A natural history study in Limb Girdle Muscular Dystrophy 2I – Magnetic Resonance imaging, spectroscopy and physical outcome measures

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Abstract

Limb Girdle Muscular Dystrophy 2I (LGMD2I) is caused by mutations in the fukutin related protein gene (*FKRP*). It is an important and frequent cause of LGMD, particularly in Northern Europe, and can range from a severe muscular dystrophy in childhood, very similar to Duchenne muscular dystrophy, to a much milder adult disease. Respiratory involvement and cardiomyopathy are common.

This thesis aims to assess the natural history of a particular subset of this rare condition across four main European centres. Thirty eight ambulant LGMD2I patients from Newcastle, London, Paris and Copenhagen were recruited. The age range was 18-64 years and disease duration, 0-49 years.

Magnetic resonance imaging (MRI) and Magnetic resonance spectroscopy (MRS) have both been performed. T1-weighted images were used to assess fat infiltration patterns using a qualitative radiological score and a quantitative 3-point Dixon technique, applied over two time points, and was performed to track the progression of fat infiltration. MRI fat infiltration has been correlated with muscle strength and function longitudinally.

In the Newcastle upon Tyne cohort, cardiac MRI was also studied assessing the cardiac involvement in this condition.

Chapter 1 presents an overview of muscular dystrophy and the current knowledge in LGMD2I and chapter 2 includes a literature review of both skeletal muscle and cardiac imaging. Chapter 3 focuses on the methodology of the study, including the patient demographics, physical assessment tools and MRI and MRS specifics. Chapters 4 -9 contain the results section; including physical and functional assessments, both cross-sectional and longitudinal, the cross sectional and longitudinal MRI results, skeletal MRS, cardiac MRI data and the FKRP registry respectively. Discussion of the results is found at the end of each chapter. Chapter 10 concludes with areas for future research.

Declaration

This was a multicentre study involving patients with LGMD2I from the following centres; Professor Hanna's group at UCL Institute of Neurology, London; Pierre Carlier's group at the Institute of Myology, Paris; Professor John Vissing's group in the Neuromuscular Research Unit, Department of Neurology, Rigshospitalet, Copenhagen and locally at the Institute of Genetic Medicine, Newcastle upon Tyne. All the protocols were written and designed by myself and ethical approval was obtained for the Newcastle upon Tyne and London sites by myself. Pierre Carlier and John Vissing had to apply for ethical approval locally.

The technical protocol for the magnetic resonance imaging and magnetic resonance spectroscopy was coordinated by Kieren Hollingsworth, Newcastle upon Tyne.

The manual for the standardised physical assessments was initially researched by myself and completed by the research physiotherapists, Michelle Eagle and Anna Mayhew. The Rasch analysis of the adapted North Star Ambulatory Assessment was completed by Anna Mayhew.

The physical and functional assessments were carried out by the physiotherapists at each of the sites and the results sent to me for analysis on all the patients.

The cardiac Cine-MRI was analysed by Ben Dixon based at the Newcastle Magnetic Resonance Centre (NMRC).

The radiographers at the NMRC Mrs Louise Morris, Mrs Carol Smith and Mr Tim Hodgson handled the equipment during the magnetic resonance spectroscopy and imaging measurements.

The participants' recruitment locally, their care during the studies and all other aspects apart from those mentioned previously were undertaken by me.

The FKRP registry technical support has been provided by Marcel Kiel and Ricarda Kiel. The submission to the Ethics Committee was coordinated by Dr Maggie Walter from the Friedrich Baur Institute at the Ludwig Maximillian University in Munich, as this is where the server for the registry is hosted. Thanks also goes to Brigitta von

Rekowski who helped with the proof reading of the final version of the various registry pages.

The composition of this thesis is my work. The research contained within the thesis has not been submitted elsewhere for an MD.

Acknowledgments

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I would like to thanks all the collaborators that have made this MRI study and international FKRP patient registry possible. Their input and support have been invaluable.

I would also like to thank all the participants in the study, both patients and controls. I had the pleasure of getting to know the participants from Newcastle upon Tyne (and those further afield) very well. They did not fail to surprise me with their enthusiasm to take part and willingness to return for further assessments. Whilst I have not met the other participants, I do feel that I know them fairly well from all the comments on the medical and physical report forms.

Lastly but not least I would like to thank my family, to my children for being without their mummy on weekends whilst writing but mostly to my husband who has been a control subject for me, a proof reader and supported me throughout this whole process. Without him I would not have completed this.

Abbreviations

6MWD – six minute walk distance

Abd – abduction

ACE inhibitors - angiotensin-converting enzyme inhibitors

AD - autosomal dominant

Add – adduction

ADG - α-dystroglycan

ADP – adenosine diphosphate

ALS – amyotrophic lateral sclerosis

AR - autosomal recessive

ATP - adenosine triphosphate

BDG - β-dystroglycan

BFLH - Biceps Femoris long head

BFSH -Biceps Femoris short head

BL - basal lamina

BMD - Becker muscular dystrophy

CCD – central core disease

b.p. – base pair

CK – creatine kinase

CMD - congenital muscular dystrophy

CMR – cardiac magnetic resonance imaging

CPC – category probability curves

CSPAMM - complementary spatial modulation and magnetization

CT – computerised tomography

DGC - dystrophin glycoprotein complex

DMD - Duchenne muscular dystrophy

DPG – 2,3-diphosphoglycerate

ECG - electrocardiogram

ECHO - echocardiogram

ECM - extracellular matrix

EDMD – Emery-Dreifuss muscular dystrophy

EDV - end-systolic volume

ENMC – European Neuromuscular Centre

ER - endoplasmic reticulum

Ext – extension

DIF – differential item functioning

DF - dorsiflexion

EU – European union

FCMD - Fukuyama congenital muscular dystrophy

FKRP - fukutin related protein

Flex – flexion

FP6 - sixth framework programme

FRM – fit residual mean

FSHD - facioscapulohumeral dystrophy

FVC - forced vital capacity

GRAC –Gracilis

ICC – item characterisation curves

ICCo – intraclass correlation coefficient

IRT – item response theory

L – left

LG -Lateral Gastrocnemius

LGMD - limb girdle muscular dystrophies

LGMD2I - limb girdle muscular dystrophy 2I

LON – London

LV – left ventricular

LVEF - left ventricular ejection fraction

MD - muscular dystrophies

MDC1C - congenital muscular dystrophy 1C

NMD – neuromuscular disorders

MEB - Muscle-Eye –Brain disease

MG -Medial Gastrocnemius

MHC I molecules – major histocompatability class 1 molecules

MmD – multi-minicore disease

MMT – manual muscle testing

MRI - Magnetic Resonance Imaging

mRNA - messenger ribonucleic acid

MRC – medical research council

MRS – magnetic resonance spectroscopy

M_Z – magnetisation

NCL – Newcastle

NIV - non-invasive ventilation

NMD – neuromuscular disease

NMR – nuclear magnetic resonance

MPS I – mucopolysaccharidosis type I

MPS II – mucopolysaccharidosis type II

MVC – maximum voluntary contraction

NSAA – North Star Ambulatory Assessment

US – ultrasound

^{31}P – phosphorus 31

PCr - phosphocreatine

PDE – phosphdiesters

Pi – inorganic phosphate

PL -Peroneus Longus

PME – phosphomonoesters

POMGnT1 - protein O-linked mannose beta1,2-N-acetylglucosaminyltransferase

POMT1 - protein-O- mannosyltransferase 1

POMT2 - protein-O- mannosyltransferase

PSI – person separation index

QMT – quantitative muscle testing

R – right

RF -Rectus Femoris

r.f. – radio frequency

RSMD1 – congenital muscular dystrophy with early rigidity of the spine

ROI – regions of interest

RTE – real time ultrasound elastrography

RUMM – Rasch Unidimensional Measurement Model

RYR1 – ryanidine receptor

SAR – Sartorius

SD – standard deviation

SEPN1 – selenoprotein N

SLS – single leg stance

SM – Semimembranosis

SMA – spinal muscular atrophy

SNR – signal to noise ratio

SOL –Soleus

SPSS – statistical package for the social sciences

ST – Semitendinosis

T_1w images - T_1weighted images

TA -Tibialis Anterior

T_E – echo time

TSR - torsion to endocardial strain ratio

T_R – repetition time

TR – recycle time

TREAT NMD - Translational Research in Europe-Assessment and Treatment of Neuromuscular Diseases

TUG – Timed up and Go

UPR - unfolded protein response

US – ultrasound

VL -Vastus Lateralis

VM -Vastus Medialis

WWS - Walker-Warburg syndrome

Contents

Chapter 1 – Introduction

Limb Girdle muscular dystrophy 2I (LGMD2I) is an autosomal recessive disease caused by mutations in the fukutin related protein gene (*FKRP*). Its rate of progression is variable in different individuals and it is associated with cardiomyopathy and respiratory failure. Currently there is no treatment for this rare disease and its natural history is not fully understood.

