an alternative isozyme.

All participants were expected to show presence of the brain and/or liver glycogen phosphorylase enzyme in skeletal muscle following treatment use. The brain and/or glycogen phosphorylase enzyme is expected to be functional, and for this reason, all participants were expected to have an improvement in their aerobic capacity. Raised levels of plasma lactate would confirm the restoration of the glycolytic pathway in trial participants.

As a primary outcome measure, the changes in  $VO_{2peak}$  were assessed by an incremental cycle test at the baseline and after three and six months of VPA treatment. The main aim of the primary outcome was to assess whether treatment intake improved the  $VO_{2peak}$  of people with McArdle disease. Exercise performance was also assessed as a secondary outcome measure by comparing the total walked distance during the 12MWT at baseline, three and six months following treatment intake. To prove that improvement in exercise performance was due to VPA intake, the number of positive phosphorylase fibres was compared in pre- and post-treatment muscle biopsies as a secondary outcome measure. To confirm that the alternative isoform of the phosphorylase enzyme was able to break down stored glycogen, plasma lactate was repetitively assessed during and after the cycle test and after the non-ischaeamic forearm exercise test as a secondary outcome measure, at baseline, three and six months following treatment intake. Treatment impact in participants' quality of life was assessed by the SF-36 questionnaire at baseline, three and six months following treatment

use as a secondary outcome measure. As this is the first time VPA has been used in a clinical trial in people with McArdle disease, safety was analysed as a secondary outcome measure, which was assessed by blood tests and review of AEs.

### **Study Protocol and Ethics Statement**

This protocol was registered in ClinicalTrials.gov (NCT03112889).

This study and related documents were reviewed and approved by the UCLH Trust (R&D), by the research ethics committee (NRES Committee London - Westminster) and by the MHRA. Ethics approval documentation (REC reference 14/LO/0208) is provided in Appendix 5. Written informed consent was obtained from all participants prior to study assessments. A copy of the final study PIS and study CF are provided in Appendix 6 and Appendix 7, respectively. The study was performed under the ethical guidelines issued by UCL for clinical trials. All investigators involved in this study regularly attended Good Clinical Practice courses in the UK.

### **Study Design**

This was a proof-of-concept pilot study to assess the feasibility of running a clinical trial of VPA in McArdle disease. The main focus of this research was to explore evidence of treatment benefits in several clinically relevant outcome measures. The knowledge gained can be used in future randomised controlled studies in larger international cohorts of people with McArdle disease.

Eight participants were expected to be recruited in the UK. Professor John Vissing was independently running the same protocol at Rigshospitalet University in Denmark aiming to recruit seven participants. In this PhD thesis, only the UK data are presented.

Because McArdle disease is rare, difficulties in recruiting were expected, especially when taking into account the need to exclude all women of childbearing potential due to the high risk of teratogenicity related to VPA use. Consequently, an open-label study with a single arm was chosen, ensuring all recruited participants would receive VPA. The use of open-muscle biopsy discouraged the inclusion of a placebo arm to avoid performing invasive procedures in people who did not receive VPA treatment.

This was a phase II open-label pilot study assessing patients who fulfilled the inclusion and exclusion criteria as illustrated in Figure 17.

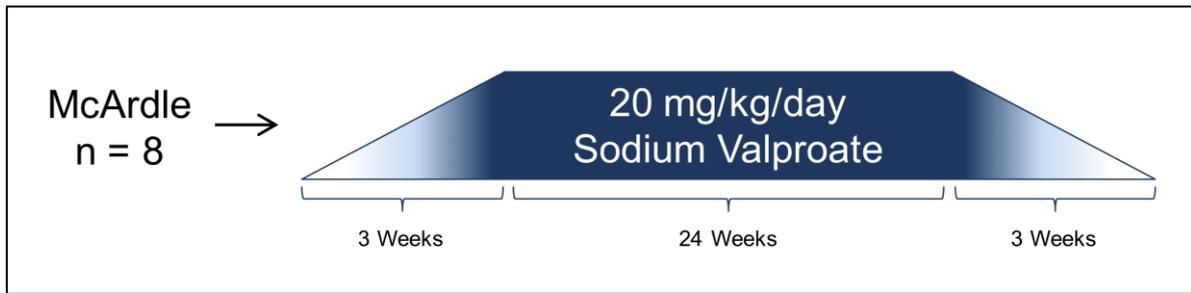


Figure 17. Schematic diagram of the overall trial design

*Sodium valproate was increased slowly up to a full treatment dose of 20 mg/kg/day. The dose was slowly decreased after six months of full dose treatment.*

### Participant Selection

Inclusion and exclusion criteria were selected based on known drug-related AEs, drug interaction with VPA, contraindications for VPA prescription, age-related variability in VPA pharmacokinetics and difficulties in performing any of the functional tests related to the study outcome measures (Table 15). As people with McArdle disease may have raised serum transaminases that originate from skeletal muscle, a previous history of liver disease was defined by an abnormal liver biopsy performed prior to study recruitment. Comorbidities precluding exercise assessments included severe unstable/untreated cardiac disease, MRC muscle score equal to or lower than three in any lower limb or pelvic girdle muscle, and lower limb fracture. In addition, sensitivity to local anaesthetics precluded muscle biopsy execution. VPA use may cause severe adverse events in the very rare instances where there is an underlying fatty acid disorder or mitochondrial disease; these scenarios were investigated at the screening visit and were part of the inclusion and exclusion criteria.

Table 15. Inclusion and exclusion criteria

**Inclusion Criteria:**

- Male subjects
- Post-menopausal or infertile females
- Diagnosed with McArdle Disease
- At least 18 years of age
- Normal serum carnitine level and acylcarnitine blood profile at screening visit

**Exclusion Criteria:**

- Children under the age of 18
- People older than 64 years of age
- Females with child-bearing potential
- A previous history of diabetes
- Inflammatory disorders (for example, systemic lupus erythematosus)
- A previous history of sensitivity/allergy to sodium valproate and/or its excipients
- Treatment with sodium valproate for epilepsy or a psychiatric disorder within the 12 months prior to screening
- A pre-existing liver disease and/or a family history of severe liver disease affecting a first degree relative
- Treatment with other anticonvulsant medication or any other medication known to interact with sodium valproate
- Sensitivity to local anaesthetics
- Any comorbid illness or disability that would prevent exercise assessment
- A previous history of porphyria or an affected first degree relative affected with porphyria
- A previous history of mitochondrial disease and/or a first degree relative with mitochondrial disease
- A previous history of abnormal acylcarnitine profile or low serum carnitine level
- Male participants unwilling to use contraception

I identified potential participants attending the specialised service for McArdle disease and related disorders at the NHNN, London, UK and contacted them by invitation letter, phone and/or in person during their routine clinic appointments at the NHNN. The study has been presented several times at the annual meetings of the Association for Glycogen Storage Disease UK. In addition, potential participants attending the McArdle Disease Clinic (NHNN) and those interested in participating were

advised to contact me for further details. The trial was also advertised on the Muscular Dystrophy UK website and by the Association for Glycogen Storage Disease UK.

All interested participants received the study PIS and were given at least 24 hours to read and discuss the study with their family members. If still interested, participants were then invited to attend a screening visit to determine study eligibility. After consenting, medical history, physical examination, vital signs, laboratory evaluation and ECG were assessed to determine individual eligibility for the study.

After recruitment, each participant undertook three study visits that followed an identical protocol. Visit 1 (V1) occurred within 28 days of the screening visit (Table 16). Subjects were reassessed at week 16 +/- seven days (V2) and week 28 +/- seven days (V3) with monthly telephone consults in between.

Table 16. Flowchart of study assessments

Visit #	Screening	Treatment Phase			Follow-up
	0	1	2	3	4
<b>Weeks</b>	-28 to -1 (days)	Week 1	Week 16 (+/- 7 days)	Week 28 (+/- 7 days)	3 month post-treatment phone call (+/- 14 days)
<b>Informed Consent</b>	X	-	-	-	-
<b>Full Medical History</b>	X	X	X	X	-
<b>ECG</b>	X	-	-	-	-
<b>Free Serum Carnitine</b>	X	-	-	-	-
<b>Acylcarnitine Profile</b>	X	-	-	-	-
<b>LFTs</b>	X	X	X	X	-
<b>U&amp;Es</b>	X	X	X	X	-
<b>Muscle Biopsy</b>	-	X*	-	X	-
<b>FBC, Coagulation</b>	X	X	X	X	-
<b>CK</b>	X	X	X	X	-
<b>Forearm Test</b>	-	X	X	X	-
<b>Adverse Events</b>	-	X	X	X	X
<b>Vital Signs</b>	X	X	X	X	-
<b>Symptom Diary</b>	-	X	X	X	-
<b>SF-36</b>	-	X	X	X	-
<b>Valproate Dispensing</b>	-	X	X	X	-
<b>Valproate Levels</b>	-	-	X	X	-
<b>Cycle Test</b>	X (no blood)	X	X	X	-
<b>12MWT</b>	-	X	X	X	-
<b>Telephone Consultation</b>	Monthly telephone consultations were performed following visit 1 and visit 2 (+/- 5 days) A final follow up telephone consultation was performed 3 months following visit 3. Total number of telephone consultations: 5				

One month indicated a 28-day schedule. \*Initial muscle biopsy unless the subject had had a previous muscle biopsy that was available for analysis. ECG: electrocardiogram; LFT: liver function tests; U&E: urea and electrolytes; FBC: full blood count; CK: creatine kinase; SF-36: The Short Form 36 health survey questionnaire; Valproate Levels: serum valproate levels; 12MWT: 12-minute walk test.

## Study Visit Assessments

To avoid inter-rater variability, all trial-related procedures were performed by the same investigator (me), who was supported by the research team described in 'Acknowledgements Related to the PhD Projects'. Standardised baseline assessments were performed during all study visits in order to ensure that participants met inclusion and exclusion criteria. Participants were excluded if they developed any comorbid illness included within the exclusion criteria. They were also excluded if compliance for drug ingestion or attendance was less than 90%, if they could not tolerate the treatment, if they had a significant AE or failed to attend the study assessments. A list of all baseline assessments is presented in Table 17.

Table 17. Baseline assessments performed on visit 1, visit 2 and visit 3

<b>Baseline Assessments</b>
<ul style="list-style-type: none"><li>• Medical history</li><li>• History of myoglobinuria</li><li>• Concomitant medication history</li><li>• Physical examination (general, skin, cardiac, pulmonary, chest, abdomen, extremities, neurological, other as required)</li><li>• Vital signs (blood pressure and pulse)</li><li>• Weight</li><li>• Height</li><li>• Methods of birth control (male participants)</li><li>• Cannulation</li><li>• Laboratory: FBC, CK, LFT, U&amp;E, coagulation, ammonia, plasma lactate, glucose and VPA drug levels</li></ul>

*Baseline bloods included full blood count (FBC), creatine kinase (CK), liver function test (LFT), urea and electrolytes (U&E), coagulation profile, baseline ammonia, baseline plasma lactate, baseline glucose and sodium valproate (VPA) drug levels (V2 and V3).*

Participants followed a standardised sequence of exercise assessments during all trial visits: the non-*ischaemic forearm exercise test*, followed by the *cycle test*, rest on a chair and finally the *12MWT*. Each visit took approximately four hours.

### *Cycle Ergometer Test*

During this trial, all participants performed cycle tests on a calibrated ergometer. Two different protocols were used, one for the screening visit and one for the trial visits following recruitment (V1,

V2 and V3). Oxygen consumption and heart rate were continuously monitored by cardiac monitor (SureSigns VM 4 Patient Monitor, Philips) and a facemask attached to the Cortex ergospirometry system (Cortex Metalyzer II, Cortex Biophysik GmbH, Leipzig, Germany). The RPE was recorded every minute during the test.

#### Screening Visit – Incremental Cycle Ergometer Test

All participants who met the inclusion and exclusion criteria performed a baseline cycle ergometer test at the screening visit. The incremental test was performed to determine their  $VO_{2peak}$  and to identify the cycle ergometer workload associated with 65% of maximal oxygen uptake. All participants underwent the same protocol at the screening visit, cycling a starting power of zero and increasing to 20 W in the first minute, increasing by 5 W every other minute.

#### Study Visit – Trial Cycle Ergometer Test

This test was performed during V1, V2 and V3 and comprised a constant workload period and an incremental workload period.

During the first 15 minutes of testing, participants cycled at the constant workload represented by 65% of the workload at each participant's  $VO_{2peak}$  as assessed during their screening visit. The constant workload period served as a warm-up to enable participants to achieve the *second wind*.

The incremental workload period occurred immediately after the first 15 minutes of cycling. From minute 16 onwards, there was a 5 W increase in load per minute until exhaustion, when the  $VO_{2peak}$  was determined.

#### Cycle Test Protocol

Each participant underwent a personalised protocol based on the workload at 65% of the  $VO_{2peak}$  assessed at the screening visit. The same personalised protocol was repeated during V1, V2 and V3.

#### Blood Sampling

During the trial cycle ergometer test, plasma lactate, serum glucose and serum ammonia levels were collected at zero, five, 10 and 15 minutes and at exhaustion.

Figure 18 shows a schematic representation of the screening visit and trial visit cycle tests. No blood samples were collected during the screening visit cycle test.

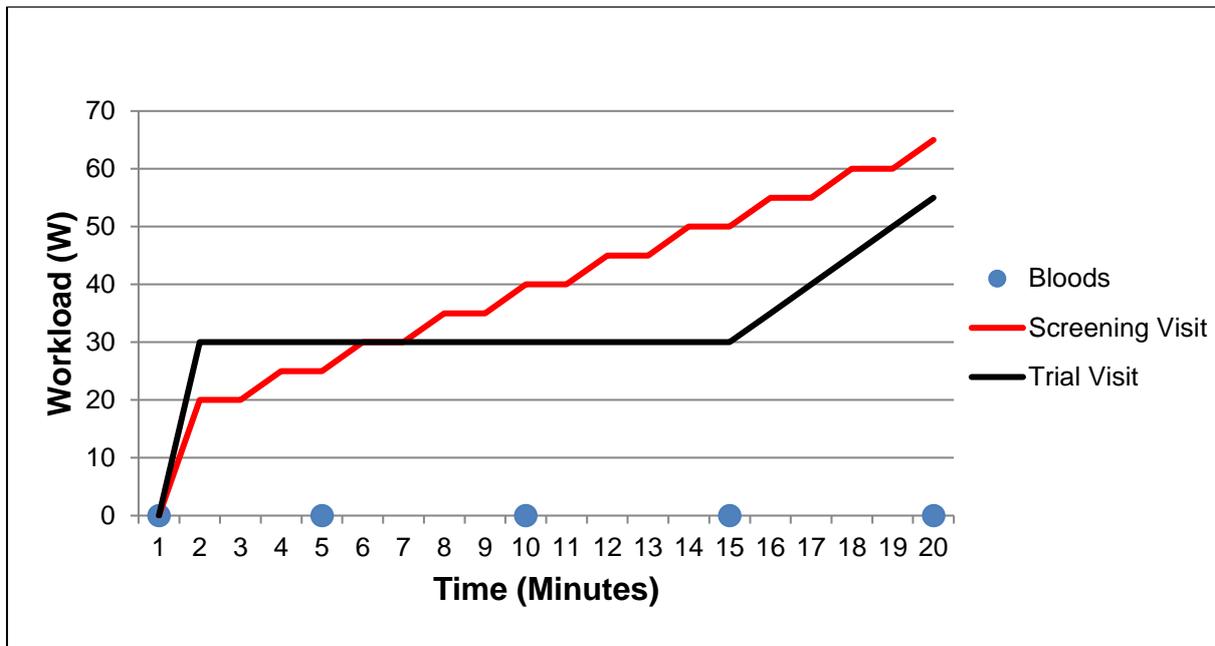


Figure 18. Schematic representation of the cycle test

*Trial Visit: from minute one to minute 15: participants cycled at the workload represented by 65% of their  $VO_{2peak}$  in this instance, 30 W. Screening Visit protocol was used during the screening assessments; Trial Visit protocol was used at V1, V2 V3. W: watt.*

### Fasting

External factors such as diet influence participant performance during exercise assessments. For this reason, all participants were required to fast prior to the study V1, V2 and V3; ensuring pre-exercise glucose intake would not increase blood-borne glucose availability at the time of the exercise assessments. This was performed to document a true VPA effect. However, at the screening visit participants were allowed to eat prior to cycling. The goal of the incremental test performed at the screening visit was to determine the participants' true maximum  $VO_{2peak}$  value.

### *Forearm Exercise Test*

The non-ischaemic forearm exercise test consisted of repetitive maximal handgrip contractions every other second for one minute (30 contractions in total). Handgrip strength was measured at baseline and every 10 seconds using a CITEC calibrated hand-held dynamometer. The handgrip exercise phase was followed by a five-minute resting phase, when participants were advised not to move the exercised hand and the respective upper limb. The test was repeated during V1, V2 and V3. The same side/hand was exercised in all trial visits.

### Blood Sampling

A cannula was inserted into the forearm as part of the baseline procedures (Table 17). The cannulation was performed in a vein on the same side as the exercised hand. During the resting phase, blood samples were systematically taken at zero, two and five minutes following exercise to measure serum ammonia levels and plasma lactate. Bloods were processed at the NHNN laboratories.

### *12-minute Walk Test*

The 12MWT was performed at least 45 minutes after the end of the cycle test to ensure participants were out of the *second wind* induced by cycling. Participants were requested to complete as many 10 metres shuttle walks as possible for 12 minutes on a 10 metres marked corridor. HR, total walked distance and RPP were collected every minute for the duration of the test, the latter was assessed using the Borg RPP scale (Borg, 1970). All participants completed the 12MWT during V1, V2 and V3.

### *SF-36*

All participants completed SF-36 questionnaires during V1, V2 and V3. Collected data were scored using the QualityMetric Health Outcomes™ Scoring Software. Scores were normed according to age and gender as per SF-36 scoring software using the 2009 population norms.

### *Muscle Biopsy*

In this trial, all participants had two skeletal muscle biopsies. A pre-treatment muscle biopsy was performed following trial recruitment and prior to V1. A post-treatment muscle biopsy was performed after participants had completed the treatment period, comprising six months on a full VPA dose (20 mg/kg/day). The pre-treatment biopsy was not required if the study participant had had a previous muscle biopsy that results of which were available for trial analysis. Both pre- and post-treatment biopsies were performed in the same muscle group, mostly quadriceps. Upper-limb-muscle biopsies were performed if required.

### Muscle Biopsy Staining

All fresh-frozen sections were handled and stored as standardised at the Division of Neuropathology, which provides the diagnostic pathology service for the NHNN. All samples, pre- and post-treatment, were stained on the same day at the end of the trial. A healthy muscle control was stained alongside the trial samples to assess technical quality. Phosphorylase staining was used to confirm muscle fibre

positivity. To confirm whether the enzyme expression resulted from VPA use and not from muscle regeneration following local damage, neonatal myosin immunohistochemistry was used to evaluate the presence of regenerating fibres. Phosphorylase staining and neonatal staining are part of the Neuropathology Department diagnostic routine as previously described (Dubowitz et al., 2013).

Muscle biopsy analysis was performed at the end of the study following the last participant's post-treatment biopsy. The muscle biopsy fibre count was performed for both neonatal myosin immunohistochemistry and phosphorylase stained slides using Image J software in a blinded fashion. The following were assessed and compared:

- The total number and percentage of phosphorylase positive fibres
- The total number and percentage of neonatal positive fibres

### **Study Diary**

During V1, all participants received a study diary and were requested to complete the diary on a weekly basis to include general information of wellbeing. Participants were also advised to record on a daily basis any episodes of AEs, myoglobinuria, severe cramps, allergic reactions, bleeding/purpuric skin eruptions/bruising, severe symptoms, significant worsening of McArdle disease, VPA overdose, missed dose of VPA, concomitant medication intake and physical injuries. Home diary completion was reviewed by monthly phone calls and at V2 and V3. At the end of the study, participants posted the diary to the study centre. Study diaries were stored with the Trial Master File.

### **Telephone Consultations**

Participants were phoned four and eight weeks (+/- five days) after study V1 and V2, and three months (+/- 14 days) after V3. The final telephone consultation was defined as visit 4 (V4): the last trial assessment for trial participants. Extra phone calls were made if needed to ensure safety. In each telephone consultation, participants were asked about AEs, study drug compliance, overdose, method of birth control (male), concomitant medication intake, general wellbeing, episodes of dark urine, hospital admission and specific questions relating to potential drug toxicity. If abnormal symptoms were reported, the research team discussed and decided, based on clinical judgement, whether the participant was required to attend an unscheduled visit to further assess patient safety, or to attend a local GP or accident and emergency (A&E) unit for medical evaluation and laboratory tests.