In this first chapter I will provide an overview of the muscular dystrophies as a whole. I will describe how muscle structure and function is affected in these disorders and will specifically focus on the pathogenesis of LGMD2I. This will include a clinical description of patients with LGMD2I, highlighting their cardiac, respiratory and skeletal muscle phenotype. I will then progress to explain the underlying genetic defect of this condition and will summarize our current knowledge of the role FKRP in LGMD2I based on work in animal models.

In order to provide appropriate care to patients with LGMD2I, it is imperative that the natural history of the condition is fully understood. This knowledge will therefore assist in developing clinical guidelines and standards of care that can be used to manage these patients clinically in a timely fashion. In preparing for clinical trials, objective, reliable and sensitive outcome measures are necessary. Many clinical trials use subjective measures as outcome measures, such as the six minute walk distance (6MWD) (McDonald et al. 2010). Magnetic Resonance Imaging (MRI) is an objective measure and has been used in the past for delineating patterns of muscle involvements in muscular dystrophy patients. Most of these studies have used T_1weighted (T_1w) images and a visual scoring technique (Mercuri et al. 2002a).

In order to learn more about the muscle pathology in patients with LGMD2I I coordinated a multicentre study with a focus on clinical assessment, muscle MRI and MRS and cardiac MRI in a subset of ambulatory adult patients with LGMD2I. I have analysed the MRI images both qualitatively (T_1w) and quantitatively using the 3 point Dixon technique (Dixon, 1984) in a large cohort (n=38) of patients with LGMD2I. The purpose of the study was to firstly compare the historical qualitative scoring with the quantitative MRI fat fraction, and secondly assess whether quantitative MRI was a

sensitive enough tool to detect changes in the fat fraction longitudinally, over a 12 month period. These results were then compared to the clinical and functional state of the patient.

Two of the centres were able to perform Magnetic Resonance Spectroscopy (MRS) and this was performed in just over half of the cohort (n=20) of the patients in order to assess whether there was any metabolic abnormalities detected in these patients muscles. In particular I wanted to assess whether any change detected predated the appearance on the MRI images and clinically, which could then potentially highlight MRS as an early biomarker.

One centre also performed cardiac MRI on the patients (n=10). It has been reported that cardiac MRI is more sensitive than conventional measures (Gaul et al. 2006), such as echocardiograms that are commonly used as a screening tool for cardiomyopathy in LGMD2I. These results were compared against the echocardiograms available.

The standard of care for patients with LGMD2I tends to be variable around the world, and little is known about the natural history of this condition. Part of my work on this project has been to address this issue by designing and launching the international FKRP patient registry (Appendix F), which will be discussed further in Chapter 9. This registry will enable the natural history and progression of this disorder to be studied in a large cohort internationally. As well as the development of appropriate standards of care and treatment guidelines, the registry will also provide an important resource for developing well designed trials and defining sensitive outcome measures and biomarkers.

One of the main objectives of my study was to contribute to the "trial readiness" of LGMD2I patients. With further knowledge on the natural history of the condition that can be obtained from the registry and longitudinal studies, care standards can be created and implemented internationally. With these in place, clinical audit can be undertaken and comparisons made. The knowledge of how ambulant patients with LGMD2I perform on standardised physical testing and the relevance of the tests for this group of patients, contributes to the further preparation of designing a good clinical trial. Whilst the physical and functional tests are subjective, MRI is objective. As with any outcome

measure, it not only has to be objective but clinically meaningful and sensitive to change. Thus studying the MRI data both cross sectional and longitudinal and comparing this to the physical and functional tests is important in order to address this.

As one prepares for "trial readiness", it is important to realise that there is however no 'one' test that can fully assess this complicated and heterogeneous group of patients. A combination of functional assessments, strength measurements and MRI need to be considered.

1.1 – Background and overview of the research project

In this section I will be covering an overview of the muscular dystrophies as a whole. I will describe how muscle structure and function is affected in these disorders and will specifically focus on the pathogenesis of LGMD2I. This will include a clinical description of patients with LGMD2I, highlighting their cardiac, respiratory and skeletal muscle phenotype.

1.1.1 - Overview of muscular dystrophy

The disorders known as the muscular dystrophies (MDs) are a group of hereditary conditions that is characterised by progressive weakness and degeneration of skeletal muscle. The pattern of these abnormalities shows a variation in both distribution and severity. The diseases can be subdivided into several disease types. These subdivisions are largely based upon the age of the patient and the distribution and pattern of weakness (**figure 1.1**) (Emery, 2002). They include the dystrophinopathies, Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD), Emery-Dreifuss muscular dystrophy, distal muscular dystrophy, congenital muscular dystrophy (CMD), facioscapulohumeral dystrophy (FSHD), myotonic dystrophy, oculopharyngeal muscular dystrophy, and limb girdle muscular dystrophy (LGMD). LGMD represent the most heterogeneous group.

MDs are caused by a wide range of gene mutations, ranging from genes encoding structural proteins such as dystrophin in DMD, to sarcomeric proteins such as telethonin, and membrane regulators such as dysferlin.

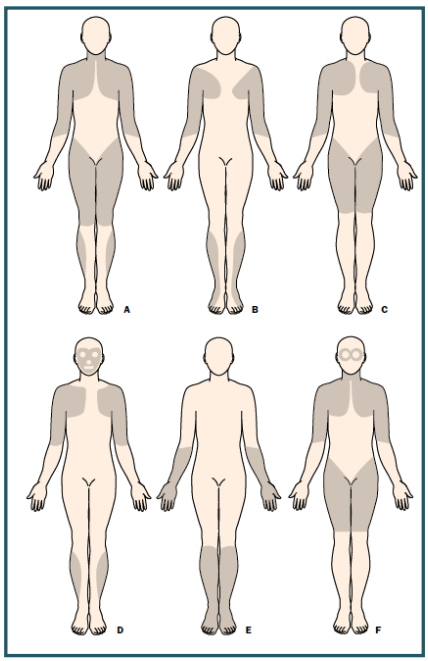


Figure 1.1: Distribution of predominant muscle weakness in different types of dystrophy.

A; DMD and BMD type, B; Emery-Dreifuss, C; LGMD, D; facioscapulohumeral, E; distal, F; oculopharyngeal. Shaded = affected areas (Emery, 2002).

Function of normal muscle

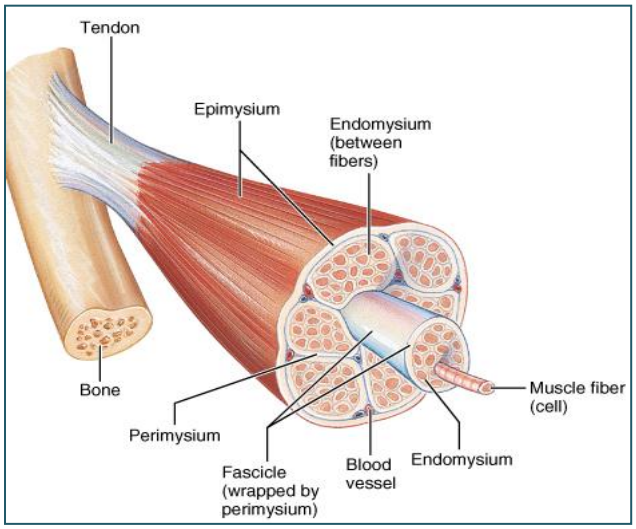
Skeletal muscles are essentially collections of muscle units bound by connective tissue. During development, undifferentiated myoblasts fuse to form long, cylindrical multinucleated cells which make up the muscle fibre. The muscle fibre is made up of bundles of myofibrils, and the myofibrils in turn consist of bundles of myofilaments. These myofilaments are primarily made up of the two proteins, actin and myosin (Engel and Franzini-Armstrong, 2004).

When viewed under the microscope, a distinctive banding pattern is evident within the muscle due to the arrangement of the cytoskeletal elements in the muscle cytoplasm, also called sarcoplasm. Actin and myosin also referred to as ‘thin’ and ‘thick’ filaments respectively are arranged in a repeated unit, called the sarcomere. Surrounding each myofibril is the sarcoplasmic reticulum, which holds a reserve of calcium ions required for muscle contraction. The interaction of actin and myosin in the myofilaments and the release of calcium, signalled by the action potential, are responsible for muscle contractions (Saladin, 2010).

Skeletal muscle is required to withstand repeated cycles of contraction and relaxation throughout a lifetime. In order to maintain stability a highly specialised system of

linkages between the subsarcolemmal cytoskeleton and the components of the extracellular matrix are required. Many muscular dystrophies are caused by either a disruption at some point in the link between the extracellular matrix and the cytoskeleton or by abnormalities in the specific matrix components themselves (Campbell, 1995). A patient with MD does not have normal muscle function and stability.

(a)



(b)

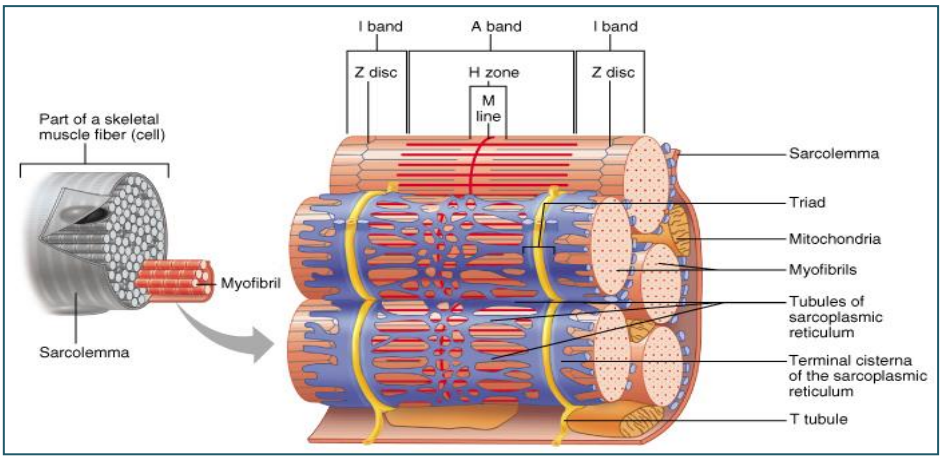


Figure 1.2: Diagram of skeletal muscle. (a) Skeletal muscle surrounded by epimysium and composed of fascicles made up of myofibres. Each muscle fibre is surrounded by endomysium. (b) A enlargement of a myofibril, which is a cylindrical bundle of contractile proteins found within the muscle cell. Myofibrils are composed of individual contractile proteins called myofilaments. These myofilaments are generally divided into thick and thin myofilaments. It is the arrangement of the contractile proteins within the myofibril that cause the striated appearance of skeletal and cardiac muscle.
http://faculty.etsu.edu/forsman/Histologyofmuscleforweb.htm

The Dystrophin Glycoprotein Complex (DGC)

In skeletal muscle the dystrophin glycoprotein complex (DGC) works as a transmembrane linkage between the extracellular matrix and the cytoskeleton (**figure 1.3**) and hence is important in normal muscle stability. Dystroglycan is a central component of the DGC and is made up of two types of protein, alpha-dystroglycan (ADG) and β-dystroglycan (BDG). The ADG is a peripheral membrane protein and the receptor for laminin-211 in the basement membrane. BDG is a transmembrane protein that binds to dystrophin (Campbell, 1995, Bushby 1999).

Dystrophin is a large rod-like cytoskeleton protein, absent in DMD patients. Dystrophin binds to intracellular actin cables. In this way, the DGC, links the actin cytoskeleton of a muscle cell with extracellular ligands, such as laminin and provides structural integrity in muscle tissues. The DGC is also known to serve as an agrin receptor in muscle, where it may regulate agrin-induced acetylcholine receptor clustering at the neuromuscular junction. This illustrates how the function of individual components of this complex works synergistically to achieve muscle and membrane stability (Engel and Franzini-Armstrong, 2004).

Integral to the stability of the DGC is the correct glycosylation of ADG. POMT1 (protein-O- mannosyltransferase 1), POMT2 (protein-O- mannosyltransferase 2) and POMGnT1 (protein O-linked mannose beta1, 2-N-acetylglucosaminyltransferase) have been shown to catalyze specific steps of O-linked glycosylation of ADG and are known to cause CMD. The function of the proteins fukutin, FKRP (fukutin related protein) and LARGE have not been fully explained (Brown et al. 2005, Xiong et al. 2006, de Paula et al. 2003, Brockington et al. 2005). The function of FKRP will be covered in Chapter 1.1.5 as it is a mutation in the FKRP gene that causes LGMD2I, our study cohort.

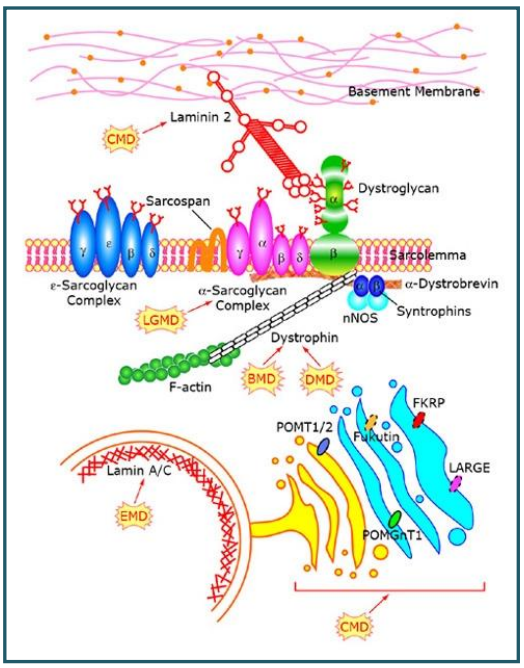


Figure 1.3: Current interaction model of the proteins in the Dystrophin Glycoprotein Complex (DGC). Proteins affected in muscular dystrophies are indicated.

http://www.sanger.ac.uk/Teams/Team31/muscle.shtml

Limb Girdle Muscular Dystrophy

The diagnostic entity of 'Limb Girdle Muscular Dystrophy' (LGMD) was first assigned by Walton and Nattrass in 1954. Initially the diagnosis was controversial. The controversy was mainly due to a lack of clarity and definition. It was assigned to a heterogeneous group of patients who typically presented in the late first or second decade of life (Bushby et al. 2009a).

The disease was described as presenting in either male or female patients genetically inherited usually in either an autosomal recessive (AR) way and less frequently autosomal dominant (AD). The pattern of muscle involvement was predominantly proximal muscle weakness with shoulder and hip girdle involvement and facial sparing. The disease had a progressive course with loss of ambulation after 20-30 years with elevated creatine kinase levels and dystrophic features on muscle biopsy.

With the development of molecular genetics and improved diagnostic methods this group of disorders have been more clearly defined especially over the last 15-20 years. In 1995, this led to further categorisation of the LGMDs, with the AD groups assigned LGMD1 and the AR group assigned LGMD2.

As different genetic subtypes have been described, the group of LGMDs recognised has expanded and can now be distinguished by specialised diagnostic techniques. Currently at least 21 types of different genetically defined subtypes of LGMDs are recognised and can be seen in **table 1.1** (Bushby et al. 2009a).

Table 1.1: This table summarises the current limb girdle muscular dystrophy classification (Bushby et al. 2009a)

LGMD forms	Chromosome	Protein	Important Complications	Other diseases associated with this gene
Autosomal Dominant				
LGMD1A	5q31	Myotilin	Other forms of myofibrillar myopathies more associated with cardiac and respiratory complications	Myofibrillar myopathies, spheroid body myopathy.
LGMD1B	1q11-21	Lamin A/C	High risk of arrhythmia with requirement for an implantable defibrillator, cardiomyopathy, respiratory failure.	Many including AD Emery-Dreifuss MD and dilated cardiomyopathy
LGMD1C	3p25	Caveolin 3		Rippling muscle disease, hyperCKaemia, myalgia, hypertrophic cardiomyopathy
LGMD1D	7q	?		
LGMD1E	6q23	?		Cardiomyopathy and conduction defect
LGMD1F	7q32	?		
LGMD1G	4p21	?		
Autosomal Recessive				
LGMD2A	15q15.1	Calpain 3		
LGMD2B	2p13	Dysferlin		Miyoshi myopathy
LGMD2C	13q12	γ- sarcoglycan	Cardiomyopathy and respiratory impairment.	
LGMD2D	17q21	α- sarcoglycan	Cardiomyopathy and respiratory impairment.	
LGMD2E	4q12	β- sarcoglycan	Cardiomyopathy and respiratory impairment.	
LGMD2F	5q33	δ- sarcoglycan	Cardiomyopathy and respiratory impairment.	
LGMD2G	17q12	Telethonin		
LGMD2H	7q31-q33	TRIM 32		Sarcotubular myopathy
LGMD2I	19q	FKRP	Cardiomyopathy and respiratory impairment; diaphragmatic involvement may cause respiratory insufficiency whilst still ambulant.	Congenital muscular dystrophy type 1C (MDC1C), Walker-Warburg syndrome
LGMD2J	2q24.2	Titin		Heterozygous mutations cause AD tibial MD
LGMD2K	9q34	POMT1		Walker-Warburg syndrome
LGMD2L	11p13-12?	Anoctamin 5		
LGMD2M	9q31	Fukutin		Fukuyama muscular dystrophy
LGMD2N	14q24	POMT2		Walker-Warburg syndrome
CK, creatine kinase; LGMD, limb girdle muscular dystrophy; POMT, protein O-mannosyltransferase; MD, muscular dystrophy; AD, Autosomal dominant.				