## **Treatment Administration**

All participants received extended-release tablets of VPA as Epilim Chrono by Sanofi-Aventis in its normal formulation and packaging. VPA was administered by mouth once daily. Tablets are manufactured in 200 mg, 300 mg and 500 mg strengths and participants were advised that they should be swallowed whole in the evening.

### *VPA Dosage*

To try and ensure tolerability during the first month of treatment, the dose was escalated at weekly intervals. Weaning occurred over a three-week period.

The medication was introduced slowly, following an escalating protocol as follows: 1) week 1: 5 mg/kg/day; 2) week 2: 10 mg/kg/day; 3) week 3: 15 mg/kg/day; 4) full-dose treatment (six months): 20 mg/kg/day. After all the study assessments were complete, including the post-treatment muscle biopsy performed after six months on the full dose therapy, weaning comprised a 5 mg/kg/day reduction each week for three weeks, when treatment was stopped. The daily dose was rounded up to the nearest tablet strength according to the participant's weight. Monitoring during the dose escalation period was not required as this was usual practice. The maximum dose was 2 g/day to avoid toxicity.

VPA tablets were dispensed during the following visits:

- V1: titration phase + full-dose treatment
- V2: full-dose treatment
- V3: weaning phase

### *Study Compliance*

Compliance included adherence to VPA treatment and protocol study procedures.

Drug accountability and study compliance were assessed during each monthly follow-up phone call, by counting the remaining tablets returned on V2 and V3, by home diary review at V2 and V3, by medical history review and by assessing serum levels of VPA at V2 and V3.

The level of VPA compliance required for participants to continue on the trial was >90%.

## **Data Collection**

Clinical and paraclinical data from participants were abstracted into a standardised CRF (reproduced in Appendix 8). The collected data plus any other relevant information were recorded in participants' source folders, participants' medical notes and participants' CRFs. All data was recorded in a specifically designed database.

## **Safety Procedures**

Participants were seen as day cases at the MRC Centre for Neuromuscular Diseases at the NHNN. They were under close medical supervision during all trial procedures. Safety of VPA was assessed by: medical history, vital signs, physical examination, reported AEs, home diary evaluation, blood sampling, serum VPA levels and assessment of birth control methods (male participants). Participants received a study card containing emergency contact details and were advised to contact a member of the trial staff immediately in case of allergic reactions, coagulation problems or signs of liver disorder. Signs and symptoms of such disorders were explained on V1, were reviewed on V2 and V3 and were specifically discussed during monthly telephone consultations. Participants were also advised to immediately stop VPA and to visit an A&E unit in case of SAEs or allergic reaction.

An emergency/resuscitation trolley was available in the clinical trial assessment room for immediate advanced cardiac life support. All exercise tests were performed with at least two co-investigators present in the assessment room to ensure safety.

### *Pregnancy*

VPA is known to have a teratogenic effect in women (Guveli et al., 2017). Thus female participants with childbearing potential were not recruited. Only women who were infertile or post-menopausal were able to take part in the study. Male participants used accepted methods of birth control for the duration of the trial. Male participants were asked about contraceptive methods compliance during all study visits and telephone consultations.

### *Concomitant Medication*

VPA interacts with other medications and for this reason participants were requested to register in the study diary any concomitant medication taken during the trial. Participants were advised to avoid certain medications for safety reasons (Table 18). The combination of VPA use and any medicine listed in Table 18 resulted in exclusion from the trial.

Table 18. Prohibited medications

<b>Prohibited Medications</b>		
Anticoagulants	Antidepressants	Aspirin
Chloroquine	Cimetidine	Colestyramine
Diazepam	Erythromycin	Imipenem
Mefloquine	Meropenem	Olanzapine
Other anticonvulsant	Ranitidine	Temozolomide
Zidovudine		

Participants were also asked to refrain from drinking alcohol for the first month and then to drink only in moderation thereafter to avoid drowsiness.

#### *Reported Adverse Events*

AEs were assessed at V1, V2 and V3, by telephone consultation and by home diary review.

#### **Statistical Analysis**

This was a pilot study to assess the feasibility of performing a multi-centre large-scale clinical trial of VPA in McArdle disease. Because the trial was designed as a pilot study, it was not powered to detect statistically significant changes in outcomes. Due to this underpowering, all analysis was descriptive in nature, with no hypothesis tests performed. The direction and magnitude of the changes observed should help determine continuation to the main trial.

The primary outcome was  $VO_{2peak}$ , measured during exercise on a cycle ergometer at exhaustion. Summaries at each time point were produced, in addition to summaries of the changes from baseline recorded during both V2 and V3. Changes from baseline were also calculated as a percentage of the baseline. A clinically important increase in  $VO_{2peak}$  was predefined as one that is greater than 10% of the baseline value. The clinical importance of any effects was compared to this fixed value.

Continuous variables were summarised by the mean and standard deviation and data range if found to follow a normal distribution, and by the median and inter-quartile range, and data range if not

normally distributed. Categorical variables were summarised by the frequency and percentage of values in each category.

Secondary outcomes included change in total distance walked in the 12MWT during V2 and V3 compared to V1, maximum blood lactate response from rest during V2 and V3 measured during the cycle test and the forearm test compared to V1, changes in SF-36 questionnaire scores measured during V1, V2 and V3, the percentage of fibres that stained positively for muscle glycogen phosphorylase at baseline and during V3. Safety was assessed by blood test results and AEs.

As with the primary outcome, secondary outcome analyses were descriptive only. Summary statistics were calculated at each time point, and also for the changes from baseline to V2 and V3.

#### *Post-hoc Analysis*

The analysis examined how changes in  $\text{VO}_2\text{peak}$  were associated with changes in other study parameters. Although the sample size was small, there were no obvious outlying values, and so Pearson correlation was used for the analyses. Corresponding p-values were calculated in addition to the correlation coefficients.

Some caution should be exercised when interpreting these values, as due to the small sample size a lack of a significant result does not necessarily imply a lack of association. Lack of significance could be due to lack of power due to small numbers. Therefore, the size of the correlation is of more importance than the size of the p-value.

## Results

I contacted and invited 49 people to take part in this clinical trial. Eleven participants agreed to attend a screening visit. The first participant was screened in January 2015. The last trial visit, defined as the last follow-up phone call to the 8<sup>th</sup> participant, took place in April 2017, when the study was ended. Two people failed the screening procedure: one failed to meet the inclusion criteria, the other presented with very raised CK levels (41,000 IU/L) as illustrated in Figure 19.

A total of nine participants were recruited. Following study enrolment, one participant was excluded as he failed to attend the pre-treatment muscle biopsy appointment. Data related to the excluded participant was not analysed in this PhD thesis as he did not attend V1 nor did he receive VPA.

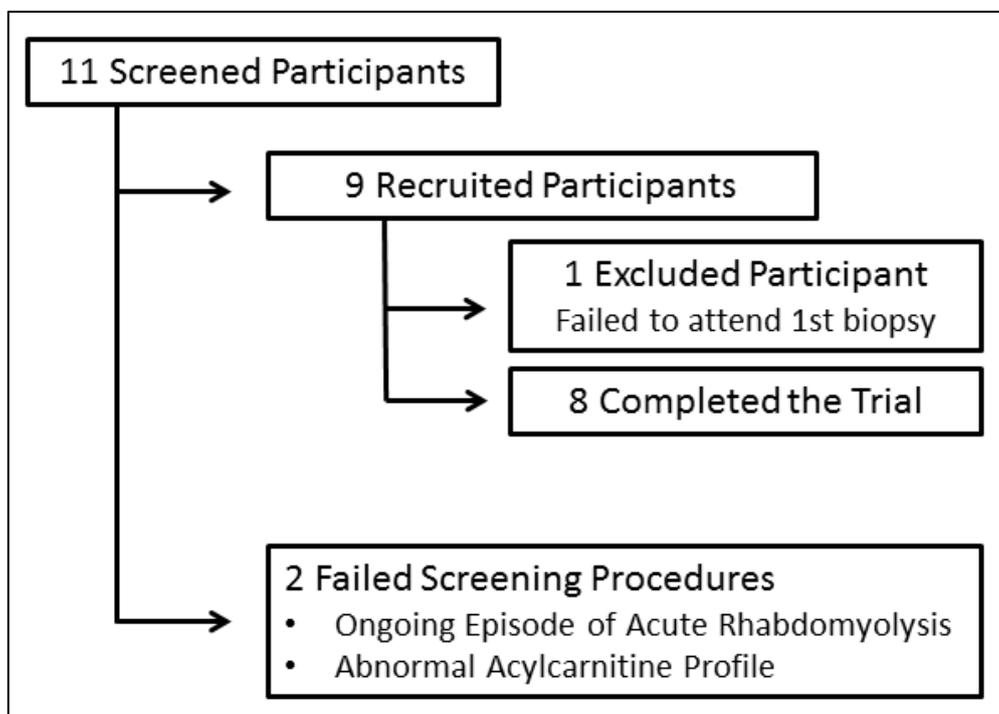


Figure 19. Study enrolment

Eight participants completed all trial procedures. The mean age was 46.2 years (range: 35–59 years). Baseline findings for all trial participants are summarised in Table 19. CK levels varied and were above the normal limits in all participants at both the screening visit and V1. Data from anthropometric assessments indicated one female participant and three male participants had a body mass index (BMI) of more than 25 kg/m<sup>2</sup>, and for this reason were classified as overweight or obese. Of the seven participants who have previously undergone a muscle biopsy, four had to undergo repeat biopsy for

technical reasons (no frozen muscle/slides available for analysis; poor quality of the previous material).

Table 19. Baseline characteristics for recruited participants who completed all trial assessments

Characteristics	P1	P2	P3	P4	P5	P6	P7	P8
<b>Gender</b>	Male	Male	Female	Male	Female	Female	Male	Male
<b>Mutation 1</b>	R50X	R50X	R50X	G567P	G205S	R50X	R50X	R50X
<b>Mutation 2</b>	G205S	R270X	R50X	G567P	425- 26A>G	R50X	R50X	c.345+2T>A
<b>RM*</b>	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
<b>BMI</b> (kg/m <sup>2</sup> )	23.9	22.1	23.9	27	18.7	39.6	30.9	25.9
<b>CK Screening</b> (IU/L)	839	425	1493	1420	317	235	948	4961
<b>CK Visit 1</b> (IU/L)	1072	4107	684	1052	421	303	1803	658
<b>Previous Biopsy</b>	2002	1975	1992	2005	1982	-	2010	2007

\* History of a previous episode of rhabdomyolysis. RM: rhabdomyolysis; BMI: body mass index; CK: creatine kinase.

### Study Compliance

The VPA compliance was 98.4% (95–100%) at V3. Eight participants attended all trial visits and were available for all telephone consultations.

#### *Protocol Deviation Affecting Outcome Measures*

In three study visits, participants had not fasted for at least four hours prior to cycling (one at V1 and two at V3).

One participant was not able to perform the forearm exercise test during V3 due to symptomatic nerve entrapment, most probably moderate to severe carpal tunnel syndrome. In this thesis, forearm exercise test results are reported for all participants on V1 and V2, and for seven participants on V3.

One post-treatment muscle biopsy was not suitable for analysis for technical reasons.

## Primary Outcome Analysis

The primary outcome measure was change in VO<sub>2</sub>peak, measured during exercise on a cycle ergometer. The VO<sub>2</sub>peak increased by 9% from V1 to V2, and by ~13% from V1 to V3. Figure 20 and Table 20 illustrate primary outcome data.

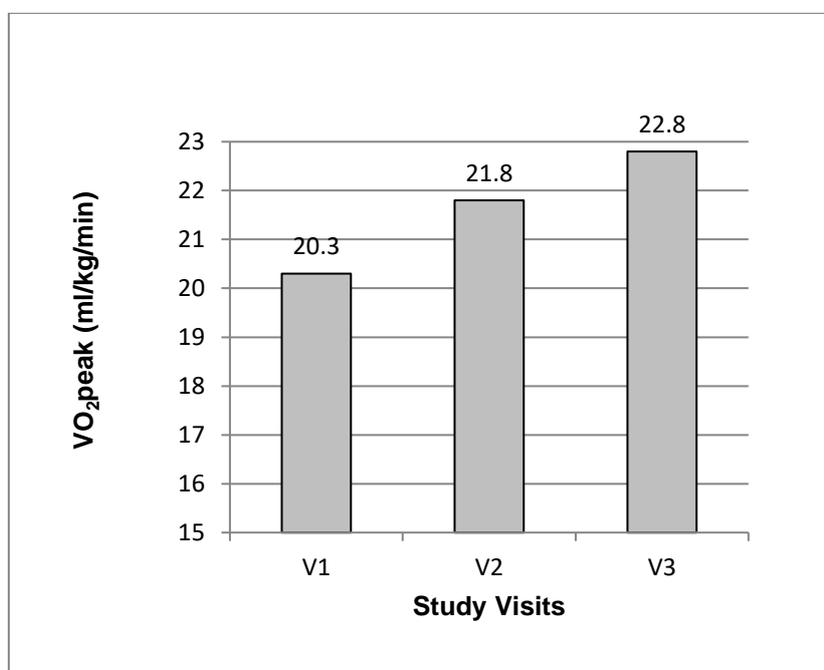


Figure 20. Mean VO<sub>2</sub>peak for all participants who completed all trial assessments in the UK. Standard deviation in V1, V2 and V3 were, respectively,  $\pm 4.6$ ,  $\pm 4.5$  and  $\pm 5.5$ . V1: visit 1; V2: visit 2; V3: visit 3.

Table 20. Summary of VO<sub>2</sub>peak (ml/kg/min)

Primary Outcome	Mean $\pm$ SD	95% CI for Mean	Range
Change V1 to V2 (ml/kg/min)	1.5 $\pm$ 2.9	-0.9, 3.9	-1–7
Change V1 to V2 (%)	9.1 $\pm$ 15.4	-3.8, 22.0	-4.8–33.3
Change V1 to V3 (ml/kg/min)	2.5 $\pm$ 2.7	0.3, 4.7	-2–5
Change V1 to V3 (%)	12.9 $\pm$ 13.9	1.3, 24.6	-9.5–26.3

V1: visit 1; V2: visit 2; V3: visit 3; SD: standard deviation; CI: confidence interval.

### *Post-hoc Analysis*

Table 21 shows the maximum HR recorded during the cycle test, serum glucose levels and the workload change in V1, V2 and V3. Figure 21 shows the median HR variation during the first 15 minutes of the constant workload phase of the cycle test.

Table 21. Cycle test assessments

Assessment	Time Point	n	Mean $\pm$ SD	Range
<b>Maximum Heart Rate</b> (bpm)	V1	8	161 $\pm$ 17	135–182
	V2	8	164 $\pm$ 20	125–190
	V3	8	162 $\pm$ 16	132–183
	Change V1 to V2	8	4 $\pm$ 215	-20–18
	Change V1 to V3	8	1 $\pm$ 6	-9–10
<b>Maximum Workload</b> (*) (W)	V1	8	33.1 $\pm$ 7.6	20–40
	V2	8	36.3 $\pm$ 6.9	25–40
	V3	8	38.8 $\pm$ 2.3	35–40
	Change V1 to V2	8	3.1 $\pm$ 8.8	-10–20
	Change V1 to V3	8	5.6 $\pm$ 7.8	0–20
<b>Glucose</b> (**) (mmol/L)	V1	8	4.51 $\pm$ 0.41	4.13–5.33
	V2	8	4.38 $\pm$ 0.30	3.83–4.85
	V3	8	4.54 $\pm$ 0.37	4.10–5.18
	Change V1 to V2	8	-0.14 $\pm$ 0.38	-0.75–0.47
	Change V1 to V3	8	0.03 $\pm$ 0.59	-0.97–0.80

(\*) Defined as maximum change from rest; (\*\*) Defined as mean of post-rest values. V1: visit 1; V2: visit 2; V3: visit 3; SD: standard deviation.

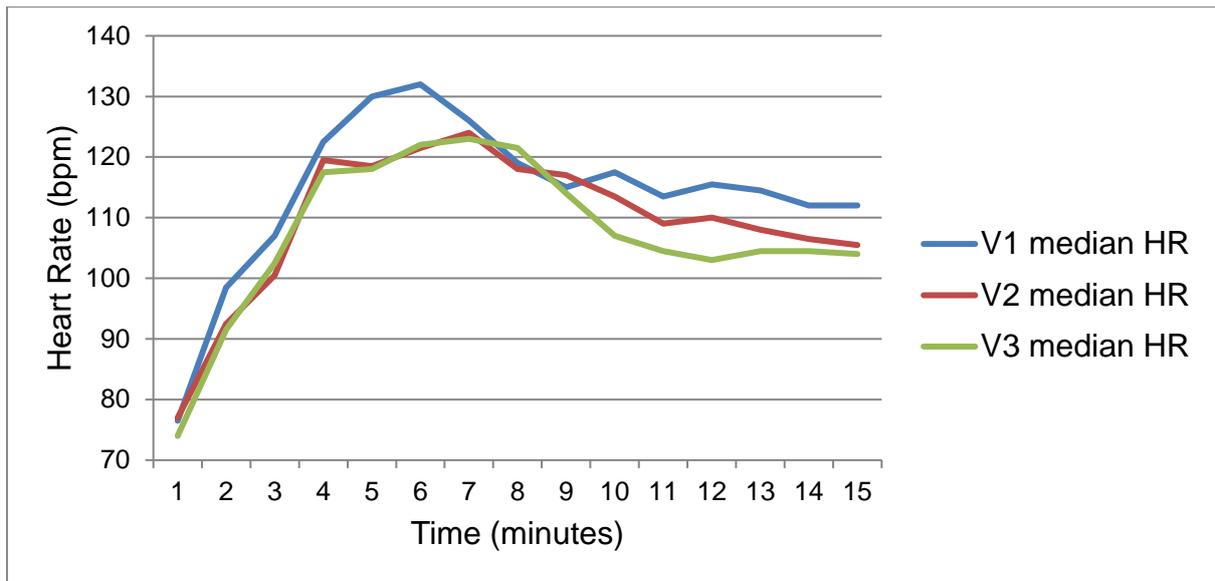


Figure 21. Heart rate variation in the cycle test

Median HR calculated for eight participants for each study visit. V1: visit 1; V2: visit 2; V3: visit 3; HR: heart rate.

### Secondary Outcomes Analyses

#### *Blood Lactate Response to Exercise*

Blood lactate response to exercise was measured at V1, V2 and V3 during the cycle test and the forearm exercise test. Mean plasma lactate values varied little during the forearm test and cycle test, as shown in Figure 22 and Figure 23, respectively. The mean lactate change from the maximum value from rest for V1 to V3 measured by the forearm exercise test and the cycle test were 0.21 mmol/L (SD:  $\pm 0.45$ ) and 0.10 mmol/L (SD:  $\pm 0.50$ ), respectively.

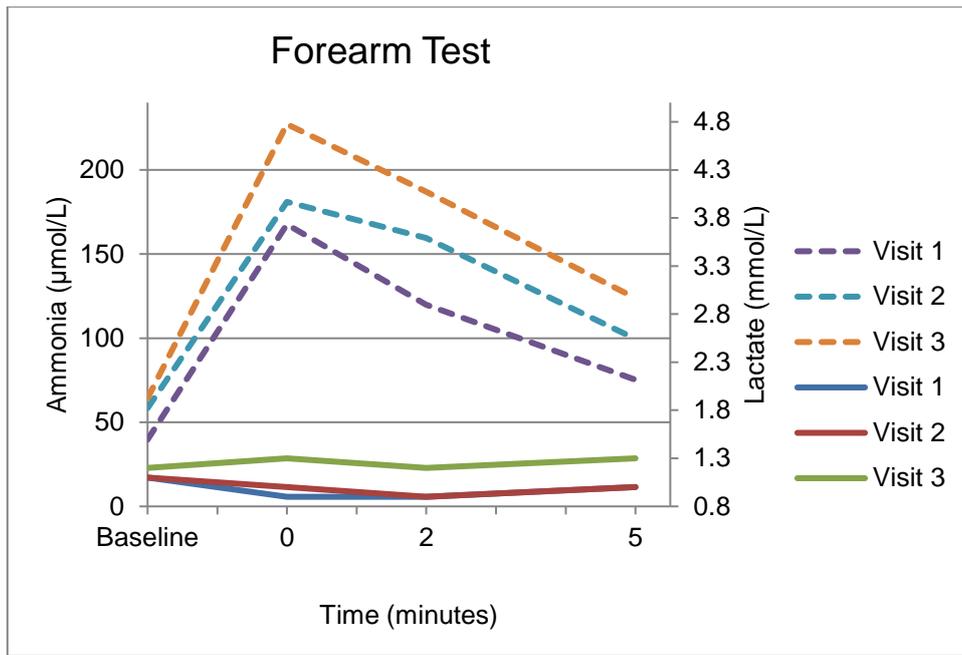


Figure 22. Lactate and ammonia changes during the non-ischaemic forearm exercise test

Bloods were collected at baseline (resting) and at zero, two and five minutes following exercise. During V3, only seven participants performed the forearm exercise test. V1: visit 1; V2: visit 2; V3: visit 3. Dashed lines represent ammonia values.