The overall prevalence of all the LGMDs has been estimated at 5-70 per million populations in several countries; however different populations have different frequencies of the various LGMDs. Norwood et al. (2009) studied the North of England's neuromuscular population and found out of 1105 patients registered, 68 were classified as having LGMD, representing a population prevalence of 2.27/100,000.

The autosomal recessive LGMDs are more common with only approximately 10% of the total accounted for by autosomal dominant LGMDs (Norwood et al. 2009). In all studies conducted, LGMD2A appears to be the commonest LGMD accounting for approximately 8-26% of all cases (van der Kooi et al. 2007, Balci et al. 2006), although this figure is 5-6 times lower in the ethnic Danes (Duno et al. 2008). LGMD2B accounts for 3-19%. Sarcoglycanopathies (LGMD2C-F) as a group account for 3-18%, however individually they are relatively rare. Within the sarcoglycanopathy group, LGMD2D (α-sarcoglycanopathy) is the commonest, and overall this type is twice as common as LGMD2C (γ-sarcoglycanopathy) and LGMD2E (β-sarcoglycanopathy) with LGMD2F (δ-sarcoglycanopathy) the rarest. These figures apply to Europe only.

The prevalence of LGMD2I worldwide is 3-8% of LGMDs, however in certain parts of Northern Europe, including Denmark and parts of England, the prevalence of LGMD2I is higher. Sveen et al. (2006) showed a high population of 38% of LGMDs with LGMD2I, and in the North of England accounted for 19.1% of the LGMD group, a prevalence of 0.43/100,000 (Norwood et al. 2009). This reflects possible previous migration from Denmark to the Northern region of England.

Overall morbidity and mortality rates depend upon associated features, such as respiratory or cardiac involvement. The earlier the onset of the disease, generally a more rapid and precipitous course is observed. This is in contrast to the late onset cases of LGMD where a more indolent course is seen. Variability is seen both within the LGMD subtype and within families with the same mutation. Some patients present in early childhood with an aggressive or congenital muscular dystrophy phenotype, whereas other family members may be asymptomatic with hyperCKaemia.

1.1.2 – The clinical phenotype of LGMD2I

In this subchapter I will cover the clinical phenotype of LGMD2I and highlight the involvement seen in the skeletal muscles, cardiac muscles and respiratory system.

Skeletal muscle phenotype

Whilst the limb girdle muscular dystrophy phenotypes present with a high degree of overlap, a definitive diagnosis can be made in the majority of cases. This is based on a combination of clinical signs and symptoms, immunohistochemistry on the muscle biopsy, genetic testing and more recently imaging. With all these modalities a more defined differential diagnosis can be achieved, eventually leading to a precise diagnosis and hence correct management and prognosis (Bushby, 2009a).

LGMD2I is a heterogeneous condition with age of presentation varying between early childhood and mid adulthood (Poppe et al. 2003). These patients can present with either an early onset DMD like phenotype with rapid deterioration and loss of ambulation in their teens or present later in adulthood with a less severe phenotype (Poppe et al. 2004). In the study by Poppe et al. (2004) approximately 1/3 of the cohort reported symptoms in childhood, although only one had actual delayed motor milestones (mild), the others reported that they were poor at sport, unable to run fast or were toe walkers. The majority of the cohort had their onset of symptoms during the 2^{nd} to the 4^{th} decade. The difficulties experienced were mainly related to proximal muscle weakness and included; walking, particularly up slopes, stair climbing and a waddling gait. Patients generally reported an indolent period from between 5-45 years followed by a more noticeable deterioration over 5 to 10 years.

The pattern of skeletal involvement is similar to the other LGMDs with proximal weakness in both the pelvic and shoulder girdle, however distinctions can be made. Early onset DMD phenotypes can present with greater degrees of weakness in their shoulder girdle compared to other early onset LGMDs, as in the sarcoglycanopathies (Mercuri et al. 2003). The weakness tends to be limited to the proximal muscles with clinically sparing of the distal muscles, and good strength in the hands and also the ankles in plantarflexion, dorsiflexion, inversion and eversion. There tends to be demonstrable asymmetry but not to a large extent. Facial weakness is not generally

recognised, however tongue hypertrophy and dysphagia has been reported (Mercuri et al. 2003). Generally there is no scapular winging or neck weakness, but if present it is usually mild (grade 4 out of 5 on the MRC (Medical Research Council) scale). Poppe et al. (2003) reported that in patients with LGMD2I the quadriceps muscles are generally weaker than the hamstrings and this is reflected in hip flexion which is very weak as is hip adduction and abduction, more so than hip extension.

In the upper limbs there is no distal weakness and in general the biceps muscles are stronger than the triceps and shoulder abduction was stronger than adduction (Poppe et al. 2003).

Contractures are not commonly seen in this group of patients, but some early onset DMD like phenotypes can have tendon Achilles tightness and contractures (Poppe et al. 2003, Mercuri et al. 2003).Whilst most patients have a lumbar lordosis, scoliosis or rigid spine is variable. In Poppe's study (2003), only one patient had a scoliosis, compared to Mercuri's study (2003), where nine of the cohort had a scoliosis. Although the study numbers were similar, 16 and 18, respectively, 13 out of the 16 studied by Poppe et al. were homozygous for the common mutation (C826A/Leu279Ile) compared to only 1 out of the 18 studied by Mercuri et al. (2003). The majority of patients tend to have calf hypertrophy and can have hypertrophy of other muscles including the quadriceps and more unusually the tongue (Poppe et al. 2003, Mercuri et al. 2003).

Respiratory phenotype

Patients with LGMD2I are prone to early respiratory involvement. This tends to be independent of their skeletal or cardiac muscle involvement. These patients can still be ambulant and require nocturnal non-invasive ventilation (NIV) with the earliest sign of involvement being diaphragmatic, with a drop in their lying forced vital capacity (FVC) compared to their sitting. This has been illustrated in the study by Poppe et al. (2004), where 44.4% of the patients exhibited respiratory compromise, and 5 out of the 8 patients ventilated were still ambulant.

In a previous study, Poppe et al. (2003) demonstrated in a LGMD2I cohort that the FVC ranged from 19-102% (corrected for height) In 2/3 patients there was a drop in lying from 8-22% suggesting diaphragmatic involvement. Over time a gradual deterioration

was observed in 7 patients over 5 years. Respiratory failure was documented in 25% of the cohort and NIV instigated with relief of symptoms. Half of the patients on NIV were still ambulant.

Cardiac phenotype

The relationship between cardiac and skeletal phenotypes is complex. The association of cardiac disease, whether cardiac dysrhythmias or cardiomyopathy, is individual depending upon the gene mutation. Some muscular dystrophies tend to have a higher relative risk. This has important implications for management. Cardiac involvement in patients with LGMD2I has been reported as being between 10% (Walter et al. 2003) and 55% (Poppe et al. 2004). Poppe and colleagues (2004) conducted a multicentre study with LGMD2I patients, who had an overall prevalence of 55.3% when assessed. The cardiac involvement was more frequent in men, with 83% affected compared to 42.3% of women. There was however a higher proportion of compound heterozygotes in the male group compared with the women who were predominantly homozygotes. Using the Kaplan-Meier analysis this suggests that 66% of heterozygotes would have cardiac involvement by the age of 20 years and 100% by 39 years, compared to the homozygotes where only 4.4% would have cardiac involvement by 20 years and 100% would have cardiac involvement by 58 years. At the time of the study 83% of the patients were still ambulant (Poppe et al. 2004).