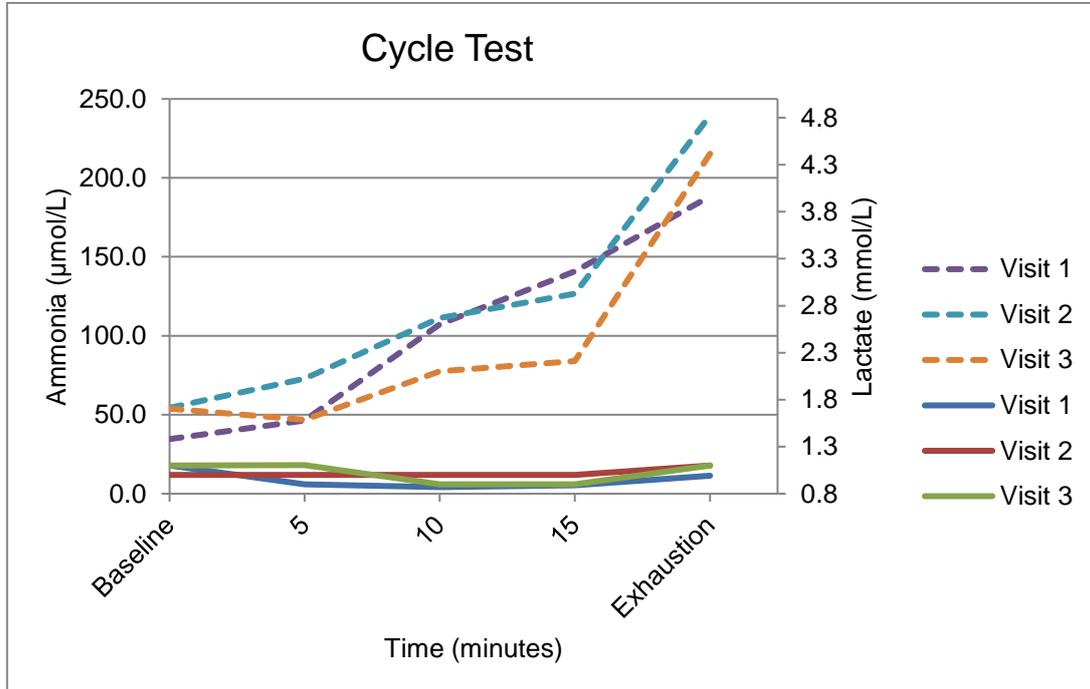


Figure 23. Lactate and ammonia changes during the trial cycle ergometer test

Bloods were collected at baseline (resting) and at 5, 10 and 15 minutes during the exercise, and at exhaustion. All participants completed the cycle test in all study visits. V1: visit 1; V2: visit 2; V3: visit 3. Dashed lines represent ammonia values.

### Post-hoc Analysis

Handgrip strength was assessed during the exercise phase of the forearm exercise test. There was an increment in handgrip strength. Changes in maximum grip strength measurement between V1 and V3 are illustrated in Table 22.

Table 22. Maximum handgrip strength assessed during the non-ischaemic forearm exercise test

<b>Outcome</b>	<b>TimePoint</b>	<b>n</b>	<b>Mean ± SD</b>	<b>Range</b>
<b>Max grip strength (N)</b>	V1	8	71 ± 35	11–121
	V2	8	84 ± 25	46–112
	V3	7	93 ± 31	59–136
	Change V1 to V2	8	12 ± 31	-15–86
	Change V1 to V3	7	22 ± 24	4–72

*During V3, only seven participants performed the forearm exercise test. V1: visit 1; V2: visit 2; V3: visit 3; SD: standard deviation; Max: maximum.*

### *Phosphorylase Expression in Skeletal Muscle*

The presence of phosphorylase-positive fibres was assessed at baseline, before treatment intake, and after six months of full dose treatment. One post-treatment muscle biopsy was unsuitable for analysis for technical issues. The median percentage of phosphorylase-positive fibres for V1 and V3 were 0% and 0.3%, respectively. The mean percentage of regenerating fibres expressing neonatal myosin for V1 and V3 were 2.2% and 2.8%, respectively.

### *Change in Total Walked Distance*

The total walked distance was analysed during V1, V2 and V3. The mean variation in the total walked distance for V1 to V3 was 34 m. Table 23 shows the 12MWT descriptive data.

Table 23. Total walked distance assessed by the 12-minute Walk Test

<b>Outcome</b>	<b>Time Point</b>	<b>Mean ± SD</b>	<b>Range</b>
<b>Total Walked Distance (m)</b>	V1	933 ± 133	683–1100
	V2	958 ± 166	660–1213
	V3	966 ± 207	606–1253
	Change V1 to V2	25 ± 215	-41–113
	Change V1 to V3	34 ± 85	-77–153

V1: visit 1; V2: visit 2; V3: visit 3; SD: standard deviation.

### SF-36

There were small changes in the two main SF-36 components as illustrated in Table 24. Table 25 illustrates eight health domain scales.

Table 24. Mental and physical component results assessed using the SF-36 questionnaire

<b>SF-36 Component</b>	<b>TimePoint</b>	<b>Mean <math>\pm</math> SD</b>	<b>Range</b>
<b>Mental Component Score</b>	V1	59 $\pm$ 4	51–65
	V2	57 $\pm$ 5	50–64
	V3	58 $\pm$ 6	44–64
	Change V1 to V2	-2 $\pm$ 2	-7–1
	Change V1 to V3	-1 $\pm$ 5	-7–7
<b>Physical Component Score</b>	V1	45 $\pm$ 7	36–56
	V2	47 $\pm$ 7	33–55
	V3	46 $\pm$ 8	32–54
	Change V1 to V2	2 $\pm$ 5	-3–11
	Change V1 to V3	2 $\pm$ 7	-8–11

V1: visit 1; V2: visit 2; V3: visit 3; SD: standard deviation.

Table 25. Eight health domain scales assessed by the SF-36 questionnaire

SF-36 Health Domain	Time Point	n	Mean ± SD	Range
<b>Physical Functioning</b>	V1	8	42 ± 7	33–50
	V2	8	42 ± 7	27–50
	V3	8	45 ± 6	35–54
	Change V1 to V2	8	0 ± 5	-6–8
	Change V1 to V3	8	3 ± 3	-2–8
<b>Role Physical</b>	V1	8	50 ± 5	44–57
	V2	8	52 ± 4	46–57
	V3	8	52 ± 4	46–57
	Change V1 to V2	8	2 ± 2	0–6
	Change V1 to V3	8	1 ± 6	7–13
<b>Bodily Pain</b>	V1	8	44 ± 10	22–56
	V2	8	48 ± 6	38–56
	V3	8	46 ± 10	34–62
	Change V1 to V2	8	3 ± 9	-9–20
	Change V1 to V3	8	2 ± 11	-17–16
<b>General Health</b>	V1	8	56 ± 6	46–65
	V2	8	57 ± 10	34–67
	V3	8	57 ± 8	39–65
	Change V1 to V2	8	1 ± 6	-12–8
	Change V1 to V3	8	0 ± 4	-7–5
<b>Vitality</b>	V1	8	53 ± 8	41–62
	V2	8	54 ± 6	44–64
	V3	8	51 ± 8	41–62
	Change V1 to V2	8	1 ± 9	-15–15
	Change V1 to V3	8	-2 ± 5	-12–6

<b>SF-36</b>	<b>Time Point</b>	<b>n</b>	<b>Mean ± SD</b>	<b>Range</b>
<b>Health Domain</b>				
<b>Social Functioning</b>	V1	8	55 ± 4	47–57
	V2	8	53 ± 7	42–57
	V3	8	53 ± 6	42–57
	Change V1 to V2	8	-2 ± 7	-15–5
	Change V1 to V3	8	-2 ± 6	-10–5
<b>Role Emotional</b>	V1	8	55 ± 2	49–56
	V2	8	54 ± 4	46–56
	V3	8	55 ± 4	46–56
	Change V1 to V2	8	-1 ± 3	-7–0
	Change V1 to V3	8	0 ± 1	-3–0
<b>Mental Health</b>	V1	8	54 ± 4	46–61
	V2	8	54 ± 5	48–61
	V3	8	57 ± 7	43–64
	Change V1 to V2	8	0 ± 2	-3, 3
	Change V1 to V3	8	3 ± 6	-3–13

V1: visit 1; V2: visit 2; V3: visit 3; SD: standard deviation.

#### Post-hoc Analysis

The association between changes in VO<sub>2</sub>peak and changes in other parameters were examined. A summary of the analysis results is summarised in Table 26.

Table 26. The association between changes in VO<sub>2</sub>peak and changes in other parameters

Variable	Correlation Coefficient	P-value
<b>SF-36 Mental Component</b>	-0.61	0.11
<b>SF-36 Physical Component</b>	0.58	0.13
<b>SF-36 Physical Functioning</b>	0.33	0.43
<b>SF-36 Role Physical</b>	0.26	0.54
<b>SF-36 Bodily Pain</b>	0.29	0.49
<b>SF-36 General Health</b>	0.86	<b>0.006</b>
<b>SF-36 Vitality</b>	0.19	0.66
<b>SF-36 Social Functioning</b>	-0.07	0.87
<b>SF-36 Role Emotional</b>	-0.38	0.35
<b>SF-36 Mental Health</b>	-0.63	0.10
<b>Distance Walked</b>	0.42	0.29

### Safety and Adverse Events

No participant reported any episode of myoglobinuria during this trial. Median CK levels were 868 IU/L (303–4,107) at V1, 561 IU/L (230–2,463) at V2 and 648 IU/L (161–2,646) at V3. Average weight was 74.4 kg (46–97 kg) at V1, 76.1 kg (48–98 kg) at V2 and 77 kg (50–101 kg) at V3.

### *Drug Levels*

VPA levels measured during V2 and V3 were 73 mg/L (34–93) and 76 mg/L (28–101), respectively.

### *Adverse Events*

There were 123 AEs for the eight participants. The most common category was musculoskeletal, comprising 30% of all AEs. This was followed by 'other' (25%) and central nervous system (23%). McArdle symptoms were the most commonly occurring AEs. Almost two-thirds of AEs were deemed not to be related to the study drug. Tiredness and sleepiness were the most common type of AE that was either definitely or probably drug related.

### Severe Adverse Event

Of the 123 AEs, only one was classed as being an SAE. One participant was admitted to hospital to investigate knee pain after driving for more than 16 hours. The admission lasted 14 hours. His CK was 915 IU/L on that occasion. His symptoms resolved completely.

The SAE was not related to VPA intake. As per protocol definitions, all hospital admissions were considered SAEs.

Table 27 and Table 28 review the reported AEs.

Table 27. Adverse events, by category

<b>Outcome</b>	<b>Category</b>	<b>Total (%)</b>
Adverse Events	Total	123
<b>Adverse Events Category</b>		
	Central Nervous System	28 (23%)
	Gastrointestinal	22 (18%)
	Infection	5 (4%)
	Musculoskeletal	37 (30%)
	Other	31 (25%)
<b>Seriousness</b>		
	Not serious	122 (99%)
	Serious adverse event	1 (1%)
<b>Severity</b>		
	Mild	77 (64%)
	Moderate	28 (23%)
	Severe	16 (13%)
<b>Relationship to Drug</b>		
	Definitely	1 (1%)
	Probably	18 (15%)
	Possibly	15 (12%)
	Unlikely	9 (7%)
	Not related	79 (65%)

Table 28. Adverse events reported by eight British participants following VPA intake

<b>AE Category</b>	<b>AE Details</b>	<b>Total Number</b>	<b>Number Definitely/ Probably Drug Related</b>	<b>Number Possibly Drug Related</b>
<b>Central Nervous System</b>	Confusion	1		
	Dizziness	3		
	Forgetfulness	1		1
	Headache	2		
	Migraine	2		
	Mood changes	3		
	Tiredness and sleepiness	12	5	5
	Vertigo	3		
	Vivid dreams	1		
<b>Gastrointestinal</b>				
<b>Gastrointestinal</b>	Anal fissure	1		
	Diarrhoea	6		4
	Gum problems	1		1
	Increased appetite	3	2	
	Nausea and/or vomiting	8	3	2
	Stomach upset	2		

<b>AE Category</b>	<b>AE Details</b>	<b>Total Number</b>	<b>Number Definitely/ Probably Drug Related</b>	<b>Number Possibly Drug Related</b>
<b>Infection</b>	URTI	5		
<b>Musculoskeletal</b>				
	Joint pain	3		
	McArdle symptoms	33	1	
	Swollen ankle	1		
<b>Other</b>				
<b>Other</b>	Anaemia	1		
<b>AE Category</b>				
<b>AE Category</b>	<b>AE Details</b>	<b>Total Number</b>	<b>Number Definitely/ Probably Drug Related</b>	<b>Number Possibly Drug Related</b>
<b>Other</b>	Bleeding	2		
	Fall	1		
	Hair loss	4	4	
	Heavy pain	1		
	Increased weight	5	4	1
	Itching	1		1
	Nerve entrapment	2		
	Orange colour urine	1		
	Post-biopsy complications	5		
	Skin lesion	2		
	Tingling (hands & toes)	1		

	Ventricular extrasystole	5		
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*URTI: upper respiratory tract infection.*

## Discussion

The innovative potential of this PhD project involved identifying a drug treatment for McArdle disease while exploring different outcome measures to be used in clinical trials for drug development for this condition. To date, this is the first clinical trial to have explored the effects of VPA in people with McArdle disease. It is also the first time the role of the 12MWT has been analysed as an outcome measure in a clinical trial.

In this chapter, challenges related to protocol development and study findings are discussed in two separate sections: the first section covers the protocol development aspects, while the second one further explores drug safety, efficacy and analysis of outcome measures.

### Protocol Development

VPA is a well-known drug prescribed as a treatment option for epilepsy and migraine (Linde et al., 2013). Its efficacy has also been evaluated for other conditions, including bipolar disorders and schizophrenia (Wang et al., 2016, Cipriani et al., 2013). More recently, studies in the field of neuromuscular disease have explored the role of VPA as a histone deacetylase inhibitor. Even though *in vitro* studies have indicated a beneficial effect of VPA in spinal muscular atrophy, similar efficacy has not been confirmed by clinical trials performed in humans with the condition (Kissel et al., 2014, Krosschell et al., 2018, Kissel et al., 2011). A Phase III study of VPA in amyotrophic lateral sclerosis also failed to demonstrate effectiveness in disease progression and survival (Piepers et al., 2009). A recent study suggested that VPA may be of potential value in oculopharyngeal muscular dystrophy, but there has been no clinical trial in humans with the condition (Abu-Baker et al., 2018).

It was not possible to predict the effect of VPA in humans with McArdle disease based on previous research into other neuromuscular conditions because they have different pathophysiologies. However, for McArdle disease, both *in vitro* and *in vivo* research in animal models has provided strong evidence that VPA has a beneficial effect, supporting further study of VPA in humans affected by the condition. One way of obtaining further evidence would have been to perform an *in vivo* study in the McArdle mouse model. However, the best way to confirm early VPA efficacy data in humans was to undertake a proof-of-concept study in people affected by the condition. Therefore, a phase II pilot study was developed.

## *Translating Knowledge from Animal Model Studies to Humans with McArdle Disease: Anticipated Challenges*

### Poor Recruitment

Because McArdle disease is rare, difficulties in recruitment were expected, especially because all female participants with childbearing potential were prohibited from taking part in this study. Other factors that were expected to negatively affect study recruitment were patients' concerns regarding cycling, VPA-related AEs and risks associated with muscle biopsy, such as severe pain, infection and scarring.

To address recruitment limitations, it was pre-established that a small sample would be recruited in an open-label single-arm trial. All participants were exposed to the intervention. The same protocol was run in Denmark, where seven participants were recruited. For data analysis purposes the sample size was increased by combining the British and Danish samples within a single database, to give a total sample size of 15. In this PhD only the British data are presented because the trials were run independently.

### Treatment Dose

Two previous studies have explored the benefits of VPA in animal models of McArdle disease (Howell et al., 2015, de Luna et al., 2015). In the *in vivo* study, McArdle sheep received increasing doses of enteric administration of 20–60 mg/kg/day VPA for up to 20 weeks (Howell et al., 2015). Biopsies were performed at different times during the treatment phase, and were taken from different muscle groups. In the sheep model, treatment benefits increased with escalating doses and a prolonged duration of exposure. An increase in the number of phosphorylase-positive fibres was seen in post-treatment muscle biopsies, but neonatal myosin immunohistochemistry to confirm whether the phosphorylase-positive fibres were related to regenerating fibres was not reported. The investigators also explored the local effects of intramuscular injections of VPA in a sub-group of sheep. It is not possible to completely exclude a local toxic effect of intramuscular VPA injection, which could have triggered muscle regeneration and thus the expression of foetal isozyme in injected muscles. An *in vitro* study analysed muscle cultures from KI mice exposed to VPA for 72 hours at one, two and 5 mM (de Luna et al., 2015). The study provided evidence that VPA activated the expression of the brain isozyme in muscle cultures. A therapeutic functional benefit was suggested by confirming the

reduction in intracellular glycogen content following VPA exposure. The treatment effect was dose dependent (de Luna et al., 2015).

In humans, however, high VPA doses may be associated with an increased number of AEs or worsening severity of AEs. The main challenge of this trial was to select a dose that would be, in theory, strong enough to improve McArdle symptoms but within the safety range for humans. The 20 mg/kg/day dose was selected based on the evidence available for VPA use as a treatment option for epilepsy (NICE, 2018). The suggested maintenance dose for epilepsy treatment is 20–30 mg/kg/day (NICE, 2018). Thus the lowest recommended therapeutic dose for epilepsy was chosen.

To reduce the risks of having a negative trial due to short treatment duration, a six-month treatment period was selected; slightly longer than the treatment period used in the ovine model study.

Poor treatment compliance negatively affects trial outcomes as it underestimates treatment efficacy. Poor compliance, therefore, was a criterion for trial exclusion. Unfortunately, there is no single tool that reliably assesses drug compliance (Pullar et al., 1989). Different procedures have different limitations. Pill count, for instance, is more useful in identifying poor compliance than confirming treatment adherence (Pullar and Feely, 1990). In this study, a series of procedures were combined to evaluate treatment compliance. Home diary information helped with assessing compliance of participants who failed to return their tablets, thereby precluding pill count. Regular phone call assessments helped reduce memory bias.

#### Adverse Events and Safety

VPA use is associated with several AEs, such as weight gain and hair loss. Expected and common VPA-related AEs are usually reversible after treatment interruption. For this reason, no long-term complication of VPA use was expected after trial completion.

VPA dose was slowly increased as is standard in clinical practice. Somnolence is an expected AE and is seen mostly during treatment initiation. To alleviate the inconvenience of somnolence, all participants were requested to take VPA as a slow-release preparation at bedtime. Participants were also advised to avoid alcohol intake during the first few weeks of treatment initiation, particularly if they experienced somnolence.

Some infrequent VPA AEs have more important clinical significance. Initial challenges included identifying the AEs of special clinical concern, developing strategies to prevent their occurrence and standardising assessment to enable detection of AEs at an early stage.

A small proportion of patients may experience drug-induced hypersensitivity syndrome, such as Steven-Johnson syndrome (Hamm, 2011). Steven-Johnson syndrome is a pharmaco-induced hypersensitivity reaction, which is rarely reported in association with VPA monotherapy (Naveen et al., 2012). Affected people require in-hospital treatment and, occasionally, intensive therapy unit care. All participants were advised to contact the study team or their local A&E department in the event of rash or mucosa changes, or allergy reaction symptoms. Skin changes and allergy symptoms were assessed during all study visits and asked about during telephone consultations. Because polypharmacy is a predisposing factor for drug-induced hypersensitivity syndrome, a list containing drugs that could increase the risks of developing pharmaco-induced hypersensitivity reactions if combined with VPA therapy was generated (Hamm, 2011). Participants were requested to avoid these medicines for the duration of the trial. VPA may potentiate the effect of certain medicines; for this reason, these were also listed in the prohibited concomitant medication list.