In a previous study by Poppe et al. (2003) it was shown that the incidence of cardiac involvement was 38%. This study had a small cohort of LGMD2I patients and the majority of patients were homozygous for the common mutation (L276I) and hence less phenotypically severe.

Due to the high prevalence of cardiac involvement in LGMD2I, it is therefore important to assess the patients regularly and start treatment early as this may have a cardioprotective effect (Duboc et al. 2007). Cardiac involvement in LGMD2I is amenable to treatment with ACE (angiotensin-converting-enzyme) inhibitors. In a large study by SOLVD investigators in 1992, it was demonstrated that left ventricular dysfunction improved and stabilised with the use of ACE inhibitors. This study was on patients without muscular dystrophy.

1.1.3 - The genotype of LGMD2I

More than 70 different FKRP mutations have been identified, of which approximately 40 cause LGMD2I (Human Genome Mutation Database). The *FKRP* gene consists of four exons containing a 1,488 base pair (bp) open reading frame that encodes a 495 amino acid protein. Mutations in the *FKRP* gene not only cause LGMD2I but more severe congenital muscular dystrophies such as congenital muscular dystrophy 1C (MDC1C), muscle eye brain disease (MEB) and Walker-Warburg syndrome (WWS) (de Paula et al. 2003). Whilst LGMD2I and MDC1C are allelic, they are clinically, pathologically and genetically diverse. Different types of mutations cause MDC1C, whereas the majority of LGMD2I are caused by the common C826A missense mutation in the *FKRP* gene (Mercuri et al. 2003, Wickland and Hilton-Jones 2003, Brown et al. 2004).

(i) The LGMD phenotype

The c.826C>A/Leu276Ile mutation is the most frequent among LGMD2I patients in Northern Europe. Patients homozygous for this common mutation tended to have a milder phenotype compared to those patients with a compound heterozygous mutation who presented with the earlier more severe DMD like phenotype (Mercuri et al. 2003, Sveen et al. 2006). In Northern Europe, LGMD2I appears to be one of the most common forms of LGMD (Poppe et al. 2003, Brockington et al. 2001a, Brockington et al. 2001b, Driss et al. 2003, Walter et al. 2004, Beltran Valero de Baernab et al. 2004, and Norwood et al. 2009). Sveen et al. (2006) identified FKRP mutations in 38 of 99 Danish individuals with a clinical diagnosis of limb-girdle muscular dystrophy. Of the 38 individuals, 27 were homozygous for the Leu276Ile mutation, and 11 were compound heterozygous for the Leu276Ile mutation and another pathogenic FKRP mutation. The homozygous patients had later onset, milder clinical progression, and less muscle weakness compared to compound heterozygous patients, all of whom were wheelchair-bound by their mid-twenties. Cardiac and respiratory involvement was found in both groups. Nine were homozygous for the Leu276Ile mutation, but no compound heterozygous, patients had initial symptoms of exertional myoglobinuria. The Leu276Ile variant was identified in 1 of 200 control alleles.

In Norway, 87 live patients were identified with FKRP mutations in 2008 (Stensland et al. 2011). This corresponded to a minimum point prevalence of 1/54,000 and a carrier rate of 1/116. The allele frequency for c.826C>A has previously been reported as 1/200 for Denmark and the UK (Beltran Valero de Baernabe et al. 2004, Brockington et al. 2001b) and 1/600 for Germany (Walter et al. 2004).

In Southern Europe higher frequencies of other *FKRP* mutations have been found (de Paula et al. 2003, Boito et al. 2005, Harel et al. 2004). In 16 patients with LGMD from 13 Brazilian families, de Paula et al. (2003) identified 10 distinct mutations, including 9 novel mutations, in the *FKRP* gene. The most common mutation, Leu276Ile, was identified in 9 of 26 alleles. Like those patients who carry the c.826C>A mutation, there was substantial variability seen those that carry the same genotype (Harel et al. 2004), and mildly affected patients other than those homozygous for the c.826C>A mutation have been described (de Paula et al 2003, Harel et al. 2004).

(ii)The CMD phenotype

Mutations in the *FKRP* gene can cause the more severe phenotypes, MEB and WWS. Beltran-Valero de Bernabe et al. (2004) identified homozygous mutations in the *FKRP* gene in 2 unrelated patients with muscle-eye-brain disease and Walker-Warburg syndrome, respectively. Both disorders are characterized by severe disruption of brain and eye structure in addition to muscular dystrophy. Mercuri et al. (2009) identified *FKRP* mutations in 7 (9%) of 81 Italian patients with a dystroglycanopathy. Three had MEB and 4 had a less severe congenital muscular dystrophy. Three patients had normal brain MRI. The findings expanded the phenotypic spectrum of disorders associated with mutation in the *FKRP* gene.

In a retrospective review of brain MRI in patients with *FKRP* mutations, Mercuri et al. (2006) found a range of various patterns. Five of 13 patients had normal imaging results and normal neurologic function. Three patients had isolated cerebellar cysts and mental retardation without other abnormal brain structure. Of the 5 remaining patients, 2 had features of MEB disease, one had features of WWS, and 2 had cerebellar cysts with nodular heterotopia and cerebellar dysplasia, respectively. Topaloglu et al. (2003) also demonstrated cerebellar cyts and mental retardation in patients with CMD and *FKRP*

mutations. There was no correlation with severity of the neurologic involvement and *FKRP* mutation. Mercuri et al. (2006) postulated that the variability may be related to the severity of disruption of alpha-dystroglycan glycosylation.

1.1.4 – Care standards in LGMD2I

The ideal model of care for patients with neuromuscular disease is a holistic one, based on medical, social and psychological elements. These patients require a multidisciplinary approach and the level of care and treatment given should be universal. As severe LGMD2I patients have a phenotype similar to BMD and DMD, the standards of care, recently published (Bushby et al. 2010) for DMD, should be adhered to as these have led to improved care and outcomes in terms of morbidity and survival in these patients. The care guidelines direct clinicians towards a more proactive management of complications rather than a reactive one. As in DMD and BMD, LGMD2I patients are at risk of both cardiomyopathy and type II respiratory failure, this therefore needs to be monitored and treated early to prevent further morbidity.

As part of the initial assessment of these patients, a structured history and family history should be taken and a physical examination conducted by a physician who is experienced in neuromuscular disorders, with the focus on the strength, motor function and any resultant functional impairment. Regular assessments should be obtained at each visit, monitoring the disease progression, such as strength, range of motion, timed tests and gait. Functional assessments should also be applied to assess the level of ability to cope with activities of daily living and psychological adjustments to any loss of function.

The suggested range of neuromuscular assessments for patients with DMD could be applied to the LGMD2I patient as described in **table 1.2**;

Table 1.2: Suggested neuromuscular assessments for patients with DMD. (Bushby et al. 2009)

	Method	Aim of testing	Ambulatory	Non-ambulatory
Strength testing	Manual muscle testing (MRC scale) Quantitative myometry (beneficial if muscle strength 3-5 on MRC scale)	Serial assessment; to identify outliers from expected clinical course; to monitor disease progression and predict functional losses; to assess response to treatment; and to monitor muscle imbalance.	Test lower extremity strength by manual muscle testing every 6 months.	Early stages; Test upper and lower extremity strength by manual muscle testing every 6 months. Later stages; value of testing is less certain.
Range of motion	Goniometry	Baseline: to identify emerging muscle hypo-extensibility and joint contractures that might contribute and lead to functional deterioration or musculoskeletal or integumentary problems. To identify need for additional or altered therapeutic/surgical intervention (ie orthoses, splinting, use of standers, iliotibial band lengthening)	Lower extremities; hip, knee, ankle joints, iliotibial band, hamstrings, gastrocnemius.	Lower extremities; hip, knee, ankle joints, iliotibial band, hamstrings, gastrocnemius. Upper extremities; elbow, wrist, long finger flexors.
Timed testing	Standardised use of timed function tests	Easy and relevant measure of daily functional status; responsive to change.	Timed 10m walk, timed Gowers' manoeuvre, time to climb 4 stairs, time to rise from a chair, 6 minute walk distance. Time to put on a shirt maybe relevant in late ambulatory phase.	Time to put on a shirt maybe relevant in early non-ambulatory phase. Timed tests not applicable in the late non-ambulatory phase.
Activities of daily living	Assessment of impairment in daily activities in the home, school and community settings.	Highly relevant to targeted input with aids, adaptations and access to environmental controls	Frequency of falls, step activity monitoring, self-care skills, writing, computer use. Functioning in school and community setting.	Self-care skills, writing, computer use, control of manual and electric wheelchair. Functioning in school and community setting.
Motor function scales	Assessment of motor function in specific domains to give a composite score	Allows monitoring of progression and response to therapy	Vignos lower extremity scale, North Star ambulatory assessment, motor function measure.	Brooke upper extremity scale, Egen Klassification functional assessment, Hammersmith motor scales, motor function measure.
Routine clinic appointments should be every 6 months, unless otherwise specified. Specialist physical and occupational therapy assessments are recommended every 4 months. MRC UK Medical Research Council. Although the panel found these tests to be appropriate assessment tools they are used more typically in research than in clinical settings.				