Blood sampling was performed as a safety assessment to detect certain AEs at early stages. Blood test selection was based on expected AEs and on the monitoring requirements for VPA use established by the National Institute for Health and Care Excellence (NICE) (NICE, 2018). Total bilirubin was used as a screening test for liver impairment as people with McArdle disease may have abnormal transaminase enzyme levels related to the underlying skeletal muscle disease.

VPA use may cause abnormal homeostasis and coagulopathy (Abdallah, 2014). For this reason, during all study visits and telephone consultations participants were asked about bleeding and bruising. Coagulation profile and platelet count were assessed during all study visits.

At the end of the trial, the VPA dose was slowly reduced based on NICE recommendations, avoiding an abrupt withdrawal (NICE, 2018).

Exclusion criteria were selected based on contraindications for VPA use and expected AEs (NICE, 2018). Pharmacokinetics of Epilim are modified in the elderly. VPA use in the elderly may require

dose adjustment, especially in the presence of kidney dysfunction. For this reason, people older than 64 years were not recruited.

Acute intoxication with VPA was also a special concern. Serum levels of VPA were collected to detect overdosage. Information on extra tablet intake was also regularly assessed during all trial visits and telephone consultations.

### Pregnancy

VPA use in pregnancy is associated with foetal malformation. Foetal toxicity is more noticeable when VPA doses are greater than one gram daily (NICE, 2018). Major malformations include, but are not limited to, neural tube defects, hypospadias and cardiac malformation (Tanoshima et al., 2015, Gooneratne et al., 2017).

VPA is an enzyme-inducing anti-epileptic drug. It increases the activity of the hepatic cytochrome enzyme P450. This accelerates the metabolism of some hormonal contraceptive methods including combined pills (Gooneratne et al., 2017). VPA use, therefore, may increase the failure rate of certain birth control methods.

For this reason, extraordinary considerations were taken to prevent conception. In this trial, being a female participant with childbearing potential was an exclusion criterion, while infertility in women was an inclusion criterion. Similar criteria were used both as an inclusion and an exclusion criterion to greatly reduce the chances of an unexpected pregnancy. In the UK, recruiting a female participant with childbearing potential would be a breach of protocol.

The teratogenic effects of VPA have not been confirmed if the father, and not the pregnant mother, is on treatment at the time of conception (EpilepsySociety, 2018). To ensure safety, all male participants were required to use an accepted method of birth control for the duration of the trial as described in the participant information sheet. This was discussed further during the screening visit.

### McArdle Disease Management

General care provided for all trial participants did not differ from the recommended standards of care for this condition. All participants received advice similar to that given to patients with McArdle disease attending the specialised clinic. This mainly included advice on taking regular exercise, which is

currently the best treatment/management approach for the condition (Quinlivan et al., 2014a, Quinlivan et al., 2011, Quinlivan et al., 2017).

It is known that exercising on a regular basis can increase the  $VO_2$ peak (Kubukeli et al., 2002). This means the general advice on fitness given to trial participants as part of their regular care could potentially have influenced the primary outcome measure of this trial. However, asking participants not to change their lifestyle if sedentary, or to keep an unchanged exercise routine would not be best medical practice.

A limitation of this study was the open-label design, as it is difficult to confirm, just by the primary outcome measure analysis, whether the  $VO_2$ peak increase was due to a real VPA effect or due to the benefit of regular physical activity and/or a learning component. To address this expected challenge, a series of secondary outcome measures were combined to further assess VPA efficacy.

#### Outcome Measures

Overall, the trial looked at the following: 1) whether an alternative phosphorylase isozyme was expressed in skeletal muscles, 2) whether the expressed enzyme broke down glycogen and, by doing so, increased plasma lactate levels during exercise, 3) whether the expressed enzyme resulted in functional benefits to patients, 4) whether the combination of those findings actually improved participants' wellbeing and quality of life, and 5) whether this treatment was safe for this population.

There was no single outcome measure able to provide all the information needed to support VPA use. The assessments selected in this project aimed to address outcome measure limitations. For clinical trial interpretation, the combination of all outcome measure results were examined, providing the evidence required to assess VPA treatment efficacy.

#### Primary Outcome Measure

The main aim of the primary outcome measure was to assess whether or not VPA treatment improved the individual's  $VO_2$ peak.

During the first few seconds of cycling, the skeletal muscle relies on the phosphagen system for ATP production. Without VPA, or in the absence of a positive effect, this is followed by a metabolic crisis in people with McArdle disease, as the glycolytic system is impaired. In the final minutes of the cycle test, the mitochondrial respiration provides the required energy to skeletal muscles. This metabolic

system is used until exhaustion, when the glycolytic system would be required for a sprint finish (Draper and Marshall, 2013).

The initial hypothesis was that VPA treatment would restore the glycogen breakdown in skeletal muscles. As a result, all participants would have a higher  $VO_2$ peak, as they would have this extra energy at the very end of the cycle test for a sprint finish. A functional glycolytic system would also improve mitochondrial respiration by providing glycogen-derived pyruvate for oxidative phosphorylation (Haller and Vissing, 2002). Such enhancements on a daily basis would allow individuals to exercise more vigorously, thus contributing to participants'  $VO_2$ peak increase.

The evidence supporting this theory is based on previous studies assessing glucose supplementation in McArdle disease. Intravenous glucose infusion reduced the intensity of the *second wind* effect when compared to placebo (Andersen et al., 2009). In addition, glucose infusion increased patients'  $VO_2$ peak by 20% (Haller and Vissing, 2002). This occurred because the ability to use blood-borne glucose as an energy source in skeletal muscle is not impaired in McArdle disease. Glucose-derived pyruvate from the extra glucose supplementation increases muscle oxidative phosphorylation, thus improving patients' exercise capacity (Haller and Vissing, 2002). Further research in the McArdle mouse model showed that improvement in  $VO_2$ peak following exercise training occurred up to a certain level, which was below the improvements seen in the wild-type mouse, supporting the important role that glycogen breakdown has in sustaining the maximal amount of physical exertion a mammal can tolerate (Fiuza-Luces et al., 2018). Intravenous infusion of lipid in people with McArdle disease, however, did not increase fatty acid oxidation and did not weaken the *second wind* phenomenon (Andersen et al., 2009). The negative impact of a 'reduced' glycolytic system for intense exercise performance has also been identified in elite athletes: ketogenic diet (low carbohydrate / high fat) reduced athletes' exercise performance when compared to a high carbohydrate diet (Burke et al., 2017). The results of this study were compared with the McArdle disease pathophysiology, further supporting the essential role of glycogen for endurance training (Nogales-Gadea et al., 2017).

In view of this, external factors such as diet were expected to influence participants' performance in the exercise assessments. For this reason, all participants were required to fast prior to the study visits, ensuring pre-exercise glucose intake would not increase blood-borne glucose availability at the time of the exercise assessments. This was done in order to enable identification of a genuine VPA

effect. However, at the screening visit, participants were allowed to eat prior to the cycle test as the aim was to reach a genuine maximum  $VO_2$ peak. As mentioned above, glucose availability may increase  $VO_2$ peak in patients with McArdle disease (Haller and Vissing, 2002).

It was not clear whether VPA affects the mitochondrial respiratory activity of people with McArdle disease, thereby compromising their aerobic metabolism. *In vitro* studies have shown a negative effect of VPA in the mitochondrial metabolism (Komulainen et al., 2015). VPA may inhibit pyruvate uptake and oxidative phosphorylation, leading to oxidative stress and cell death (Komulainen et al., 2015, Aires et al., 2008). VPA use is contraindicated in people with mitochondrial disorders as its effects in a person with compromised mitochondrial function may increase VPA toxicity, increasing the risks of complications such as liver failure. If the same happens in people with McArdle disease, it was hypothesised that a reduction in their  $VO_2$ peak would be detected by the cycle test, as both the glycolytic and mitochondrial respiratory systems would be impaired, resulting in a more prominent metabolic crisis.

#### Secondary Outcome Measures

There were no major concerns related to the 12MWT execution. This walking test, when first used to study McArdle disease, was performed in a 20-metre-long corridor (Buckley et al., 2014). In this trial, a 10-metre-long corridor was used. It was hypothesised that participants would cover longer distances if VPA treatment was beneficial. The ability to break down glycogen would provide the energy required for the first minutes of walking, thus eliminating the *second wind* phenomenon.

The *second wind* phenomenon is a potential bias to be considered when performing exercise assessments in people with McArdle disease, particularly a cycle test followed by a walking test. If the walk test is performed when the patient is in *second wind*, drug efficacy may be overestimated as participants would be able to walk long distances without the need to slow down. Thus, it was important to ensure participants were out of the *second wind* before starting each of the exercise tests.

There has never been a formal study to investigate the time it takes for the *second wind* to fade. Thus the selection of a minimum rest period between tests was based on patients' reported experience. According to patients who attend the UK clinic, it takes approximately 30 minutes' rest for the *second wind* to disappear (Wakelin, 2013, IAMGSD, 2014). After 45 minutes' rest, people with McArdle

disease would need to warm up again in order to get into the *second wind*. Thus, a minimum of 45 minutes' rest was taken between exercise assessments in this study.

Muscle biopsy was used to confirm whether VPA induced the expression of an alternative isozyme. An expected challenge related to the muscle biopsy technique is the fact that the phosphorylase stain fades over time (Sato, 2002). This technical issue influences trial outcomes: a faded phosphorylase stain could bias the results as absence of the enzyme leads to underestimates of VPA efficacy. To address this expected technical issue, all sections were stained together at the end of the trial, and an external control was used to confirm the quality of the histochemical stain. All slides were reviewed immediately after staining. To avoid bias, all slides (pre- and post-treatment) were analysed by the same investigator in a blinded way.

Other expected challenges were related to muscle damage. Temporary brain glycogen phosphorylase expression in regenerating skeletal muscle fibres is a normal response following injury. The presence of regenerating fibres could lead to overestimates of VPA efficacy. Thus, positive phosphorylase fibres in this context would be unrelated to treatment effect. Neonatal myosin immunohistochemistry was used to identify regenerating muscle fibres. The comparison between positive phosphorylase fibres and positive neonatal myosin fibres was used to prevent overestimates of VPA efficacy due to an expected physiological process.

In the sheep study, the positive muscle biopsy findings were patchy, and not all fibres of similar muscle groups showed positive phosphorylase staining (Howell et al., 2015). In view of this, muscle biopsy was considered a secondary outcome measure rather than a primary outcome measure. Therefore, a limitation of this method was the risk of a false negative result related to a patchy effect, and consequent underestimates of treatment benefits.

Lactate was measured during the cycle test and during the non-ischaemic forearm exercise test. This measurement can give further evidence of an ongoing glycogen breakdown during exercise. Glucose administration may raise plasma lactate, which could lead to overestimates of treatment benefits. To address this limitation, all participants were required to fast for at least four hours before exercising.

There were no major concerns related to completion of the SF-36 questionnaire.

## **Developing New Treatment Options for Rare Diseases**

This drug repurposing study attempted to identify whether or not VPA stimulated the expression of the brain and/or liver isozymes in skeletal muscle of people with genetically confirmed McArdle disease, and if so to assess the functional benefits of the treatment in this population.

### *Sodium Valproate Safety*

VPA was well tolerated. The study compliance was above 90%. Common VPA-related AEs reported in this study were expected and previously reported in the Sanofi Summary of Product Characteristics (SPC). Blood sample abnormalities had no clinical significance. VPA use did not cause liver dysfunction in the study participants. There was one SAE during this trial. This was not related to VPA intake. All VPA-related AEs resolved following treatment interruption: there was no ongoing VPA-related AE at the time of the final trial visit.

In this study, VPA use did not limit participants'  $\text{VO}_2\text{peak}$ , as it increased above 10% after six months' treatment. This finding suggests that VPA does not negatively interact with the mitochondrial metabolism of people with McArdle disease. Further studies to properly investigate the toxic effects of VPA in mitochondrial respiration in this patient population were not performed as this was not the aim of this project.

No participant conceived a child during this trial. The precautions taken to prevent conception while on VPA treatment succeeded in preventing an unexpected pregnancy. Repeating such precautions should be considered in future trials that assess VPA therapy.

Therefore, this study shows for the first time that a prescription of 20 mg/kg/day VPA orally for six months was safe in a small sample of people with McArdle disease when known contraindications for VPA use were excluded.

One limitation of this study in terms of assessing drug safety was the small sample size. This study was not powered to assess safety. Rare AEs were unlikely to be revealed in such a small group of patients. The translation of the safety knowledge gained in this pilot study to the community could be related to the general use of VPA for epilepsy and other conditions. This study confirmed that McArdle disease should not be considered a formal contraindication for VPA prescription.

### *Sodium Valproate Efficacy*

In this project, secondary outcome measure analysis provided sufficient data to prove a lack of efficacy of VPA treatment in humans with McArdle disease. Post-treatment muscle biopsies did not show significant changes in phosphorylase expression, confirming VPA intake did not stimulate the expression of the brain and/or liver glycogen phosphorylase in skeletal muscle. The non-significant/blunt increase in plasma lactate during cycling and following the non-ischaemic forearm exercise test confirmed the lack of an active enzyme in skeletal muscle, illustrating a severe metabolic block in the skeletal muscle glycolytic system. No major changes were seen in the total distances walked assessed by the 12MWT. There was no clinically relevant change in the SF-36 health domain scales over the course of the trial.

Serum ammonia increase during exercise, which is typically seen in McArdle disease, was still present following VPA treatment. Even though ammonia increase was not considered an independent outcome measure in this study, it gives further evidence to support the presence of an impaired glycolytic system (Kazemi-Esfarjani et al., 2002). Reported McArdle symptoms were not considered an outcome measure either; however, the presence of typical muscle contractures even with treatment administration also illustrated the lack of VPA efficacy. Median CK levels were abnormal in all study visits, as expected in symptomatic McArdle disease. HR analysis of the first few minutes of cycling under a constant workload confirmed that *second wind* phenomenon, the hallmark of McArdle disease, occurred in all study visits.

The change seen in the primary outcome of this study, the improvement in participants'  $VO_2$ peak, was considered clinically relevant. Improvements greater than 10% were pre-defined as clinically important (as further detailed in 'Statistical Analysis', Methods section). The  $VO_2$ peak increase from baseline to V2 did not meet this threshold, but the mean increase from baseline to V3 was ~13% in the British sample. It is noted, however, that the mean change over time had relatively wide confidence intervals, which is likely to be due to the small sample size of this study. When analysing data from both the Danish and British centres, a mean decrease of 2% from baseline to V3 was seen in the primary outcome ( $VO_2$ peak), confirming this to be a negative trial. In other words, when assessed as an international multi-centre study, this is a negative trial: it provides strong evidence VPA is ineffective for McArdle disease, precluding further studies of this treatment in the condition.

However, when assessed as a single-centre trial, the primary outcome improved in the British participants.

Possible explanations for this finding relate to the external factors influencing the  $VO_2$ peak and not to treatment efficacy. From a cultural perspective, people in Denmark are more used to cycling on a daily basis. Also, the cycle test is commonly used in Denmark by Professor Vissing's team in a population of subjects affected by McArdle disease who have also participated in previous studies. Consequently, the negative finding in Denmark more reliably illustrates the lack of efficacy of VPA in this patient population, as there was no learning component. In the UK, a learning component and/or regular exercise most probably contributed to the  $VO_2$ peak improvement. The British participants had not previously been exposed to the cycle test, and most participants were initially reluctant to cycle because of the potential risk of muscle damage. It is possible that participants felt more comfortable in performing the cycle test during V3 than V1.

Since physical activity is currently the best management approach for McArdle disease, people with this condition are encouraged to exercise when they attend routine clinic appointments and by the peer support provided by the Association for Glycogen Storage Disease UK ([agsd.org.uk](http://agsd.org.uk)). Participants may have felt more confident to exercise while on treatment and increased their levels of physical activity during the trial period, which possibly caused the increase in their  $VO_2$ peak. When analysing the constant workload phase of the cycle test, the *second wind* seemed to alleviate at V3 as compared to the previous visits. The HR at minute 15 was reduced at V3, suggesting better conditioning. A limitation of this study is the fact that physical activity was not assessed using a validated questionnaire. For this reason, it is impossible to confirm to which extent regular exercise contributed to the  $VO_2$ peak improvement.

The improvement in the trial primary outcome measure (positive trial) in the absence of a real pharmaceutical effect may be a result of the study limitation related to the open-label design: the absence of a placebo arm. The use of a placebo arm could have contributed in terms of primary outcome data analysis and interpretation.

It is very difficult to confirm the extent to which the protocol deviation related to participants who failed to fast (three out of 24 study visits) affected the primary outcome analysis as the sample size is very small.

In summary, this study provides sufficient evidence to show that VPA is not effective in inducing brain and/or liver isozyme expression in skeletal muscle in McArdle disease.

A possible explanation for the lack of efficacy compared with the animal studies may be the fact that sheep may be more sensitive to VPA. However, some limitations should be considered when analysing the animal study results. One limitation of the sheep study is the fact that it is not possible to confirm whether the positive phosphorylase fibres seen in the post-treatment biopsy were related to regenerating fibres, as neonatal myosin immunohistochemistry was not reported. Based on the information provided, it is not entirely possible to confirm a true effect of VPA, as the muscle biopsy findings could potentially be explained by muscle regeneration. In the *in vitro* study, the mice muscle cells were analysed out of a normal environment, where the gene expression profile may change. It is not possible to confirm whether an *in vivo* effect would also be seen in the McArdle mouse model as such study has never been performed/published.

Another possible hypothesis could be related to the VPA dose: potentially, humans may require a higher VPA dose to effectively stimulate gene-expression. However, increasing the dose in humans is likely to increase toxicity and may not achieve the expected effect.

An alternative explanation could be related to the muscle biopsy technique. In the sheep study, the positive muscle biopsy findings were patchy: the investigators performed multiple biopsies to confirm positive results. Also, not all fibres of similar muscle groups showed positive phosphorylase staining (Howell et al., 2015). As only one post-treatment muscle biopsy was assessed per patient, in an attempt to avoid performing unnecessary invasive and painful procedures, a possible explanation for the absent phosphorylase staining in this study could be related to a patchy effect in small biopsies in a small study sample. However, the totality of the results indicates that this was not the case.

Other clinical trials exploring the HDACI effect of VPA in humans with neuromuscular disorders following pre-clinical research have also failed to show its effectiveness, but a close comparison may not be possible as such conditions have different disease mechanisms. Future studies to develop an associated therapy containing an agent/molecule designed to potentiate the VPA HDACI effect may improve treatment efficacy in genetic disorders like McArdle disease. Other HDACI agents could have different effects, but further research is needed to address this.

## Outcome Measures

### *The Cycle Test*

The cycle test did not cause muscle damage: no participant experienced rhabdomyolysis or any episode of myoglobinuria during this trial. This cycle test was safe in the studied sample: it followed a four-hour fasting period and included a 15-minute warm-up, when participants cycled according to a personalised protocol with workload corresponding to 65% of the workload at each participant's  $VO_2$ peak.

The cycle test succeeded in documenting an improvement in  $VO_2$ peak. Two factors could have contributed to the  $VO_2$ peak increase: a learning component and/or physical training. Because data presented here suggest the *second wind* progressively alleviated from V1 to V3, and the HR at minute 15 progressively decreased from V1 to V3, it is more likely that the trial participants performed more physical activity and exercised more often during the trial, thus improving patients' exercise capacity. Regular exercise training improves muscle blood flow, oxygen utilisation, mitochondrial respiration, pulmonary function, muscle power, cardiac output and more (Gremeaux et al., 2012). More specific to McArdle disease, a mouse model study showed moderate-intensity endurance exercise training provoked muscle tissue adaptations related to muscle plasticity (Fiuza-Luces et al., 2018), which may explain the fact that, compared to sedentary patients, patients who are physically active experience less-severe symptoms (Lucia et al., 2012). Previous research in McArdle disease has shown that exercise training increases  $VO_2$ peak and mitochondrial enzyme activity and reduces serum CK levels (Kitaoka, 2014).

When using the cycle test as an outcome measure for drug development studies, researchers should consider planning a placebo arm and ensuring trial subjects are fasted prior to assessment. Planning a meaningful placebo arm in small studies for rare diseases such as McArdle disease may be difficult though. Including a physical-activity diary and questionnaires may contribute to study data interpretation.

### *The 12-minute Walk Test*

This is the first time the 12MWT has been used as an outcome measure in a clinical trial in McArdle disease registered in ClinicalTrials.gov. All participants were familiar with this test as it is routinely

performed as part of the British clinic assessments, and for this reason no learning component was expected in the studied sample.

The results suggested a moderate correlation (around 0.4) between change in  $VO_2$ peak and change in distance walked. This suggests those who increase  $VO_2$ peak also tend to increase distance walked. However, the results did not reach statistical significance. Even though the participants'  $VO_2$ peak increased significantly, the total distance walked did not increase greatly.

This trial suggests that  $VO_2$ peak improvement, assessed by the cycle test, did not dramatically improve the total distance walked, as assessed by the 12MWT, in people with McArdle disease. This is an interesting finding as it suggests that either regular exercise may not significantly influence the total walked distance assessed by the 12MWT in people with genetically confirmed McArdle disease with clear *second wind* or that a ceiling effect played a part. Lack of significance could also be due to lack of power due to small numbers. Further studies should investigate the extent to which exercise training influences the total distance walked during the 12MWT in this patient population.

The 12MWT is a useful screening tool to assess the *second wind* phenomenon. During the 12MWT patients reduce their walking speed to manage symptoms whilst trying to achieve the *second wind* (Buckley et al., 2014, Scalco et al., 2014a). The total distance walked may increase if treatment restores the glycolytic system, permitting patients to walk faster during the early minutes of the test. As a negative trial, it was not possible to perform such analysis. Further studies are needed to assess whether the total distance walked assessed by the 12MWT correlates with drug treatment effects. The 12MWT may be a useful tool to assess people with contraindications for the cycle test, such as fixed muscle weakness in lower limbs. Further studies are needed to assess the limitations of performing this test in people with McArdle disease and concomitant severe cardiovascular disease. Currently, ongoing research is being performed to compare the 12MWT performed in a corridor with the 12MWT on a treadmill (Chatfield et al., 2018). Results of this project should address the cons of this test related to room space. Pros and cons of the 12MWT are represented in Table 29.

Table 29. Pros and Cons of the 12-minute Walk Test for McArdle Disease

Pros	Cons
<ul style="list-style-type: none"> <li>• Self-paced test – accommodates participants' limitations</li> <li>• Low risk of harm to participants</li> <li>• Cheap – minimal equipment required</li> <li>• Easy to administer</li> <li>• Easy to replicate</li> <li>• Mimics real-life activity</li> </ul>	<ul style="list-style-type: none"> <li>• Does not assess VO<sub>2</sub>peak</li> <li>• Not a patient-reported outcome measure</li> <li>• Its value to assess drug efficacy is still unknown</li> <li>• Requires sufficient space</li> <li>• Total walked distance may vary according to a number of factors (e.g.: patients' motivation)</li> <li>• ? Ceiling effect</li> </ul>

### *Plasma Lactate*

The non-ischaemic forearm exercise test reliably confirmed the lack of lactate rise in McArdle disease. It was easily reproducible and relatively safe. No major complications were associated with this test. In one visit, one participant did not perform the test due to symptomatic nerve entrapment. The absence of such data did not influence the trial results, as lactate data for this participant was collected during the cycle test.

Plasma lactate did not rise during the cycle test or at exhaustion. It reliably demonstrated the effect of the glycolytic block in patients when exercise was performed after a period of fast. VO<sub>2</sub>peak variation did not influence lactate rise in McArdle disease, thus this assessment complemented other measures of functional outcomes. Lactate rise may be considered a primary outcome measure in future clinical trial in McArdle disease assessing new molecules that may activate the impaired glycolytic system. An external factor that may influence lactate rise is food and fluid intake, as previously described, which could lead to overestimate of treatment benefits. For this reason, fasting prior to assessment should be considered in future drug development research when this outcome is used. In this trial, three participants forgot to fast prior to three trial visits. There was no clear procedure to encourage fasting. Such procedures should be considered in future studies, such as including a phone call the day before each study visit to remind all participants about fasting. Exercise intensity may also influence the rise in lactate, highlighting the importance of following standardised protocols.

Participants' handgrip strength increased during this trial. It is not possible to confirm whether the increased strength was due to a learning effect, or even to a placebo effect. During V1, the assessed participants were concerned about developing muscle contractures during the forearm exercise test. They may have felt more confident in applying stronger handgrips while on treatment (placebo effect) or after having noted during previous study visits that the forearm exercise test did not cause muscle contractures.

#### *The SF-36 Questionnaire*

No clinically relevant change was seen in the health domain scales of the SF-36 questionnaire. However, it is very difficult to gauge the real impact of the small changes seen in the studied sample, as a few factors may have affected it, including VPA-related AEs, the underlying symptomatic disease and change in  $VO_2$ peak. A total of 123 AEs were reported during the trial; only 65% of them were definitely not related to VPA treatment. Physical component scores, which were lower than the general population scores, may have illustrated underlying McArdle disease, symptoms of which were unchanged by VPA treatment, further supporting the lack of efficacy. Thus, VPA AEs and an underlying untreated metabolic disease may have negatively affected the participants' quality of life, counterbalancing the benefits of physical conditioning.

#### Post-hoc Analysis: $VO_2$ peak X SF-36 Scores

An increase in aerobic fitness was related to an improvement in quality of life in people with McArdle disease (Munguia-Izquierdo et al., 2015). When correlating  $VO_2$ peak and SF-36 scores, just one health domain score reached statistical significance, the general health measure of the SF-36. The positive correlation suggested that a greater change in  $VO_2$ peak was associated with a greater change in this measure. Despite not reaching statistical significance, there was also a reasonably large positive correlation between changes in  $VO_2$ peak and change in the SF-36 physical component. Contrary to the previous results, there was a reasonably large negative correlation between change in  $VO_2$ peak and change in the SF-36 mental component and mental health values. The results did not reach statistical significance, but the correlations were relatively large (approximately -0.6). The negative correlations suggested that a greater change in  $VO_2$ peak was associated with a lesser change in these parameters.

## Conclusions

As a proof-of-concept study, this clinical study was set up to be a small and brief trial to give insights for future multi-centre clinical trials.

Based on this study, 20 mg/kg/day VPA was not shown to induce brain and/or liver glycogen phosphorylase activity in people with McArdle disease. VPA was shown to be safe in the reported sample. Limitations for the safety data analysis were the small sample size and the descriptive analysis, as this study was not powered for such an outcome. As this study has shown VPA to be an ineffective drug for treating McArdle disease, further research is discouraged. Also, it is reasonable to say that McArdle disease should not be considered a formal contraindication for VPA use in patients with a strong clinical indication for its prescription and when known contraindications for VPA use are excluded. Therefore, the results of this project are of benefit to people with McArdle disease who suffer from other conditions such as epilepsy.

In this study, VPA use did not limit participants'  $\text{VO}_2\text{peak}$ , as it increased by more than 10% after six months' treatment. This finding suggests that VPA does not negatively interact with the mitochondrial metabolism of people with McArdle disease. Further studies to properly investigate VPA toxic effects in mitochondrial respiration in this patient population were not performed, as this was not the aim of this translational research project.

Performing a cycle test following four hours' fasting was safe in McArdle disease when a personalised protocol was used. The incremental phase of the cycle test was performed after a 15-minute warm-up. The cycle test data ( $\text{VO}_2\text{peak}$ , HR variation and maximum workload) were most probably influenced by physical training and/or learning effect. A placebo-controlled study should have addressed this limitation, and should be considered when planning future trials using the  $\text{VO}_2\text{peak}$  changes as a primary outcome measure. Including a physical-activity diary and/or questionnaires assessing physical activity levels may also contribute to data interpretation. Plasma lactate levels may confirm efficacy when treatment benefits are expected to activate the impaired glycolytic system.

The 12MWT was well tolerated. The role of the 12MWT as an outcome measure in clinical trials is promising, but further studies are required to assess whether the 12MWT can reliably confirm treatment efficacy. Ceiling effect and the test relation with cardiorespiratory fitness and conditioning should be investigated in larger cohorts of people with McArdle disease.

Muscle biopsy was a reliable outcome measure. However, its use may affect trial recruitment.

### **Next Steps**

Further studies must determine whether other HDACI agents may increase phosphorylase activity in skeletal muscle of people with McArdle disease and thus improve outcomes of direct relevance to patients.

Following this clinical trial, next-step research could be a clinical project designed to assess the 12MWT in a larger cohort of people with McArdle disease. Correlating the heart rate change, walking speed and total walking distance to the  $VO_2$ peak may provide further evidence on which variant better correlates with patients'  $VO_2$ peak. Such study may also provide further data on the test limitations, such as the effects of height and body mass index on the total distance walked. Comparing data from healthy controls with a larger cohort of McArdle disease patients may give valuable information on this assessment tool, in particular the likely level of a ceiling effect.

The most important clinical research required in this field is the development of a patient-reported outcome measure. Unfortunately, there is no outcome measure that reliably assesses the wellbeing and quality of life of people with McArdle disease. This is actually a major challenge in terms of proving the real impact of new medicines to be used in this condition. Not addressing this major limitation at an early stage may be a great challenge in future applications for drug approvals to the regulatory bodies. Great efforts should be made by international scientific centres to address this problem. The involvement of patients and patients' associations may give valuable insights on the development of McArdle disease scales and/or questionnaires, and should be seriously considered.

## **16. Conclusions**

Currently there are few pharmacological treatments for rare neuromuscular diseases. This research attempted to address this limitation for McArdle disease and HypoPP. This thesis also explores the challenges related to T1 translation in rare disorders.

### **Improving T1 Translation**

#### **Promoting T1 Research at Academic Institutions**

Developing two investigator-initiated studies at an academic institution during a PhD programme has proven to be successful. As the funding for this research and the PhD duration were limited, drug repurposing studies using generic drugs were chosen in an attempt to identify treatment options for two myopathies within a feasible timeframe. The safety profiles of both investigational medicinal products were known, bypassing the need to develop early stage studies. The well-established summary of product characteristics also contributed to the development of specific trial procedures to reduce the occurrence of expected adverse events, ensuring trial safety.

This research was developed at a centre that provides specialised services for rare neuromuscular disorders. The British NHS model concentrates clinical services for very rare diseases into a few highly specialised centres named Highly Specialist Services (HSS) (NHS\_England, 2017). Muscle channel disorders and glycogen storage diseases are two examples of conditions supported by the HSS. One of the main commitments of the HSS is to promote research into rare conditions. The HSS for HypoPP and McArdle disease have enabled the translational research reported here.

This research confirmed the positive role an academic institution and a centre of expertise can play in promoting drug discovery in the field of rare myopathies. Involving a PhD student in the whole process of phase II clinical trial development, from very early stages of protocol writing to the final stages of data interpretation, helps overcome the ongoing issue of rarity of trained physicians with expertise in developing clinical trials in the field of rare diseases (Augustine et al., 2013, Sung et al., 2003).

## Translating Findings from Animal Models to Humans

In both clinical trials, therapeutic data evidenced in animal models were not translatable to humans with McArdle disease and HypoPP. The results of both clinical trials should support basic science research to further understand the contradictory findings.

Not infrequently, therapeutic research has failed to demonstrate similar efficacy data in animal models and humans. Several authors have suggested different strategies to improve T1 translation, including better regulation of pre-clinical research. Analysing pre-clinical research data was outside the scope of this thesis. However, the data presented here may provide insights for future research addressing the clinical value of the reported animal models.

### *Bumetanide Trial*

There has been just one *in vivo* study of bumetanide in the R528H<sup>m/m</sup> HypoPP mouse (*CACNA1S*), which tested a 0.08 mg/kg dose. A suggested pre-clinical study following the phase II clinical trial reported in this thesis would be a project developed to analyse different bumetanide doses in the HypoPP mouse model, including a dose equivalent to 2 mg bumetanide for a 70 kg person. This next *in vivo* study should improve T1 translation by analysing early evidences of a possible therapeutic range of bumetanide in HypoPP. The results of this study may confirm that 2 mg is an insufficient dose for HypoPP symptom improvement, also confirming a possible limitation of the bumetanide phase II clinical trial. An *in vivo* pre-clinical study assessing an abortive treatment strategy of bumetanide should also be performed. Such a study could also analyse the benefits of different doses.

### *Sodium Valproate Trial*

It is unclear why two animal models failed to predict treatment benefits in humans with McArdle disease. Reassessing both pre-clinical studies may provide further understanding of the advantages and limitations of each animal model, which will benefit future drug development research.

Performing an *in vivo* study of VPA in the mouse model of McArdle disease may help evaluate the value of this animal model for drug development: if such a study shows a negative effect, similar to the findings of the project reported in this thesis, it will illustrate that an *in vivo* study performed in the mouse model may be of more clinical relevance than an *in vitro* study in the mouse model or an *in vivo* study in the sheep model.

## Improving TR in Exercise-related Muscle Disorders

### Development of International Registries and Networks for International Trials

Recruiting participants for clinical trials in very rare diseases has proven to be difficult. The recruitment target was not met in the bumetanide clinical trial even though great efforts were made to address this expected challenge. It was initially expected that recruitment for the VPA project would be even more difficult as it involved prolonged use of the investigational medicinal product, more study visits (more time off work), exclusion of women of child-bearing potential and two unpleasant muscle biopsies. Surprisingly, this was not the case.

One of the main factors influencing recruitment rate was the recruitment strategy itself. The bumetanide project was developed locally by the study team, and was advertised at a specialised clinic setting, at the specialised clinic patients' meetings and online via the Muscular Dystrophy UK charity organisation. Setting up the VPA clinical trial was more complex, from initial development to final advertisement, as it involved an international registry and a patients' association for glycogen storage disorders.

#### *EUROMAC Registry*

The EUROMAC Registry for McArdle disease and related disorders was created to collect clinical data from affected people within the European Union. The registry was developed to identify the geographic distribution of patients, increase knowledge of the natural history of the investigated diseases and organise education and training activities across the European Union (Euromac, 2012, Euromac, 2016b, Euromac, 2016a, Euromac, 2016c, Quinlivan et al., 2015, Scalco et al., 2014c). The project's crucial aims are to improve diagnostic rates in Europe and to raise the awareness of McArdle disease and related disorders. The registry has also promoted research by developing and maintaining a database to be used as a platform for future multi-centre clinical trials in Europe (<http://euromacregistry.eu>).

The VPA clinical trial protocol and the selected outcome measures were reviewed and approved by two experts in the field of McArdle disease who were part of the steering committee of the EUROMAC Registry. The same research protocol was run in Denmark, contributing to data analysis. The VPA clinical trial protocol and preliminary results were also discussed at EUROMAC meetings attended by

international experts in the field, further adding to the trial safety analysis and results dissemination (Quinlivan et al., 2017). A workshop on outcome measures for McArdle disease was organised by the EUROMAC Registry to standardise data collection in clinical trials (Quinlivan et al., 2015). The researchers involved in the VPA clinical trial were trained during this workshop, which took place prior to participants' recruitment.

#### *Association for Glycogen Storage Disease UK (AGSD-UK)*

The AGSD-UK, the national McArdle disease association, is a very active organisation that has been instrumental in shaping and helping with early funding of the specialised clinic for McArdle disease based in London (agsd.org.uk). This level of activity and partnership with NHS organisations is unique among similar associations across the globe (Scalco et al., 2016a, Quinlivan, 2012). Representatives from the AGSD-UK continue to collaborate closely with NHS healthcare workers to organise group interventions for patients that benefit newly diagnosed patients and those well known to the service. The association also promotes social activities, which is a valuable opportunity for patients to meet other people with McArdle disease, sharing experiences and worries, and creating a valuable social network. The organisation also provides printed materials and is very active in social media, reaching people with McArdle disease all over the world (Wakelin, 2013).

The AGSD-UK assistance with the VPA trial helped to reach more potential participants, including people with McArdle disease who were not regular patients at the specialised clinic in London. The trial was presented several times at the association's annual meetings, where interested people had the opportunity to ask questions about the project in a friendly and informal environment. The VPA trial coordinator's contact details (me) were shared by the AGSD-UK, possibly contributing to the trial's successful recruitment rates. The AGSD-UK facilitated the communication between patients and the research team, bridging the gap between technical science and the general public.

The positive lessons learnt from the successful experience of the VPA trial project emphasised the importance of international registries and peer support in rare diseases. Such benefits are not restricted to symptom management, they encourage clinical research development. Having patient representatives join international experts in the field to discuss outcome measures and clinical trial projects is extremely important to ensure quality and promote multi-centre international research. The active support of the national patients' association illustrated the importance of local social interaction

to raise clinical trial awareness, as word-of-mouth was very effective. Against this background, the EUROMAC Registry is the best way to combine scientific partnership and patient networking, as it interacts directly with patients' associations and supports their initiatives. The real impact of the EUROMAC Registry will be seen in future multi-centre international trials that target larger cohorts of participants.

Further efforts are needed to improve the situation for HypoPP. Organising a European registry for HypoPP with values similar to those of the EUROMAC Registry may help improve diagnostic rates and patient care in European countries. Based on the experience gained here, it is clear that a very active patients' association may motivate affected people to share their experiences and participate in scientific research.

### **Study Design and Outcome Measures Selection**

As proof-of-concept studies, both trials contributed to addressing the proposed research questions. One study used a more complex scientific methodology to assess drug efficacy, a randomised controlled trial, while the other was developed as an open-label pilot study.

This was the first time a controlled trial has been developed to assess treatment efficacy in aborting acute attacks of weakness in HypoPP ([clinicaltrials.gov](http://clinicaltrials.gov)). Several theoretical uncertainties had to be considered at the very early stages of protocol development. The cross-over design was of great value as it expanded data collection in a small sample while reducing bias related to inter-individual variability, thereby contributing to data interpretation. The use of randomisation, a placebo arm and blinded treatments warranted class I evidence, reducing assessor and participant biases. Outcome measures assessing acute disease activity are limited in HypoPP. The need to develop a new outcome measure was particularly challenging, but has proven to be achievable. Data collected in the placebo group may be used to further understand the expected variation in this patient population, which will contribute to sample size calculation for future studies.

The availability of different diagnostic tools and functional tests facilitated the development of a clinical trial in McArdle disease, particularly because different outcome measures were reviewed recently at an international training workshop (Quinlivan and Vissing, 2007, Quinlivan et al., 2015, Quinlivan et al., 2014a). The combination of several outcome measures in the VPA study was extremely important as the project successfully confirmed a lack of efficacy of VPA treatment in McArdle disease – despite

significant improvement in participants' VO<sub>2</sub>peak, the study's primary outcome measure. The totality of the trial results counterbalanced the in-existent placebo arm. Understanding the limitations of different outcome measures was of great value, as the combination of several assessments enriched data interpretation.

Open-label trials are subjected to bias. If VPA treatment had proven to stimulate the expression of the brain and/or liver glycogen phosphorylase enzyme, a multi-centre randomised controlled trial would have been required to investigate the therapeutic benefits of VPA.

Another interesting observation was that the positive trial (VPA) was associated with an ineffective treatment, while the negative trial (bumetanide) was associated with a possibly effective treatment. Focusing on labelling trials as 'positive' or 'negative' based on the primary outcome p-value may overgeneralise the research findings (Pocock and Stone, 2016). The negative trial results did not completely exclude bumetanide efficacy. Performing a randomised controlled trial in a small sample may preclude the detection of small therapeutic effects, which is particularly relevant in the field of HypoPP (Augustine et al., 2013). In this project, other factors may also have negatively contributed to the trial results. The experience gained in this original research will definitely contribute to future studies to be performed in the field of periodic paralysis, as all factors that may have influenced the trial results will be considered when developing new study protocols. It emphasises the multi-directional cycle of the translational research structure, as new knowledge functions as feedback to previous research while inspiring future developments.

Both projects highlighted the need to develop patient-reported outcome measures. Active interaction between clinicians, scientists and patients may be invaluable in the development of these.

## **Final Considerations**

Translational research in rare diseases is a continuous process. Pilot studies are valuable as a starting point, but should be followed by multi-centre trials involving larger cohorts of patients. Nevertheless, additional safety data may only be collected after the drug is approved for commercialisation, illustrating the continuous process of translational research (Suvarna, 2010).

Networking and partnerships may contribute to protocol development and study recruitment (Conwit et al., 2011). It also contributes to pre-clinical research development, which facilitates future T1

translation (Conwit et al., 2011). For this reason, strategies to promote communication between pre-clinical research scientists and clinical trial scientists should be implemented.

The research presented in this thesis shows the importance of proof-of-concept studies in the development of treatments for rare conditions. Small pilot studies may provide valuable knowledge to the field, which can be used in future to further develop multi-centre international trials involving larger cohorts of patients. Academic institutions, when associated with centres of expertise, have the medical experience, scientific personnel and the clinical setting required to investigate very rare conditions such as exercise-related muscle disorders.

The combination of highly specialised clinical services, an academic institution, an international registry and a patients' association generated a valuable partnership promoting clinical research, which was one of the most important insights gained in this research. Such partnerships may be the foundations to overcome the lack of treatment in the field of very rare diseases.