Although not all these tests will be possible in a busy clinic setting, it is important that the assessments that are used are appropriate for the patient and completed. These assessments should ideally be done consistently and over time to delineate the progression of the disease. I will now describe some of these assessments in more detail with relevance to LGMD2I.

(i) Mobility and strength;

Stretching and Positioning Effective stretching of the musculotendinous unit requires a number of interventions including passive stretching, active stretching, active-assisted stretching as well as the use of splints and orthoses to achieve a prolonged stretch of the unit to prevent contractures. Whilst contractures are not usually a marked feature in LGMD2I daily stretching, active, passive and active-assisted is advised on at least 4-6 days per week. Stretching of the hips, knees and ankles are essential to aid ambulation and when non-ambulant to prevent contractures and ultimately difficult positioning in the wheelchair.

Orthoses are used to prevent or minimise contractures. These are particularly useful in the non-ambulant phase when a custom-moulded AFO (Ankle Foot Orthoses) can be fashioned and fabricated for comfort and optimum foot and ankle alignment.

Exercise Regular gentle exercise, such as swimming, is recommended and the evidence suggesting exercise as a treatment is just beginning to be recognised. Aerobic exercise is safe and beneficial in a number of neuromuscular diseases (van der Kooi et al. 2005). Sveen et al. (2007) found that over 12 weeks of low intensity aerobic exercise, patients with LGMD2I increased their level of fitness with documented increased endurance, leg strength and walking distances. There was no evidence of muscle damage and no increase in CK levels. Capillary densities increased with training by 18%. The capillary density prior to the exercise regime had been 55% lower than healthy controls. This phenomenon has previously been described by Olsen et al. (2003). The disease process or deconditioning may be the reason that the difference in capillary densities is seen and the lower capillary density is associated with a 43% higher fibre type area.

Endurance training is also advised by the cardiologists (Stolen et al. 2003) and therefore would be of benefit to patients with LGMD2I as they develop cardiomyopathy as part of the condition.

(ii) Cardiac health;

LGMD2I patients should have regular cardiac evaluations as part of their annual review to detect early signs of cardiomyopathy. Cardiomyopathy can develop independently to their walking ability and skeletal muscle strength (Sveen et al. 2008). Early treatment with beta blockers and ACE inhibitors is well recognised to improve cardiac function in established cardiomyopathy (SOLVD investigators, 1992). Further research is required into whether treatment with these cardio-protective drugs before the onset of cardiomyopathy in muscular dystrophy should be commenced. Further research is also needed into more sensitive markers, such as cardiac MRI, and whether this should be employed if a normal echocardiogram (ECHO) is obtained.

The current clinical practice in the care of patients with LGMD2I is the same as employed in the treatment of Duchenne and Becker muscular dystrophy (DMD/BMD) patients (Bushby et al. 2010) At diagnosis all patients should have a cardiological workup including an ECHO and electrocardiogram (ECG). This should be followed up by annual assessments depending on investigational findings and clinical symptoms. Patients should be treated with angiotensin-converting enzyme (ACE) inhibitors initially in the presence of progressive abnormalities (Ishikawa et al. 1999). Subsequently the addition of beta blockers should be considered (MacMahon et al. 1997).

(iii) Respiratory health;

LGMD2I are at risk of type II respiratory failure due to diaphragmatic weakness. As a consequence patients should be assessed on a yearly basis with at least a sitting and lying Forced Vital Capacity (FVC). If a postural drop is observed and/or symptoms of nocturnal hypoventilation are observed, then a sleep study should be requested and early referral to a respiratory physician with experience in non-invasive ventilation (NIV) made.

(iv) Steroid treatment;

In LGMD2I, there is a small amount of data concerning therapeutic steroid use. Darin et al. (2007) reported two patients, both with a DMD phenotype and compound heterozygotes. In both patients they commenced on steroids at a lower dose of 0.35mg/kg/day as per the Swedish study (Backman et al.1995), rather than the higher usual dose of 0.75mg/kg/day used in DMD (Manzur et al. 2004). Both patients, treated in their teens, made dramatic improvements in motor function and even on relatively small doses maintained motor function. In one the steroid was withdrawn as he was only on 0.07mg/kg/day, however he rapidly deteriorated but regained his previous motor function on restarting at the same dose (Darin et al.2007).

1.1.5 – The FKRP gene and its protein product

Since the discovery of the *FKRP* gene in 2001 by Brockington and colleagues, there has been much debate as to the function of FKRP and its role in LGMD2I and MDC1C. In this subchapter I will describe the process of glycosylation, which affects the stability of the DGC and hence the muscle in patients with LGMD2I and the role of FKRP in this process.

(i) Glycosylation

Glycosylation, the enzymatic addition of carbohydrates to lipids or proteins, is the most common and most complex post translational modification (Wopereis et al. 2006). Approximately 1% of human genes are required for this process (Lowe and Marth, 2003) and it is estimated that almost half of all proteins are glycosylated (Apweiler et al. 1999). Glycosylation can be divided into three main groups, N-linked, O-linked and C-linked glycosylation (Lowe and Marth, 2003).

Glycans possess the potential for huge structural diversity, they have many branches and can form glycosidic linkages with sugar residues in α or β configuration. O-linked glycans are classified by the first sugar attached to a Ser, Thr or Lys residue in a protein. There are 7 O-glycans identified in humans and O-Mannosyl glycans represent the least common type, of which ADG is one (Wopereis et al. 2006)

The biosynthesis of complex O-linked and N-linked glycans is located in the secretory pathway (Wells and Hart, 2003). Proteins are made by the ribosomes and then directed to the rough endoplasmic reticulum (ER) following which protein folding occurs and then transportation by the transport vesicle to the Golgi apparatus (Matlack et al. 1998, Rapoport et al. 1996)

The O-linked glycans are found to have functions involving protein structure and stability, immunity, receptor mediated signalling, non-specific protein interactions and protein expression and processing (Wopereis et al. 2006). The sialylated O-mannosyl glycans of ADG serves as binding sites for laminin in both muscle and brain (Endo, 2004).

(ii) Glycosylation defects of dystroglycan.

Recently there has been recognition that a number of congenital muscular dystrophies and LGMDs have been associated with abnormal glycosylation of the DGC (Muntoni et al. 2002, Muntoni et al. 2004, Martin 2007, Barresi and Campbell 2006). As stated earlier the DGC is a large protein complex, derived from post translational cleavage of a precursor polypeptide encoded by the DAG1 gene (Henry and Campbell 1999, Winder 2001, Ervasti and Campbell 1993), that is integral to maintaining the stability of the sarcolemma by connecting components of the extracellular matrix to the internal cytoskeleton of the muscle fibre (**figure 1.3**).

In congenital muscular dystrophy mutations in six genes have so far been identified. These include *POMT1, POMT2, POMGnT1, LARGE, fukutin* and *FKRP* (Brockington et al. 2001a, Beltran-Valero de Bernabe et al. 2002, van Reeuwijk et al. 2005, Yoshida et al.2001, Kabayshi et al. 1998, Longman et al. 2003). They cause a spectrum of diseases from severe congenital muscular dystrophies, usually associated with structural brain malformations and eye involvement to a variable extent. Fukuyama congenital muscular dystrophy (FCMD), muscle-eye–brain disease (MEB) and Walker-Warburg syndrome (WWS) represent the severe CMDs, (Muntoni et al 2004) whilst MDC1C is milder and does not have any associated brain involvement. As with the other CMDs, patients with MDC1C present early in life, usually before 6 months, with hypotonia and weakness, which is predominantly limb girdle in distribution and associated with severe

wasting in these muscle groups. Patients are unable to walk and also develop a severe restrictive respiratory defect progressing to failure in the second decade. Cardiomyopathy is also observed in some patients (Brockington et al. 2001a), however the brain is rarely involved, although severe mutations can lead to cerebellar changes (Topaloglu et al. 2003, Mercuri et al. 2006) or more extensive changes as seen in WWS and MEB (Longman et al. 2003).

The LGMD type 2 variants include LGMD2I, LGMD2L and LGMD2N.