## 17. References

- ABDALLAH, C. 2014. Considerations in perioperative assessment of valproic acid coagulopathy. *J Anaesthesiol Clin Pharmacol*, 30, 7-9.
- ABU-BAKER, A., PARKER, A., RAMALINGAM, S., LAGANIERE, J., BRAIS, B., NERI, C., DION, P. & ROULEAU, G. 2018. Valproic acid is protective in cellular and worm models of oculopharyngeal muscular dystrophy. *Neurology*.
- AGSD-UK. 1986. *Association for Glycogen Storage Disease (UK)* [Online]. UK. Available: <http://www.agsd.org.uk> [Accessed 20/06/2016 2016].
- AIRES, C. C., SOVERAL, G., LUIS, P. B., TEN BRINK, H. J., DE ALMEIDA, I. T., DURAN, M., WANDERS, R. J. & SILVA, M. F. 2008. Pyruvate uptake is inhibited by valproic acid and metabolites in mitochondrial membranes. *FEBS Lett*, 582, 3359-66.
- ALKAABI, J. M., MUSHTAQ, A., AL-MASKARI, F. N., MOUSSA, N. A. & GARIBALLA, S. 2010. Hypokalemic periodic paralysis: a case series, review of the literature and update of management. *Eur J Emerg Med*, 17, 45-7.
- ALMEIDA, O. D., JR. & SPINNATO, J. A. 1989. Maternal Bartter's syndrome and pregnancy. *Am J Obstet Gynecol*, 160, 1225-6.
- ANDERSEN, S. T., JEPPESEN, T. D., TAIVASSALO, T., SVEEN, M. L., HEINICKE, K., HALLER, R. G. & VISSING, J. 2009. Effect of changes in fat availability on exercise capacity in McArdle disease. *Arch Neurol*, 66, 762-6.
- ANDREU, A. L., NOGALES-GADEA, G., CASSANDRINI, D., ARENAS, J. & BRUNO, C. 2007. McArdle disease: molecular genetic update. *Acta Myol*, 26, 53-7.
- ASBURY, M. J., GATENBY, P. B., O'SULLIVAN, S. & BOURKE, E. 1972. Bumetanide: potent new "loop" diuretic. *Br Med J*, 1, 211-3.
- ASHBURN, T. T. & THOR, K. B. 2004. Drug repositioning: identifying and developing new uses for existing drugs. *Nat Rev Drug Discov*, 3, 673-83.
- ASK, K. & KOLB, M. R. 2015. Drug development for chronic lung disease--mission impossible? *Respirology*, 20, 13-4.
- ATTWOOD, M. M., RASK-ANDERSEN, M. & SCHIOTH, H. B. 2018. Orphan Drugs and Their Impact on Pharmaceutical Development. *Trends Pharmacol Sci*, 39, 525-535.
- AUGUSTINE, E. F., ADAMS, H. R. & MINK, J. W. 2013. Clinical trials in rare disease: challenges and opportunities. *J Child Neurol*, 28, 1142-50.
- BAKER, J. S., MCCORMICK, M. C. & ROBERGS, R. A. 2010. Interaction among Skeletal Muscle Metabolic Energy Systems during Intense Exercise. *J Nutr Metab*, 2010, 905612.
- BARTRAM, C., EDWARDS, R. H. & BEYNON, R. J. 1995. McArdle's disease-muscle glycogen phosphorylase deficiency. *Biochim Biophys Acta*, 1272, 1-13.
- BARTRAM, C., EDWARDS, R. H., CLAGUE, J. & BEYNON, R. J. 1993. McArdle's disease: a nonsense mutation in exon 1 of the muscle glycogen phosphorylase gene explains some but not all cases. *Hum Mol Genet*, 2, 1291-3.
- BERG, J., TYMOCZKO, J. & STRYER, L. 2002. *Each Organ Has a Unique Metabolic Profile* [Online]. Biochemistry. Available: <https://www.ncbi.nlm.nih.gov/books/NBK22436/> [Accessed 08/07/2018 2018].
- BORG, G. 1970. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med*, 2, 92-8.
- BRAAKHEKKE, J. P., DE BRUIN, M. I., STEGEMAN, D. F., WEVERS, R. A., BINKHORST, R. A. & JOOSTEN, E. M. 1986. The second wind phenomenon in McArdle's disease. *Brain*, 109 ( Pt 6), 1087-101.
- BRADY, S., GODFREY, R., SCALCO, R. S. & QUINLIVAN, R. M. 2014a. Emotionally-intense situations can result in rhabdomyolysis in McArdle disease. *BMJ case reports*, 2014.
- BRADY, S., MELATH, S., SCALCO, R. S. & PENN, H. 2014b. Fatal cardiac involvement complicating antisynthetase syndrome. *BMJ Case Rep*, 2014.
- BRODIE, S. A. & BRANDES, J. C. 2014. Could valproic acid be an effective anticancer agent? The evidence so far. *Expert Rev Anticancer Ther*, 14, 1097-100.
- BRUNO, C., CASSANDRINI, D., MARTINUZZI, A., TOSCANO, A., MOGGIO, M., MORANDI, L., SERVIDEI, S., MONGINI, T., ANGELINI, C., MUSUMECI, O., COMI, G. P., LAMPERTI, C., FILOSTO, M., ZARA, F. & MINETTI, C. 2006. McArdle disease: the mutation spectrum of PYGM in a large Italian cohort. *Hum Mutat*, 27, 718.

- BUCKLEY, J. P., QUINLIVAN, R. M., SIM, J., ESTON, R. G. & SHORT, D. S. 2014. Heart rate and perceived muscle pain responses to a functional walking test in McArdle disease. *J Sports Sci*, 32, 1561-9.
- BURGE, J. A. & HANNA, M. G. 2012. Novel insights into the pathomechanisms of skeletal muscle channelopathies. *Curr Neurol Neurosci Rep*, 12, 62-9.
- BURKE, L. M., ROSS, M. L., GARVICAN-LEWIS, L. A., WELVAERT, M., HEIKURA, I. A., FORBES, S. G., MIRTSCHIN, J. G., CATO, L. E., STROBEL, N., SHARMA, A. P. & HAWLEY, J. A. 2017. Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *J Physiol*, 595, 2785-2807.
- CARR, A. S., PELAYO-NEGRO, A. L., JAUNMUKTANE, Z., SCALCO, R. S., HUTT, D., EVANS, M. R., HEALLY, E., BRANDNER, S., HOLTON, J., BLAKE, J., WHELAN, C. J., WECHALEKAR, A. D., GILLMORE, J. D., HAWKINS, P. N. & REILLY, M. M. 2015. Transthyretin V122I amyloidosis with clinical and histological evidence of amyloid neuropathy and myopathy. *Neuromuscul Disord*, 25, 511-5.
- CASTAÑEDA, M. S., ZANOTELI, E., SCALCO, R. S., SCARAMUZZI, V., CALDAS, V. M., REED, U. C., SILVA, A. M. S. D., O'CALLAGHAN, B., PHADKE, R., BUGIARDINI, E., SUD, R., MCCALL, S., HANNA, M. G., POULSEN, H., MÄNNIKKÖ, R. & MATTHEWS, E. 2018. A novel ATP1A2 mutation in a patient with hypokalaemic periodic paralysis and CNS symptoms. *Brain*.
- CAVEL-GREANT, D., LEHMANN-HORN, F. & JURKAT-ROTT, K. 2012. The impact of permanent muscle weakness on quality of life in periodic paralysis: a survey of 66 patients. *Acta Myol*, 31, 126-33.
- CHATFIELD, S., BOOTH, S., GODFREY, R., PATTNI, J., SCALCO, R. & QUINLIVAN, R. 2018. Evaluating the 12-minute walk test in McArdle disease. *Neuromuscular Disorders*, 28, S38.
- CHATZIZISIS, Y. S., MISIRLI, G., HATZITOLIOS, A. I. & GIANNOGLOU, G. D. 2008. The syndrome of rhabdomyolysis: complications and treatment. *Eur J Intern Med*, 19, 568-74.
- CIPRIANI, A., REID, K., YOUNG, A. H., MACCRITCHIE, K. & GEDDES, J. 2013. Valproic acid, valproate and divalproex in the maintenance treatment of bipolar disorder. *Cochrane Database Syst Rev*, Cd003196.
- CLABORN, M. K., STEVENS, D. L., WALKER, C. K. & GILDON, B. L. 2018. Nusinersen: A Treatment for Spinal Muscular Atrophy. *Ann Pharmacother*, 1060028018789956.
- CLINICALTRIALS.GOV. 2000. *ClinicalTrials* [Online]. NIH and the Food and Drug Administration (FDA) Available: <https://www.clinicaltrials.gov/> [Accessed 02/07/2018 2018].
- CONWIT, R. A., BHANUSHALI, M. J., PORTER, J. D., KAUFMANN, P. & GUTMANN, L. 2011. Adding more muscle and nerve to clinical trials. *Muscle Nerve*, 44, 695-702.
- COSTA, R., CASTRO, R., COSTA, A., TAIPA, R., VIZCAÍNO, R. & MORGADO, T. 2013. McArdle disease presenting with rhabdomyolysis and acute kidney injury. *Acta Medica Portuguesa*, 26.
- CRERAR, M. M., KARLSSON, O., FLETTERICK, R. J. & HWANG, P. K. 1995. Chimeric muscle and brain glycogen phosphorylases define protein domains governing isozyme-specific responses to allosteric activation. *J Biol Chem*, 270, 13748-56.
- CUMMINGS, J. L., MORSTORF, T. & ZHONG, K. 2014. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimers Res Ther*, 6, 37.
- DE LUNA, N., BRULL, A., GUIU, J. M., LUCIA, A., MARTIN, M. A., ARENAS, J., MARTI, R., ANDREU, A. L. & PINOS, T. 2015. Sodium valproate increases the brain isoform of glycogen phosphorylase: looking for a compensation mechanism in McArdle disease using a mouse primary skeletal-muscle culture in vitro. *Dis Model Mech*, 8, 467-72.
- DESIKAN, M., SCALCO, R. S., MANOLE, A., GARDINER, A. R., SCHAPIRA, A. H., LACHMANN, R. H., HOULDEN, H., HOLTON, J. L., PHADKE, R. & QUINLIVAN, R. 2018. GYG1 causing progressive limb girdle myopathy with onset during teenage years (polyglucosan body myopathy 2). *Neuromuscul Disord*, 28, 346-349.
- DIMAURO, S., ARNOLD, S., MIRANDA, A. & ROWLAND, L. P. 1977. McArdle disease: the mysterious appearance of phosphorylase activity in cells that ought to lack the genetic program. A fetal isoenzyme? *Trans Am Neurol Assoc*, 102, 112-5.
- DRAPER, N. & MARSHALL, H. 2013. General concepts for applied exercise physiology. In: DRAPER, N. & MARSHALL, H. (eds.) *Exercise Physiology: For Health and Sports Performance*. Barcelona, Spain: Routledge.
- DUBOWITZ, V., SEWRY, C. & OLDFOR, A. 2013. *Muscle Biopsy: A Practical Approach*, United Kingdom, Saunders, Elsevier.

- DUENAS-GONZALEZ, A., CORONEL, J., CETINA, L., GONZALEZ-FIERRO, A., CHAVEZ-BLANCO, A. & TAJA-CHAYEB, L. 2014. Hydralazine-valproate: a repositioned drug combination for the epigenetic therapy of cancer. *Expert Opin Drug Metab Toxicol*, 10, 1433-44.
- EPILEPSYSOCIETY. 2018. *Concerns around sodium valproate* [Online]. Epilepsy Society. Available: <https://www.epilepsysociety.org.uk/concerns-around-sodium-valproate-0#.W1rX0clna71> [Accessed 27/07/2018 2018].
- EUROMAC. 2012. *EUROMAC registry of patients with McArdle disease and other rare glycogenolytic disorders with exercise intolerance* [Online]. Available: [www.euromacregistry.eu](http://www.euromacregistry.eu) [Accessed 06/05/2018 2018].
- EUROMAC. 2016a. *Euromac Symposium* [Online]. Available: <http://www.euromacregistry.eu/portal1/content.asp?contentid=1217> [Accessed 06/05/2018 2018].
- EUROMAC. 2016b. *EUROMAC TEACHING COURSE: DIAGNOSIS AND MANAGEMENT OF MCARDLE DISEASE AND RARE MUSCLE GLYCOGENOSES* [Online]. Available: <http://www.euromacregistry.eu/portal1/content.asp?contentid=1221&c=EUROMAC%20TEACHING%20COURSE> [Accessed 05/05/2018 2018].
- EUROMAC. 2016c. *McArdle Study Day* [Online]. Available: <http://www.euromacregistry.eu/portal1/content.asp?contentid=1219&c=McArdle%20Study%20Day> [Accessed 06/05/2018 2018].
- EUROPEANMEDICINESAGENCY. 2017. *Orphan Incentives* [Online]. EMA: EMA. Available: [http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general\\_content\\_000393.jsp&mid=WC0b01ac058061f017](http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000393.jsp&mid=WC0b01ac058061f017) [Accessed 04/08/2018 2018].
- EUROPEANMEDICINESAGENCY 2018. Rare diseases, orphan medicines. EMA.
- EVANGELISTA, T., WOOD, L., FERNANDEZ-TORRON, R., WILLIAMS, M., SMITH, D., LUNT, P., HUDSON, J., NORWOOD, F., ORRELL, R., WILLIS, T., HILTON-JONES, D., RAFFERTY, K., GUGLIERI, M. & LOCHMULLER, H. 2016. Design, set-up and utility of the UK facioscapulohumeral muscular dystrophy patient registry. *J Neurol*, 263, 1401-8.
- FIALHO, D., GRIGGS, R. C. & MATTHEWS, E. 2018. Periodic paralysis. *Handb Clin Neurol*, 148, 505-520.
- FIKRI-BENBRAHIM, O., CAZALLA-CADENAS, F. & VADILLO-BEMEJO, A. 2013. From acute renal failure to the diagnosis of McArdle's disease. *Nefrologia*, 33, 605-6.
- FINSTERER, J. & STOLLBERGER, C. 2014. Recurrent aborted sudden cardiac death with seizures and rhabdomyolysis due to bulimia-induced hypokalemia: report of one case. *Rev Med Chil*, 142, 799-802.
- FIUZA-LUCES, C., SANTOS-LOZANO, A., LLAVERO, F., CAMPO, R., NOGALES-GADEA, G., DIEZ-BERMEJO, J., BALADRON, C., GONZALEZ-MURILLO, A., ARENAS, J., MARTIN, M. A., ANDREU, A. L., PINOS, T., GALVEZ, B. G., LOPEZ, J. A., VAZQUEZ, J., ZUGAZA, J. L. & LUCIA, A. 2018. Muscle molecular adaptations to endurance exercise training are conditioned by glycogen availability: a proteomics-based analysis in the McArdle mouse model. *J Physiol*, 596, 1035-1061.
- FLETCHER, J. 2008. Statistical question. Interpreting an underpowered trial. *Bmj*, 337, a2957.
- FORREST, C. B., BARTEK, R. J., RUBINSTEIN, Y. & GROFT, S. C. 2011. The case for a global rare-diseases registry. *Lancet*, 377, 1057-9.
- FOURNIER, E., ARZEL, M., STERNBERG, D., VICART, S., LAFORET, P., EYMARD, B., WILLER, J. C., TABTI, N. & FONTAINE, B. 2004. Electromyography guides toward subgroups of mutations in muscle channelopathies. *Ann Neurol*, 56, 650-61.
- GARCIA-BENITEZ, S., FLECK, S. J., NACLERIO, F., MARTIN, M. A. & LUCIA, A. 2013. Resistance (weight lifting) training in an adolescent with McArdle disease. *J Child Neurol*, 28, 805-8.
- GEUKES FOPPEN, R. J., VAN MIL, H. G. & VAN HEUKELOM, J. S. 2002. Effects of chloride transport on bistable behaviour of the membrane potential in mouse skeletal muscle. *J Physiol*, 542, 181-91.
- GHODKE-PURANIK, Y., THORN, C. F., LAMBA, J. K., LEEDER, J. S., SONG, W., BIRNBAUM, A. K., ALTMAN, R. B. & KLEIND, T. E. 2013. Valproic acid pathway: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics*, 23, 236-241.
- GLAUSER, T., BEN-MENACHEM, E., BOURGEOIS, B., CNAAN, A., GUERREIRO, C., KALVIAINEN, R., MATTSO, R., FRENCH, J. A., PERUCCA, E. & TOMSON, T. 2013. Updated ILAE evidence review of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. *Epilepsia*, 54, 551-63.
- GODFREY, R. & QUINLIVAN, R. 2016. Skeletal muscle disorders of glycogenolysis and glycolysis. *Nat Rev Neurol*, 12, 393-402.

- GOEBEL, H., SEWRY, C. & WELLER, R. 2013. *Muscle Disease: Pathology and Genetics*, London, Wiley-Blackwell.
- GOODWIN, M. L., HARRIS, J. E., HERNANDEZ, A. & GLADDEN, L. B. 2007. Blood lactate measurements and analysis during exercise: a guide for clinicians. *J Diabetes Sci Technol*, 1, 558-69.
- GOONERATNE, I. K., WIMALARATNA, M., RANAWEERA, A. K. P. & WIMALARATNA, S. 2017. Contraception advice for women with epilepsy. *Bmj*, 357, j2010.
- GOTLIB, D., RAMASWAMY, R., KURLANDER, J. E., DERIGGI, A. & RIBA, M. 2017. Valproic Acid in Women and Girls of Childbearing Age. *Curr Psychiatry Rep*, 19, 58.
- GREGER, R. & WANGEMANN, P. 1987. Loop diuretics. *Ren Physiol*, 10, 174-83.
- GREMEAUX, V., GAYDA, M., LEPERS, R., SOSNER, P., JUNEAU, M. & NIGAM, A. 2012. Exercise and longevity. *Maturitas*, 73, 312-7.
- GUVELI, B. T., ROSTI, R. O., GUZELTAS, A., TUNA, E. B., ATAKLI, D., SENCER, S., YEKELER, E., KAYSERILI, H., DIRICAN, A., BEBEK, N., BAYKAN, B., GOKYIGIT, A. & GURSES, C. 2017. Teratogenicity of Antiepileptic Drugs. *Clin Psychopharmacol Neurosci*, 15, 19-27.
- HALLER, R. G., LEWIS, S. F., COOK, J. D. & BLOMQVIST, C. G. 1985. Myophosphorylase deficiency impairs muscle oxidative metabolism. *Ann Neurol*, 17, 196-9.
- HALLER, R. G. & VISSING, J. 2002. Spontaneous "second wind" and glucose-induced second "second wind" in McArdle disease: oxidative mechanisms. *Arch Neurol*, 59, 1395-402.
- HALLER, R. G., WYRICK, P., TAIVASSALO, T. & VISSING, J. 2006. Aerobic conditioning: an effective therapy in McArdle's disease. *Ann Neurol*, 59, 922-8.
- HAMM, R. L. 2011. Drug-hypersensitivity syndrome: diagnosis and treatment. *J Am Coll Clin Wound Spec*, 3, 77-81.
- HOGREL, J. Y., VAN DEN BOGAART, F., LEDOUX, I., OLLIVIER, G., PETIT, F., KOUJAH, N., BEHIN, A., STOJKOVIC, T., EYMARD, B., VOERMANS, N. & LAFORET, P. 2015. Diagnostic power of the non-ischæmic forearm exercise test in detecting glycogenosis type V. *Eur J Neurol*, 22, 933-40.
- HOPKINS, P. M. 2006. Skeletal muscle physiology. *Continuing Education in Anaesthesia Critical Care & Pain*, 6, 1-6.
- HORGA, A., RAJA RAYAN, D. L., MATTHEWS, E., SUD, R., FIALHO, D., DURRAN, S. C., BURGE, J. A., PORTARO, S., DAVIS, M. B., HAWORTH, A. & HANNA, M. G. 2013. Prevalence study of genetically defined skeletal muscle channelopathies in England. *Neurology*, 80, 1472-5.
- HOWELL, J. M., DUNTON, E., CREED, K. E., QUINLIVAN, R. & SEWRY, C. 2015. Investigating sodium valproate as a treatment for McArdle disease in sheep. *Neuromuscul Disord*, 25, 111-9.
- HOWELL, J. M., WALKER, K. R., CREED, K. E., DUNTON, E., DAVIES, L., QUINLIVAN, R. & KARPATI, G. 2014. Phosphorylase re-expression, increase in the force of contraction and decreased fatigue following notexin-induced muscle damage and regeneration in the ovine model of McArdle disease. *Neuromuscul Disord*, 24, 167-77.
- HUBER, K., DOYON, G., PLAKS, J., FYNE, E., MELLORS, J. W. & SLUIS-CREMER, N. 2011. Inhibitors of histone deacetylases: correlation between isoform specificity and reactivation of HIV type 1 (HIV-1) from latently infected cells. *J Biol Chem*, 286, 22211-8.
- IAMGSD. 2014. *Walking with McArdle's* [Online]. YouTube: IAMGSD. Available: [https://www.youtube.com/watch?v=\\_l3HFHU3ICE](https://www.youtube.com/watch?v=_l3HFHU3ICE) [Accessed 2018].
- JAUREGUI-GARRIDO, B. & JAUREGUI-LOBERA, I. 2012. Sudden death in eating disorders. *Vasc Health Risk Manag*, 8, 91-8.
- JENKINSON, C., COULTER, A. & WRIGHT, L. 1993. Short form 36 (SF36) health survey questionnaire: normative data for adults of working age. *Bmj*, 306, 1437-40.
- JONKER, C. J., KWA, M. S. G., VAN DEN BERG, H. M., HOES, A. W. & MOL, P. G. M. 2018. Drug Registries and Approval of Drugs: Promises, Placebo, or a Real Success? *Clin Ther*, 40, 768-773.
- JOPPI, R., BERTELE, V. & GARATTINI, S. 2013. Orphan drugs, orphan diseases. The first decade of orphan drug legislation in the EU. *Eur J Clin Pharmacol*, 69, 1009-24.
- JURKAT-ROTT, K., WEBER, M. A., FAULER, M., GUO, X. H., HOLZHERR, B. D., PACZULLA, A., NORDSBORG, N., JOECHLE, W. & LEHMANN-HORN, F. 2009. K<sup>+</sup>-dependent paradoxical membrane depolarization and Na<sup>+</sup> overload, major and reversible contributors to weakness by ion channel leaks. *Proc Natl Acad Sci U S A*, 106, 4036-41.
- KAHLE, K. T. & STALEY, K. J. 2008. The bumetanide-sensitive Na-K-2Cl cotransporter NKCC1 as a potential target of a novel mechanism-based treatment strategy for neonatal seizures. *Neurosurg Focus*, 25, E22.