(iii) Functional properties of FKRP

There has been much debate about the subcellular localisation of FKRP. Originally it was thought that both Fukutin and FKRP were Golgi resident proteins (Escapa et al. 2002) required for post-translational modification of dystroglycan and that mislocalisation of the mutant protein may underlie MDC1C. Later it was thought that FKRP localised to the rough ER (Escapa et al. 2005, Torelli et al. 2005) and that protein mislocalisation was not a common mechanism of disease in MDC1C and LGMD2I (Dolatshad et al. 2005, Torelli et al. 2005). More recently it has been hypothesised that FKRP is present at the cell surface, associates with the DGC and has a unique role in the ADG processing pathway (Beedle et al. 2007).

The *FKRP* gene which encodes the FKRP is thought to be a tissue specific glycosyltransferase involved in the O-mannosylation of ADG (Brockington et al. 2001a). FKRP is expressed in many tissues including skeletal muscle, placenta and heart and less predominantly in the lung, liver, kidney, pancreas and brain (Wopereis et a.l 2006).

FKRP has been shown to directly affect dystroglycan processing by altering the isoforms of ADG. A mutation in *FKRP* has been demonstrated in vitro and causes a decrease in molecular weight of the ADG (Esapa et al. 2002). This could be secondary to modification, possibly explained by the addition of terminating glycans therefore no further modification can occur, or by altering the stability of the dystroglycan rendering it more susceptible to endogenous proteases (Esapa et al. 2002). This is evident in patients; mutations in fukutin result in an absence of glycosylated ADG on muscle biopsies, whereas mutations in the *FKRP* gene cause a decrease but still detectable

levels of ADG, indicating a difference in dystroglycan processing (Brockington et al. 2001a, Brockington et al. 2001b, Boito et al. 2007, Yamamoto et al. 2008).

Dystroglycan glycosylation is important for binding to laminin and agrin in the basal lamina (Ervasti and Campbell, 1993, Gee et al. 1994, Sugiyama et al. 1994). Abnormalities in the basal lamina (BL) and reductions in laminin alpha 2 have been demonstrated in the muscle and brain of patients with FCMD. This may represent defects in the BL and extracellular matrix (ECM) that involve components of the DGC. In MDC1C, disruption of the BL due to reduced capacity to bind ADG to EC laminin may be present. MDC1C patients also have secondary laminin immunoreactivity (Brockington et al. 2001a). In LGMD2I there is evidence on ultra structural studies of a focally disorganised and thinner BL (Boito et al.2007).

Esapa et al. (2005) showed that mutant FKRP is altered in the cells and ER retained, whilst wildtype FKRP and mutant L276I that causes the milder LGMD2I were predominantly found in the Golgi apparatus. Mutant FKRP and hypoglycosylated ADG may be misfolded in the cell and detrimental to it (Boito et al. 2007). Esapa et al. (2005) also demonstrated that the ER retained proteins had a shorted half-life than the wildtype FKRP plus calnexin, an ER chaperone molecule, bound preferentially to the ER retained mutant FKRP acting as a possible quality control pathway.

Upregulation of several ER resident proteins then occurs to re-establish cell homeostasis, via a process of unfolded protein response (UPR), an intracellular signalling pathway from the ER to the nucleus after triggers from misfolded proteins (Ellgaard and Helenius, 2001). Upregulation of MHC class I molecules were also identified and this could signal UPR (Boito et al. 2007). Upregulation of class I MHC molecules may induce ER stress and lead to damage and dysfunction of the muscle fibre in a non-immune fashion. In the LGMD2I muscle biopsies it was observed that the rough ER had proliferated and modified (Boito et al. 2007).

(v) Animal model studies

At present there is no animal model for LGMD2I, however zebrafish have been used in the study of the pathogenesis of muscular dystrophies (Parsons et al. 2002a, Basset and Currie, 2003, Basset et al. 2003, Guyon et al. 2005, Nixon et al. 2005). There have been

various Zebrafish mutants studied involving an ADG binding protein, such as the candyfloss (caf) mutant associated with mutations in the laminin α-2 gene and a degenerative muscle phenotype (Hall et al. 2007). Other laminin mutants *bashful (bal), sleepy (sly)* and *grumpy (gup)* have all demonstrated phenotypes involving defects in notochord differentiation and eye development (Parsons et al. 2002b, Pollard et al. 2006).

Downregulation of *FKRP* in the zebrafish By Kawahava et al. (2010) using 2 different morpholinos resulted in a phenotypic spectrum, seen also in the human spectrum from the severe congenital muscular dystrophy and the milder limb girdle forms. The most affected mutant embryos would die within 24 hours post fertilization and those with a less severe phenotype demonstrated a range of morphological abnormalities including alterations in the somitic and sarcomeric integrity, as well as disruption to the muscle fibres and basement membrane (Kawahava et al. 2010).

In the *FKRP* morphant embryos a reduction in the glycosylation of ADG was demonstrated and this correlated with the resultant phenotype. Laminin binding was also found to be defective, as in the human disorders, suggesting a similar disruption to the extracellular matrix binding of the internal cytoskeleton.

(vi) Conclusion

In conclusion, the *FKRP* gene, encodes for a putative O-linked glycosyltranferase. Abnormalities in FKRP function result in hypoglycosylated ADG, which causes destabilisation of the DGC. In LGMD2I, this disruption appears less, compared to the more severe phenotypes seen in the congenital muscular dystrophy and this may be a result of variability in the glycosylation process. In LGMD2I there is evidence of a reduction in ADG on muscle biopsies, a focally disordered and thinner basal lamina as well as proliferated and modified rough ER. All these findings suggest that glycosylation has been affected and that modification of the ADG has been altered.

The milder LGMD2I phenotype is caused predominantly by the L276I mutation in the *FKRP* gene and in this case the FKRP is located in the Golgi and results in hypoglycosylation and protein misfolding. In the *FKRP* mutations causing the more severe phenotypes, there was ER retention and an absence of ADG on muscle biopsies.

The phenotypes associated with mutations in the *FKRP* gene range from WWS, MEB, and MDC1C to the milder LGMD2I patients and asymptomatic cases. Within LGMD2I, the phenotypes vary depending on whether the patient is homozygous or compound heterozygous for the common L276I mutation. The degree of glycosylation of ADG is crucial in the stability of the DGC and hence the integrity of the muscle.

1.1.6 - Aims and Objectives

LGMD2I represents a heterogeneous disorder caused in the majority of cases by the *FKRP* mutation. As it is one of the more frequently seen limb girdle muscular dystrophies in the Northern hemisphere, it highlighted a clinically relevant population to study in further detail.

Clinical trials and studies in neuromuscular disorders are becoming increasingly evident as pharmaceutical companies develop more 'orphan' drugs for these rare diseases. Feasibility studies using patient registries have proved invaluable for planning these studies and clinical trials. The feasibility studies establish disease population demographics and identify potential trial sites as well as numbers of patients potentially eligible to take part in such studies. Patient registries for rare diseases have become increasingly important in understanding these disorders, both within and outside the neuromuscular field.

These patient registries have already proved useful to Industry in providing data on patients with Duchenne muscular dystrophy. The registry was able to identify patients who had a specific deletion of exon 51, therefore assisting with the design and trial site location for the antisense oligonucleotide therapeutic trials. With each study and trial alterations to the protocols and changes to end points and outcome measures have been made. Changes and recommendations to future therapeutic trials are made following the experience of previous trials, natural history studies and assessments.

In order to define study parameters and outcome measures, it is essential that the population to be studied is well researched. In order to do this, natural history studies need to be undertaken, establishing the pattern of the disease. This includes the typical age of presentation and symptoms, as well as the features of progression and the rate at which this occurs. In neuromuscular conditions, and in particular, limb girdle muscular

dystrophies it is important to assess whether there is any respiratory or cardiac involvement, as well as the pattern of skeletal involvement. As referred to in chapter 1.1, when the LGMDs were first defined there was no distinction as to which LGMD would present with cardiac or respiratory involvement, however with further genotype and phenotype definition it is now known for the currently defined LGMDs. Further classification of the LGMDs will continue as new genes are found.

Skeletal muscle involvement and the particular patterns seen on muscle imaging is becoming more defined with further utilisation of this modality. The MRI studies that have been published however are still small in patient numbers and whist patterns are well established in some muscle conditions, others are less well defined. Historically the imaging modality widely used has provided a qualitative image using T_1 weighted (T_1w) images and grades assigned to the whole muscle as according to the Mercuri et al. (2002a) grading.