- KATZ, J. S., WOLFE, G. I., IANNACCONI, S., BRYAN, W. W. & BAROHN, R. J. 1999. The exercise test in Andersen syndrome. *Arch Neurol*, 56, 352-6.
- KAZEMI-ESFARJANI, P., SKOMOROWSKA, E., JENSEN, T. D., HALLER, R. G. & VISSING, J. 2002. A nonischemic forearm exercise test for McArdle disease. *Ann Neurol*, 52, 153-9.
- KE, Q., LUO, B., QI, M., DU, Y. & WU, W. 2013. Gender differences in penetrance and phenotype in hypokalemic periodic paralysis. *Muscle Nerve*, 47, 41-5.
- KISSEL, J. T., ELSHEIKH, B., KING, W. M., FREIMER, M., SCOTT, C. B., KOLB, S. J., REYNA, S. P., CRAWFORD, T. O., SIMARD, L. R., KROSSCHELL, K. J., ACSADI, G., SCHROTH, M. K., D'ANJOU, G., LASALLE, B., PRIOR, T. W., SORENSON, S., MACZULSKI, J. A. & SWOBODA, K. J. 2014. SMA valiant trial: a prospective, double-blind, placebo-controlled trial of valproic acid in ambulatory adults with spinal muscular atrophy. *Muscle Nerve*, 49, 187-92.
- KISSEL, J. T., SCOTT, C. B., REYNA, S. P., CRAWFORD, T. O., SIMARD, L. R., KROSSCHELL, K. J., ACSADI, G., ELSHEIKH, B., SCHROTH, M. K., D'ANJOU, G., LASALLE, B., PRIOR, T. W., SORENSON, S., MACZULSKI, J. A., BROMBERG, M. B., CHAN, G. M. & SWOBODA, K. J. 2011. SMA CARNIVAL TRIAL PART II: a prospective, single-armed trial of L-carnitine and valproic acid in ambulatory children with spinal muscular atrophy. *PLoS One*, 6, e21296.
- KITAOKA, Y. 2014. McArdle Disease and Exercise Physiology. *Biology (Basel)*, 3, 157-66.
- KOMULAINEN, T., LODGE, T., HINTTALA, R., BOLSZAK, M., PIETILA, M., KOIVUNEN, P., HAKKOLA, J., POULTON, J., MORTEN, K. J. & UUSIMAA, J. 2015. Sodium valproate induces mitochondrial respiration dysfunction in HepG2 in vitro cell model. *Toxicology*, 331, 47-56.
- KROSSCHELL, K. J., KISSEL, J. T., TOWNSEND, E. L., SIMEONE, S. D., ZHANG, R. Z., REYNA, S. P., CRAWFORD, T. O., SCHROTH, M. K., ACSADI, G., KISHNANI, P. S., VON KLEIST-RETZOW, J. C., HERO, B., D'ANJOU, G., SMITH, E. C., ELSHEIKH, B., SIMARD, L. R., PRIOR, T. W., SCOTT, C. B., LASALLE, B., SAKONJU, A., WIRTH, B. & SWOBODA, K. J. 2018. Clinical trial of L-Carnitine and valproic acid in spinal muscular atrophy type I. *Muscle Nerve*, 57, 193-199.
- KUBUKELI, Z. N., NOAKES, T. D. & DENNIS, S. C. 2002. Training techniques to improve endurance exercise performances. *Sports Med*, 32, 489-509.
- KUNTZER, T., FLOCARD, F., VIAL, C., KOHLER, A., MAGISTRIS, M., LABARRE-VILA, A., GONNAUD, P. M., OCHSNER, F., SOICHOT, P., CHAN, V. & MONNIER, G. 2000. Exercise test in muscle channelopathies and other muscle disorders. *Muscle Nerve*, 23, 1089-94.
- LARS, H., ERIC, H. & BENGT, S. 1967. Muscle Glycogen during Prolonged Severe Exercise. *Acta Physiologica Scandinavica*, 71, 129-139.
- LEMONNIER, E., VILLENEUVE, N., SONIE, S., SERRET, S., ROSIER, A., ROUE, M., BROSSET, P., VIELLARD, M., BERNOUX, D., RONDEAU, S., THUMMLER, S., RAVEL, D. & BEN-ARI, Y. 2017. Effects of bumetanide on neurobehavioral function in children and adolescents with autism spectrum disorders. *Transl Psychiatry*, 7, e1056.
- LEVITT, J. O. 2008. Practical aspects in the management of hypokalemic periodic paralysis. *J Transl Med*, 6, 18.
- LINDE, M., MULLENERS, W. M., CHRONICLE, E. P. & MCCRORY, D. C. 2013. Valproate (valproic acid or sodium valproate or a combination of the two) for the prophylaxis of episodic migraine in adults. *Cochrane Database Syst Rev*, Cd010611.
- LINDINGER, M. I. & SJOGAARD, G. 1991. Potassium regulation during exercise and recovery. *Sports Med*, 11, 382-401.
- LINKS, T. P., SMIT, A. J., MOLENAAR, W. M., ZWARTS, M. J. & OOSTERHUIS, H. J. 1994a. Familial hypokalemic periodic paralysis. Clinical, diagnostic and therapeutic aspects. *J Neurol Sci*, 122, 33-43.
- LINKS, T. P., VAN DER HOEVEN, J. H. & ZWARTS, M. J. 1994b. Surface EMG and muscle fibre conduction during attacks of hypokalaemic periodic paralysis. *J Neurol Neurosurg Psychiatry*, 57, 632-4.
- LUCIA, A., NOGALES-GADEA, G., PEREZ, M., MARTIN, M. A., ANDREU, A. L. & ARENAS, J. 2008. McArdle disease: what do neurologists need to know? *Nat Clin Pract Neurol*, 4, 568-77.
- LUCIA, A., QUINLIVAN, R., WAKELIN, A., MARTIN, M. A. & ANDREU, A. L. 2013. The 'McArdle paradox': exercise is a good advice for the exercise intolerant. *Br J Sports Med*, 47, 728-9.
- LUCIA, A., RUIZ, J. R., SANTALLA, A., NOGALES-GADEA, G., RUBIO, J. C., GARCIA-CONSUEGRA, I., CABELLO, A., PEREZ, M., TEIJEIRA, S., VIEITEZ, I., NAVARRO, C., ARENAS, J., MARTIN, M. A. & ANDREU, A. L. 2012. Genotypic and phenotypic features of McArdle disease: insights from the Spanish national registry. *J Neurol Neurosurg Psychiatry*, 83, 322-8.

- MAHROO, O. A., KHAN, K. N., WRIGHT, G., OCKRIM, Z., SCALCO, R. S., ROBSON, A. G., TUFAIL, A., MICHAELIDES, M., QUINLIVAN, R. & WEBSTER, A. R. 2018. Retinopathy associated with bi-allelic mutations in PYGM (McArdle disease). *Ophthalmology*.
- MANOLE, A., JAUNMUKTANE, Z., HARGREAVES, I., LUDTMANN, M. H. R., SALPIETRO, V., BELLO, O. D., POPE, S., PANDRAUD, A., HORGA, A., SCALCO, R. S., LI, A., ASHOKKUMAR, B., LOURENCO, C. M., HEALES, S., HORVATH, R., CHINNERY, P. F., TORO, C., SINGLETON, A. B., JACQUES, T. S., ABRAMOV, A. Y., MUNTONI, F., HANNA, M. G., REILLY, M. M., REVESZ, T., KULLMANN, D. M., JEPSON, J. E. C. & HOULDEN, H. 2017. Clinical, pathological and functional characterization of riboflavin-responsive neuropathy. *Brain*, 140, 2820-2837.
- MARTINUZZI, A., SCHIEVANO, G., NASCIMBENI, A. & FANIN, M. 1999. McArdle's disease. The unsolved mystery of the reappearing enzyme. *Am J Pathol*, 154, 1893-7.
- MATE-MUNOZ, J. L., MORAN, M., PEREZ, M., CHAMORRO-VINA, C., GOMEZ-GALLEGO, F., SANTIAGO, C., CHICHARRO, L., FOSTER, C., NOGALES-GADEA, G., RUBIO, J. C., ANDREU, A. L., MARTIN, M. A., ARENAS, J. & LUCIA, A. 2007. Favorable responses to acute and chronic exercise in McArdle patients. *Clin J Sport Med*, 17, 297-303.
- MATTHEWS, E. & HANNA, M. G. 2010. Muscle channelopathies: does the predicted channel gating pore offer new treatment insights for hypokalaemic periodic paralysis? *J Physiol*, 588, 1879-86.
- MATTHEWS, E., NEUWIRTH, C., JAFFER, F., SCALCO, R. S., FIALHO, D., PARTON, M., RAJA RAYAN, D., SUETTERLIN, K., SUD, R., SPIEGEL, R., MEIN, R., HOULDEN, H., SCHAEFER, A., HEALY, E., PALACE, J., QUINLIVAN, R., TREVES, S., HOLTON, J. L., JUNGBLUTH, H. & HANNA, M. G. 2018. Atypical periodic paralysis and myalgia: A novel RYR1 phenotype. *Neurology*, 90, e412-e418.
- MATTHEWS, E., PORTARO, S., KE, Q., SUD, R., HAWORTH, A., DAVIS, M. B., GRIGGS, R. C. & HANNA, M. G. 2011. Acetazolamide efficacy in hypokalemic periodic paralysis and the predictive role of genotype. *Neurology*, 77, 1960-4.
- MCA RDLE, B. 1951. Myopathy due to a defect in muscle glycogen breakdown. *Clin Sci*, 10, 13-35.
- MCCORMICK, Z., CHU, S. K., CHANG-CHIEN, G. C. & JOSEPH, P. 2013. Acute pain control challenges with buprenorphine/naloxone therapy in a patient with compartment syndrome secondary to McArdle's disease: a case report and review. *Pain Med*, 14, 1187-91.
- MCMANIS, P. G., LAMBERT, E. H. & DAUBE, J. R. 1986. The exercise test in periodic paralysis. *Muscle Nerve*, 9, 704-10.
- MEDBO, J. I. & SEJERSTED, O. M. 1990. Plasma potassium changes with high intensity exercise. *J Physiol*, 421, 105-22.
- MICHELSON, J. 2018. My Diagnostic Odyssey—A Call to Expand Access to Genomic Testing for the Next Generation. *The Hastings Center Report*, 48, S32-S34.
- MILLER, T. M., DIAS DA SILVA, M. R., MILLER, H. A., KWIECINSKI, H., MENDELL, J. R., TAWIL, R., MCMANIS, P., GRIGGS, R. C., ANGELINI, C., SERVIDEI, S., PETAJAN, J., DALAKAS, M. C., RANUM, L. P., FU, Y. H. & PTACEK, L. J. 2004. Correlating phenotype and genotype in the periodic paralyses. *Neurology*, 63, 1647-55.
- MISRA, U. K. & KALITA, J. 2006. Ulnar Nerve. *Clinical Neurophysiology*. India: Elsevier.
- MITSUMOTO, J., DORSEY, E. R., BECK, C. A., KIEBURTZ, K. & GRIGGS, R. C. 2009. Pivotal studies of orphan drugs approved for neurological diseases. *Ann Neurol*, 66, 184-90.
- MOMMAERTS, W. F., ILLINGWORTH, B., PEARSON, C. M., GUILLORY, R. J. & SERAYDARIAN, K. 1959. A FUNCTIONAL DISORDER OF MUSCLE ASSOCIATED WITH THE ABSENCE OF PHOSPHORYLASE. *Proc Natl Acad Sci U S A*, 45, 791-7.
- MUL, K., KINOSHITA, J., DAWKINS, H., VAN ENGELEN, B. & TUPLER, R. 2017. 225th ENMC international workshop:: A global FSHD registry framework, 18-20 November 2016, Heemskerk, The Netherlands. *Neuromuscul Disord*, 27, 782-790.
- MULLARD, A. 2014. New drugs cost US\$2.6 billion to develop. *Nature Reviews Drug Discovery*, 13, 877.
- MUNGUIA-IZQUIERDO, D., SANTALLA, A. & LUCIA, A. 2015. Cardiorespiratory fitness, physical activity, and quality of life in patients with McArdle disease. *Med Sci Sports Exerc*, 47, 799-808.
- NATASHA, G., BRANDOM, K. G., YOUNG, E. C. & YOUNG, P. J. 2008. Valproate and spinal muscular atrophy (Review). *Mol Med Rep*, 1, 161-5.
- NAVEEN, K., ARUNKUMAR, J., HANUMANTHAYYA, K. & PAI, V. 2012. Stevens-Johnson syndrome induced by sodium valproate monotherapy. *Int J Crit Illn Inj Sci*, 2, 44-5.

- NEWGARD, C. B., HWANG, P. K. & FLETTERICK, R. J. 1989. The family of glycogen phosphorylases: structure and function. *Crit Rev Biochem Mol Biol*, 24, 69-99.
- NHS\_ENGLAND. *Rhabdomyolysis and Metabolic Myopathies 30 Gene Panel* [Online]. NHS Genetic Testing Network: NHS England. Available: <https://ukgtn.nhs.uk/find-a-test/search-by-disorder-gene/rhabdomyolysis-and-metabolic-myopathies-30-gene-panel-780/> [Accessed 09/07/2018 2018].
- NHS\_ENGLAND. 2017. *Highly specialised services* [Online]. NHS England: NHS England. Available: <https://www.england.nhs.uk/commissioning/spec-services/highly-spec-services/> [Accessed 09/08/2018 2018].
- NICE. 2018. *Sodium Valproate* [Online]. National Institute for Health and care Excellence. Available: <https://bnf.nice.org.uk/drug/sodium-valproate.html#indicationsAndDoses> [Accessed 27/07/2018 2018].
- NOGALES-GADEA, G., ARENAS, J. & ANDREU, A. L. 2007. Molecular genetics of McArdle's disease. *Curr Neurol Neurosci Rep*, 7, 84-92.
- NOGALES-GADEA, G., SANTALLA, A., ARENAS, J., MARTIN, M. A., MORAN, M. & LUCIA, A. 2017. Low versus high carbohydrates in the diet of the world-class athlete: insights from McArdle's disease. *J Physiol*, 595, 2991-2992.
- OPREA, T. I., BAUMAN, J. E., BOLOGA, C. G., BURANDA, T., CHIGAEV, A., EDWARDS, B. S., JARVIK, J. W., GRESHAM, H. D., HAYNES, M. K., HJELLE, B., HROMAS, R., HUDSON, L., MACKENZIE, D. A., MULLER, C. Y., REED, J. C., SIMONS, P. C., SMAGLEY, Y., STROUSE, J., SURVILADZE, Z., THOMPSON, T., URSU, O., WALLER, A., WANDINGER-NESS, A., WINTER, S. S., WU, Y., YOUNG, S. M., LARSON, R. S., WILLMAN, C. & SKLAR, L. A. 2011. Drug Repurposing from an Academic Perspective. *Drug Discov Today Ther Strateg*, 8, 61-69.
- PANTZIARKA, P., PIRMOHAMED, M. & MIRZA, N. 2018. New uses for old drugs. *Bmj*, 361, k2701.
- PIEPERS, S., VELDINK, J. H., DE JONG, S. W., VAN DER TWEEL, I., VAN DER POL, W. L., UIJTENDAAL, E. V., SCHELHAAS, H. J., SCHEFFER, H., DE VISSER, M., DE JONG, J. M., WOKKE, J. H., GROENEVELD, G. J. & VAN DEN BERG, L. H. 2009. Randomized sequential trial of valproic acid in amyotrophic lateral sclerosis. *Ann Neurol*, 66, 227-34.
- PIETRUSZ, A., SCALCO, R. S. & QUINLIVAN, R. 2018. Resistance Exercise Training in McArdle Disease: Myth or Reality? *Case Reports in Neurological Medicine*, 2018, 6.
- POCOCK, S. J. & STONE, G. W. 2016. The Primary Outcome Fails - What Next? *N Engl J Med*, 375, 861-70.
- PRESSLER, R. M., BOYLAN, G. B., MARLOW, N., BLENNOW, M., CHIRON, C., CROSS, J. H., DE VRIES, L. S., HALLBERG, B., HELLSTROM-WESTAS, L., JULLIEN, V., LIVINGSTONE, V., MANGUM, B., MURPHY, B., MURRAY, D., PONS, G., RENNIE, J., SWARTE, R., TOET, M. C., VANHATALO, S. & ZOHAR, S. 2015. Bumetanide for the treatment of seizures in newborn babies with hypoxic ischaemic encephalopathy (NEMO): an open-label, dose finding, and feasibility phase 1/2 trial. *Lancet Neurol*, 14, 469-77.
- PULLAR, T. & FEELY, M. P. 1990. Reporting compliance in clinical trials. *Lancet*, 336, 1252-3.
- PULLAR, T., KUMAR, S. & FEELY, M. 1989. Compliance in clinical trials. *Ann Rheum Dis*, 48, 871-5.
- QUINLIVAN, R. 2012. *Specialised Service for Glycogen Storage Diseases* [Online]. Centre for Neuromuscular Diseases. Available: <http://www.ucl.ac.uk/cnmd/patient-services/muscle/glycogen-storage-diseases> [Accessed 03/07/2018 2018].
- QUINLIVAN, R., ANDREU, A. L. & MARTI, R. 2017. 211th ENMC International Workshop:: Development of diagnostic criteria and management strategies for McArdle Disease and related rare glycogenolytic disorders to improve standards of care. 17-19 April 2015, Naarden, The Netherlands. *Neuromuscul Disord*, 27, 1143-1151.
- QUINLIVAN, R., BUCKLEY, J., JAMES, M., TWIST, A., BALL, S., DUNO, M., VISSING, J., BRUNO, C., CASSANDRINI, D., ROBERTS, M., WINER, J., ROSE, M. & SEWRY, C. 2010. McArdle disease: a clinical review. *J Neurol Neurosurg Psychiatry*, 81, 1182-8.
- QUINLIVAN, R., LUCIA, A., SCALCO, R. S., SANTANA, A., PARINI, J., GODFREY, R., MARTI, R. & PARTICIPANTS, W. 2015. Report on the EUROMAC McArdle Exercise Testing Workshop, Madrid, Spain, 11-12 July 2014. *Neuromuscular Disorders*, 25, 739-745.
- QUINLIVAN, R., MARTINUZZI, A. & SCHOSER, B. 2014a. Pharmacological and nutritional treatment for McArdle disease (Glycogen Storage Disease type V). *Cochrane Database Syst Rev*, 11, Cd003458.
- QUINLIVAN, R., MATTHEWS, E. & HANNA, M. G. 2014b. Innovative care model for patients with complex muscle diseases. *Curr Opin Neurol*, 27, 607-13.

- QUINLIVAN, R. & VISSING, J. 2007. 144th ENMC International Workshop: Outcome Measures in McArdle Disease, 29 September-1 November 2006, Naarden, The Netherlands. *Neuromuscul Disord*, 17, 494-8.
- QUINLIVAN, R., VISSING, J., HILTON-JONES, D. & BUCKLEY, J. 2011. Physical training for McArdle disease. *Cochrane Database Syst Rev*, Cd007931.
- REASON, S. L. 2016. *Toward an understanding of McArdle disease: Implications for rare disease policy development*. Doctor of Education.
- REASON, S. L., CADZOW, R., JEGIER, B. J. & WAKELIN, A. 2018. Centre of Expertise: Comprehensive Care for Patients with McArdle Disease. *Journal Rare Disorders: Diagnosis & Therapy*, 4, 3.
- RODRIGUEZ-GOMEZ, I., SANTALLA, A., DIEZ-BERMEJO, J., MUNGUÍA-IZQUIERDO, D., ALEGRE, L. M., NOGALES-GADEA, G., ARENAS, J., MARTIN, M. A., LUCIA, A. & ARA, I. 2018. A New Condition in McArdle Disease: Poor Bone Health-Benefits of an Active Lifestyle. *Med Sci Sports Exerc*, 50, 3-10.
- RUBIO, D. M., SCHOENBAUM, E. E., LEE, L. S., SCHTEINGART, D. E., MARANTZ, P. R., ANDERSON, K. E., PLATT, L. D., BAEZ, A. & ESPOSITO, K. 2010. Defining translational research: implications for training. *Acad Med*, 85, 470-5.
- SANSONE, V., MEOLA, G., LINKS, T. P., PANZERI, M. & ROSE, M. R. 2008. Treatment for periodic paralysis. *Cochrane Database Syst Rev*, Cd005045.
- SANSONE, V. A., BURGE, J., MCDERMOTT, M. P., SMITH, P. C., HERR, B., TAWIL, R., PANDYA, S., KISSEL, J., CIAFALONI, E., SHIEH, P., RALPH, J. W., AMATO, A., CANNON, S. C., TRIVEDI, J., BAROHN, R., CRUM, B., MITSUMOTO, H., PESTRONK, A., MEOLA, G., CONWIT, R., HANNA, M. G. & GRIGGS, R. C. 2016. Randomized, placebo-controlled trials of dichlorophenamide in periodic paralysis. *Neurology*, 86, 1408-16.
- SANTALLA, A., MUNGUÍA-IZQUIERDO, D., BREA-ALEJO, L., PAGOLA-ALDAZABAL, I., DIEZ-BERMEJO, J., FLECK, S. J., ARA, I. & LUCIA, A. 2014a. Feasibility of resistance training in adult McArdle patients: clinical outcomes and muscle strength and mass benefits. *Front Aging Neurosci*, 6, 334.
- SANTALLA, A., NOGALES-GADEA, G., ENCINAR, A. B., VIEITEZ, I., GONZALEZ-QUINTANA, A., SERRANO-LORENZO, P., CONSUEGRA, I. G., ASENSIO, S., BALLESTER-LOPEZ, A., PINTOS-MORELL, G., COLL-CANTI, J., PAREJA-GALEANO, H., DIEZ-BERMEJO, J., PEREZ, M., ANDREU, A. L., PINOS, T., ARENAS, J., MARTIN, M. A. & LUCIA, A. 2017. Genotypic and phenotypic features of all Spanish patients with McArdle disease: a 2016 update. *BMC Genomics*, 18, 819.
- SANTALLA, A., NOGALES-GADEA, G., ORTENBLAD, N., BRULL, A., DE LUNA, N., PINOS, T. & LUCIA, A. 2014b. McArdle disease: a unique study model in sports medicine. *Sports Med*, 44, 1531-44.
- SATO, K., IMAI, F., HATAYAMA, I. & ROELOFS, R. I. 1977. Characterization of glycogen phosphorylase isoenzymes present in cultured skeletal muscle from patients with McArdle's disease. *Biochem Biophys Res Commun*, 78, 663-8.
- SATO, Y. 2002. Phosphorylase. In: PREEDY, V. R. & PETERS, T. J. (eds.) *Skeletal muscle : pathology, diagnosis and management of disease*. London: GMM.
- SCALCO, R. S. 2014. Umphred's Neurological Rehabilitation, Sixth Edition. Darcy Ann Umphred, Rolando T. Lazaro, Margaret L. Roller and Gordon U. Burton, Eds., ISBN: 978-0-323-07586-2 Elsevier, 2013, 1262pp. *Neuromuscular Disorders*, 24, 193.
- SCALCO, R. S., BLOOTH, S., ELLERTON, C., GODFREY, R., KAHRAMAN, A., WIGLEY, R., CHATFIELD, S., CARRUTHERS, R., PREECE, M. A., KIRK, R., TURNER, P., COSKERAN, H., WAKELIN, A., HOLTON, J., PATTNI, J. & QUINLIVAN, R. 2016a. P111 Effect of a multi-disciplinary approach to diagnosis and management for non-lysosomal skeletal muscle glycogen storage disorders. *Neuromuscular Disorders*, 26, Supplement 1, S38.
- SCALCO, R. S., BRADY, S., BECKER, J., GOMES, I., HOLTON, J. L. & STAUB, H. L. 2015a. LETTER TO THE EDITOR Atypical Granulomatous Myositis and Pulmonary Sarcoidosis. *The open rheumatology journal*, 9, 57-9.
- SCALCO, R. S., CHATFIELD, S., GODFREY, R., PATTNI, J., ELLERTON, C., BEGGS, A., BRADY, S., WAKELIN, A., HOLTON, J. L. & QUINLIVAN, R. 2014a. From exercise intolerance to functional improvement: the second wind phenomenon in the identification of McArdle disease. *Archivos De Neuro-Psiquiatria*, 72, 538-541.
- SCALCO, R. S., CHATFIELD, S., JUNEJO, M. H., BOOTH, S., PATTNI, J., GODFREY, R. & QUINLIVAN, R. 2016b. McArdle Disease Misdiagnosed as Meningitis. *Am J Case Rep*, 17, 905-908.

- SCALCO, R. S., CHATFIELD, S., PATTNI, J., ELLERTON, C., BEGGS, A., GODFREY, R., HOLTON, J. L. & QUINLIVAN, R. 2014b. T.P.26 McArdle disease misdiagnosed as meningitis: A case report. *Neuromuscular Disorders*, 24, 872.
- SCALCO, R. S., GARDINER, A. R., PITCEATHLY, R. D., HILTON-JONES, D., SCHAPIRA, A. H., TURNER, C., PARTON, M., DESIKAN, M., BARRESI, R., MARSH, J., MANZUR, A. Y., CHILDS, A. M., FENG, L., MURPHY, E., LAMONT, P. J., RAVENSCROFT, G., WALLEFELD, W., DAVIS, M. R., LAING, N. G., HOLTON, J. L., FIALHO, D., BUSHBY, K., HANNA, M. G., PHADKE, R., JUNGBLUTH, H., HOULDEN, H. & QUINLIVAN, R. 2016c. CAV3 mutations causing exercise intolerance, myalgia and rhabdomyolysis: Expanding the phenotypic spectrum of caveolinopathies. *Neuromuscul Disord*, 26, 504-10.
- SCALCO, R. S., GARDINER, A. R., PITCEATHLY, R. D., ZANOTELI, E., BECKER, J., HOLTON, J. L., HOULDEN, H., JUNGBLUTH, H. & QUINLIVAN, R. 2015b. Rhabdomyolysis: a genetic perspective. *Orphanet J Rare Dis*, 10, 51.
- SCALCO, R. S., LORENZONI, P. J., LYNCH, D. S., MARTINS, W. A., JUNGBLUTH, H., QUINLIVAN, R., BECKER, J. & HOULDEN, H. 2017a. Polymyositis without Beneficial Response to Steroid Therapy: Should Miyoshi Myopathy be a Differential Diagnosis? *Am J Case Rep*, 18, 17-21.
- SCALCO, R. S., MORROW, J., SKORUPINSKA, I., MANOLE, A., FEDERICO, R., MATTHEWS, E., HANNA, M. G. & FIALHO, D. 2017b. RCT of Bumetanide in Hypokalaemic Periodic Paralysis (HypoPP) using abductor digiti minimi compound muscle action potential (CMAP) as an objective outcome measure. *Muscle & Nerve*, n/a-n/a.
- SCALCO, R. S., MORROW, J. M., BOOTH, S., CHATFIELD, S., GODFREY, R. & QUINLIVAN, R. 2017c. Misdiagnosis is an important factor for diagnostic delay in McArdle disease. *Neuromuscul Disord*, 27, 852-855.
- SCALCO, R. S., QUINLIVAN, R., MARTIN, R., BARUCH, N., MARTIN, M., NAVARRA, C., MARTINUZZI, A., BRUNO, C., LAFORET, P., SPERBER, K., SACCONI, S., WAKELIN, A., HADJIGEORGIOU, G., VISSING, J., VORGERD, M., HALLER, R., OFLAZER, Z., POUGET, J., LUCIA, A., ANDREU, T., TOSCANO, A. & MUSUMECI, O. 2014c. Exercise related muscle disorders: The EUROMAC Registry for McArdle disease and other rare glycogenolytic disorders. *Neuromuscular Disorders*, 24, 890.
- SCALCO, R. S., SNOECK, M., QUINLIVAN, R., TREVES, S., LAFORET, P., JUNGBLUTH, H. & VOERMANS, N. C. 2016d. Exertional rhabdomyolysis: physiological response or manifestation of an underlying myopathy? *BMJ Open Sport Exerc Med*, 2, e000151.
- SCALCO, R. S., VOERMANS, N. C., PIERCY, R. J., JUNGBLUTH, H. & QUINLIVAN, R. 2016e. Dantrolene as a possible prophylactic treatment for RYR1-related rhabdomyolysis. *Eur J Neurol*, 23, e56-7.
- SCHMID, R. & MAHLER, R. 1959. Chronic progressive myopathy with myoglobinuria: demonstration of a glycogenolytic defect in the muscle. *J Clin Invest*, 38, 2044-58.
- SCHMIDT, B. 2006. Proof of Principle studies. *Epilepsy Res*, 68, 48-52.
- SCHUTZ, P. W., SCALCO, R. S., BARRESI, R., HOULDEN, H., PARTON, M. & HOLTON, J. L. 2017. Calpainopathy with macrophage-rich, regional inflammatory infiltrates. *Neuromuscul Disord*, 27, 738-741.
- SHAH, R. R. 2006. Regulatory framework for the treatment of orphan diseases. In: MEHTA A, BECK M & G, S.-P. (eds.) *Fabry Disease: Perspectives from 5 Years of FOS*. Oxford: Oxford PharmaGenesis.
- SINGH, A. K., KELLEY, K. & AGARWAL, R. 2008. Interpreting results of clinical trials: a conceptual framework. *Clin J Am Soc Nephrol*, 3, 1246-52.
- SLACK, M. K. & DRAUGALIS, J. R. 2001. Establishing the internal and external validity of experimental studies. *Am J Health Syst Pharm*, 58, 2173-81; quiz 2182-3.
- STALEY, K. J. 2006. Wrong-way chloride transport: is it a treatable cause of some intractable seizures? *Epilepsy Curr*, 6, 124-7.
- STATLAND, J. M., FONTAINE, B., HANNA, M. G., JOHNSON, N. E., KISSEL, J. T., SANSONE, V. A., SHIEH, P. B., TAWIL, R. N., TRIVEDI, J., CANNON, S. C. & GRIGGS, R. C. 2018. Review of the Diagnosis and Treatment of Periodic Paralysis. *Muscle Nerve*, 57, 522-530.
- STERNBERG, D., MAISONOBE, T., JURKAT-ROTT, K., NICOLE, S., LAUNAY, E., CHAUVEAU, D., TABTI, N., LEHMANN-HORN, F., HAINQUE, B. & FONTAINE, B. 2001. Hypokalaemic periodic paralysis type 2 caused by mutations at codon 672 in the muscle sodium channel gene SCN4A. *Brain*, 124, 1091-9.
- STONE, W. J., BENNETT, W. M. & CUTLER, R. E. 1981. Long-term bumetanide treatment of patients with edema due to renal disease. Cooperative studies. *J Clin Pharmacol*, 21, 587-980.

- STONE, C. M. & LEE JOHNSON, L. 2012. Chapter 19 - Design of Clinical Studies and Trials. *In: GALLIN, J. I. & OGNIBENE, F. P. (eds.) Principles and Practice of Clinical Research (Third Edition)*. Boston: Academic Press.
- STUNNENBERG, B. C., RAAPHORST, J., DEENEN, J. C. W., LINKS, T. P., WILDE, A. A., VERBOVE, D. J., KAMSTEEG, E. J., VAN DEN WIJNGAARD, A., FABER, C. G., VAN DER WILT, G. J., VAN ENGELEN, B. G. M., DROST, G. & GINJAAR, H. B. 2018. Prevalence and mutation spectrum of skeletal muscle channelopathies in the Netherlands. *Neuromuscul Disord*.
- SUNG, N. S., CROWLEY, W. F., JR., GENEL, M., SALBER, P., SANDY, L., SHERWOOD, L. M., JOHNSON, S. B., CATANESE, V., TILSON, H., GETZ, K., LARSON, E. L., SCHEINBERG, D., REECE, E. A., SLAVKIN, H., DOBS, A., GREBB, J., MARTINEZ, R. A., KORN, A. & RIMOIN, D. 2003. Central challenges facing the national clinical research enterprise. *Jama*, 289, 1278-87.
- SUVARNA, V. 2010. Phase IV of Drug Development. *Perspect Clin Res*, 1, 57-60.
- TAN, S. V., MATTHEWS, E., BARBER, M., BURGE, J. A., RAJAKULENDRAN, S., FIALHO, D., SUD, R., HAWORTH, A., KOLTZENBURG, M. & HANNA, M. G. 2011. Refined exercise testing can aid DNA-based diagnosis in muscle channelopathies. *Ann Neurol*, 69, 328-40.
- TANOSHIMA, M., KOBAYASHI, T., TANOSHIMA, R., BEYENE, J., KOREN, G. & ITO, S. 2015. Risks of congenital malformations in offspring exposed to valproic acid in utero: A systematic review and cumulative meta-analysis. *Clin Pharmacol Ther*, 98, 417-41.
- TARUSCIO, D., GENTILE, A. E., EVANGELISTA, T., FRAZZICA, R. G., BUSHBY, K. & MONTSERRAT, A. M. 2014. Centres of Expertise and European Reference Networks: key issues in the field of rare diseases. The EUCERD Recommendations. *Blood Transfus*, 12 Suppl 3, s621-5.
- TATA, P. N. V., VENKATARAMANAN, R. & SAHOTA, S. K. 1993. Bumetanide. *In: HARRY, G. B. (ed.) Analytical Profiles of Drug Substances and Excipients*. Academic Press.
- TAWIL, R., MCDERMOTT, M. P., BROWN, R., JR., SHAPIRO, B. C., PTACEK, L. J., MCMANIS, P. G., DALAKAS, M. C., SPECTOR, S. A., MENDELL, J. R., HAHN, A. F. & GRIGGS, R. C. 2000. Randomized trials of dichlorophenamide in the periodic paralyses. Working Group on Periodic Paralysis. *Ann Neurol*, 47, 46-53.
- TROCHIM, W., KANE, C., GRAHAM, M. J. & PINCUS, H. A. 2011. Evaluating translational research: a process marker model. *Clin Transl Sci*, 4, 153-62.
- VAN NORMAN, G. A. 2016. Drugs, Devices, and the FDA: Part 1: An Overview of Approval Processes for Drugs. *JACC: Basic to Translational Science*, 1, 170-179.
- VENANCE, S. L., CANNON, S. C., FIALHO, D., FONTAINE, B., HANNA, M. G., PTACEK, L. J., TRISTANI-FIROUZI, M., TAWIL, R. & GRIGGS, R. C. 2006. The primary periodic paralyses: diagnosis, pathogenesis and treatment. *Brain*, 129, 8-17.
- VICART, S., STERNBERG, D., ARZEL-HEZODE, M., FRANQUES, J., BENDAHHOU, S., LORY, P., HAINQUE, B., FOURNIER, E., NICOLE, S. & FONTAINE, B. 1993a. Hypokalemic Periodic Paralysis. *In: ADAM, M. P., ARDINGER, H. H., PAGON, R. A., WALLACE, S. E., BEAN, L. J. H., STEPHENS, K. & AMEMIYA, A. (eds.) GeneReviews((R))*. Seattle (WA): University of Washington, Seattle
- University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.
- VICART, S., STERNBERG, D., ARZEL-HEZODE, M., FRANQUES, J., BENDAHHOU, S., LORY, P., HAINQUE, B., FOURNIER, E., NICOLE, S. & FONTAINE, B. 1993b. Hypokalemic Periodic Paralysis. *In: PAGON, R. A., ADAM, M. P., ARDINGER, H. H., WALLACE, S. E., AMEMIYA, A., BEAN, L. J. H., BIRD, T. D., LEDBETTER, N., MEFFORD, H. C., SMITH, R. J. H. & STEPHENS, K. (eds.) GeneReviews(R)*. Seattle (WA): University of Washington, Seattle
- University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.
- VICART, S., STERNBERG, D., ARZEL-HÉZODE, M., FRANQUES, J., BENDAHHOU, S., LORY, P., HAINQUE, B., FOURNIER, E., NICOLE, S. & FONTAINE, B. 2002. Hypokalemic Periodic Paralysis. *GeneReviews@ [Internet]*.
- WAKELIN, A. 2013. *101 Tips for a good life with McArdle disease*, AGSD-UK.
- WANG, Y., XIA, J., HELFER, B., LI, C. & LEUCHT, S. 2016. Valproate for schizophrenia. *Cochrane Database Syst Rev*, 11, Cd004028.
- WARD, A. & HEEL, R. C. 1984. Bumetanide. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use. *Drugs*, 28, 426-64.

- WARGO, K. A. & BANTA, W. M. 2009. A comprehensive review of the loop diuretics: should furosemide be first line? *Ann Pharmacother*, 43, 1836-47.
- WERTSCH, J. J., OSWALD, T. A. & KINCAID, J. C. 2000. Ulnar Technique. *In: AANEM (ed.) AANEM Workshop*. AANEM.
- WISE, J. 2017. Women still not being told about pregnancy risks of valproate. *Bmj*, 358, j4426.
- WU, F., MI, W. & CANNON, S. C. 2013a. Beneficial effects of bumetanide in a CaV1.1-R528H mouse model of hypokalaemic periodic paralysis. *Brain*, 136, 3766-74.
- WU, F., MI, W. & CANNON, S. C. 2013b. Bumetanide prevents transient decreases in muscle force in murine hypokalemic periodic paralysis. *Neurology*, 80, 1110-6.
- ZHANG, X., ZHANG, Y., YE, X., GUO, X., ZHANG, T. & HE, J. 2016. Overview of phase IV clinical trials for postmarket drug safety surveillance: a status report from the ClinicalTrials.gov registry. *BMJ Open*, 6, e010643.
- ZHAO, X., LI, A., SONI, M., MURIELLO, M. J., JONES, C. H. & WHITTIER, W. L. 2017. McArdle disease: a "pediatric" disorder presenting in an adult with acute kidney injury. *CEN Case Rep*, 6, 156-160.
- ZOELLNER, J., VAN HORN, L., GLEASON, P. M. & BOUSHEY, C. J. 2015. What is translational research? Concepts and applications in nutrition and dietetics. *J Acad Nutr Diet*, 115, 1057-71.
- ZUTT, R., VAN DER KOOI, A. J., LINTHORST, G. E., WANDERS, R. J. & DE VISSER, M. 2014. Rhabdomyolysis: review of the literature. *Neuromuscul Disord*, 24, 651-9.

## **18. Academic Activities at UCL**

Available in: printed thesis

**Awards and Prizes**

**Student Supervision**

**Other Related Activities**

**Event Organisation**

**Oral Presentations**

**Scientific Journal Reviewer**

**Publications**

**Poster Presentation**

## **19. Appendix**

Available in: printed thesis

### **Appendix 1**

Ethics approval documentation – bumetanide clinical trial

### **Appendix 2**

Participant information sheet – bumetanide clinical trial

### **Appendix 3**

Consent form – bumetanide clinical trial

### **Appendix 4**

Case report form – bumetanide clinical trial

### **Appendix 5**

Ethics approval documentation – sodium valproate clinical trial

### **Appendix 6**

Participant information sheet – sodium valproate clinical trial

### **Appendix 7**

Consent form – sodium valproate clinical trial

### **Appendix 8**

Case report form – sodium valproate clinical trial