The aims and objectives of this study were therefore;

1) The first objective was threefold; firstly to define the muscle involvement on imaging using both the standardised method (T_1w) as well as a quantitative method of MRI, secondly to assess these changes longitudinally and thirdly to assess whether any metabolic changes could be demonstrated on skeletal MRS. The initial aim was to complete this over a 12 month period and assess changes demonstrated by this method. As quantitative MRI is an objective measure, I was interested to determine whether this imaging modality would be sensitive enough to detect change. And if so, imaging could be another adjunct for use in clinical trials to monitor progression of the disease process or indeed any improvement in a therapeutic trial. Initially this project was planned to be a collaborative project between Newcastle upon Tyne and London, however during the development of the protocol it became evident that Paris, France and Copenhagen, Denmark would also be interested in collaborating and had previous experience in MRI studies. This therefore meant that careful planning and quality assurance was required from each centre to ensure reliable data and patient recruitment.

2) The second objective was to develop a standardised physical examination that was both meaningful in a clinical perspective as well as acceptable to an adult patient with LGMD. Recently scales, addressing function and strength have been rigorously developed for the DMD population as there are a number of therapeutic trials underway. These, however, are not entirely suitable or mirror the difficulties encountered by adults with LGMD2I. The aims were therefore twofold, to both assess the patients in order to correlate strength and functional ability with the MRI findings but also develop a robust set of assessments that could be applied to this population and hence useful in a clinical trial setting as outcome measures. These assessments, if appropriate to measure strength and function, may be appropriate for other adult LGMD patients and therefore useful in future natural history, multicentre studies.
3) The third objective was developed during the course of the project as I was researching other MRI techniques and applications. The LGMD2I patients are known to develop cardiac complications in the form of a cardiomyopathy and this, as commented in chapter 1.3, these are conventionally screened for by an echocardiogram. In DMD there has been much debate about the use of cardio protective drugs before detection of any cardiomyopathy and certainly prompt treatment with cardio protective medication is recommended as soon as signs of a cardiomyopathy are detected. Gaul et al. (2006) demonstrated that cardiac MRI appeared more sensitive at picking up early changes not seen on conventional imaging and if this is the case then even earlier treatment could then be commenced. The objective therefore was to perform cardiac MRI on the Newcastle upon Tyne patient cohort and correlate this with their echocardiogram findings
4) The fourth objective was to set up an international patient register with *FKRP* mutations; this register would be the first international neuromuscular registry, translated into a number of languages and designed to be patient initiated. It would aim to capture the data on all patients worldwide, to establish the presenting symptom and age of onset, the rate of progression and nature of the disease progression. The registry would also record on an annual basis the change in these patients, any concurrent diseases that may be an association and track the changes in their respiratory and cardiac function. Whilst patient

initiated it would also require medical input from professionals including the genetic confirmation, cardiac and respiratory parameters, and muscle strength testing including functional timed tests. This would therefore provide longitudinal, natural history data on a large number of patients with this condition as well as their demographics, which are essential if a feasibility study was required before a clinical trial.

Chapter 2 –Skeletal and cardiac muscle imaging and spectroscopy; literature review

2.1 – Ultrasound imaging (US)

2.1.1 - Ultrasound imaging; how it works

Ultrasound imaging of the muscle was first introduced in the field of neuromuscular disorders (NMD) in 1980 (Heckmatt et al. 1980, Young et al. 1980). Muscle ultrasound has not been widely adopted in neuromuscular clinics or in diagnostic evaluation protocols, as many clinicians do not have adequate training in the use of and interpretation of US. It can be extremely useful in children as well as adults, as it is safe, painless, quick, and relatively cheap. It also has the great advantage of being portable.

Sound waves and their echoes form the basis of ultrasound images. A transducer sends out pulses of high-frequency sound waves and receives their echoes. The temporal properties, the time taken to send and receive the pulse, and acoustic properties, the amplitude, determine the position and the brightness of the image produced respectively. Biological tissues are comprised of mainly fat and water, both of which are capable of transmitting sound and have only a small difference in acoustic impedance, the combination of sound velocity through and the density of the tissue. The echo intensity, and corresponding image, is determined by the quantity of returning echoes. Bone and air produce a strong reflection, at transition, and this results in a bright spot on the image. Hardly any sound can get through to the deeper layers either and hence no structures beneath that transition can be displayed. Muscle and fascia on the other hand, produce a partial reflection; however most of the sound waves can continue through to deeper structures, which then further reflect the sound waves producing varying echo intensities depending on the structure (Pillen et al. 2008).

Normal muscle appears black on ultrasound images due to its low echo intensity. In the transverse plane muscle can appear speckled due to the echogenic sheets of perimysial connective tissue, which surrounds the muscle fibre bundles, and in the longitudinal plane this is visualised as hyperechoic lines. This means that muscle ultrasound images are distinct from surrounding structures and due to the echogenic nature of the

epimysium (fascia), and the boundaries of the muscle are well demarcated. All superficial muscles can be easily visualised, however deeper muscle can be more difficult due to the reflection or absorption of sound from more superficial tissue layers and individual small muscles can also prove difficult when multiple muscle groups overlap them (Pillen et al. 2008).

When using US in a clinical setting, it is imperative that the normal values and appearances are known in order to detect disease states. The thickness of muscle alters both in childhood and depending on gender, and normal values for these have been established (Arts et al. 2007, Heckmatt et al. 1988, Reimers et al. 1998, Schmidt and Voit, 1993, Scholten et al. 2003). During childhood the muscle thickness increases rapidly, with the main determinant being the weight of the child (Scholten et al. 2003). Gender differences do not dominate until puberty (Arts et al. 2007, Kanehisa et al. 1994, Kanehisa et al. 1995), when men start to develop thicker muscles than women. This trend continues and peaks between 25 and 50 years of age, after which a gradual decline is seen (Arts et al. 2007, Reimers et al. 1998, Kanehisa et al. 1994, Kanehisa et al. 1995, Doherty, 2003).

As well as the thickness of the muscle, echo intensity can also be evaluated. As with the thickness of the muscle, echo intensity changes with age. The echo intensity increases due to an age related increase in fat and fibrous tissue. Due to the increased number of reflecting interfaces the muscle appears whiter. This also applies to the increase in fat and fibrous tissue in neuromuscular disorders. Heckmatt and colleagues (1982) developed a visual grading scale to classify this. Grade I is normal through to grade IV, which is severely increased muscle echo intensity with total loss of bone echo. The intensity can further be described depending on whether the muscle appears homogeneous or inhomogeneous. Qualitative scales and visual grading are always subject to the experience of the observer and the settings of the machine. It has been demonstrated that visual assessment of muscle US has a low interobserver agreement ($\kappa = 0.53$), which is further lowered if an inexperienced observer interprets the images (Pillen et al. 2006a). If a quantitative gray-scale analysis is used the interobserver agreement improves ($\kappa = 0.82$)(Pillen et al. 2006a). Visual assessment still has a role, particularly in detecting focal changes and distribution of changes within a muscle, whether homogeneous or inhomogeneous.

In order for these measurements to be meaningful, adequate amounts of contact gel need to be applied to ensure optimal acoustic coupling as well as to remove the potential for pressure when scanning. If longitudinal measurements are being made then standard operating procedures need to be adopted and adhered to by individual operators. Contraction of the muscles will result in decreased echo intensity as well as an increased muscle diameter (Heckmatt et al. 1988a) and changes in the muscle fibres will alter the echo intensity, such as bending the knee when scanning the quadriceps (Heckmatt et al. 1980, Zuberi et al. 1999).

2.1.2 - Ultrasound imaging in neuromuscular disorders.

There have been a small number of studies in NMD assessing the reliability of ultrasound. In adults with idiopathic inflammatory myopathies a robust correlation between the histologic and sonographic changes has been demonstrated (Walker et al. 2004, Emery, 2002). Quantitative muscle ultrasound has been successfully used to differentiate between typical myopathies and neuropathies in adults (Mauritis et al. 2003) and there also appears to be a robust correlation with the fibrous tissue content (Pillen et al. 2008). Whilst there has only been one prospective study in 28 adults there have been a few in children evaluating the role of muscle ultrasound. The study by Mauritis and colleagues in the adult population addressed the issue of quantitative muscle ultrasound, and whilst these quantitative techniques were able to distinguish between healthy and affected musculature, there was failure to determine distinct disease entities. Patients with dystrophic myopathies, inflammatory myopathies and motor neurone disease (ALS) were enrolled, however the study size was small at n=28 and many parameters that have previously been used to diagnose these patients were not used, including the presence of fasciculation in ALS and oedema that can be demonstrated in inflammatory myopathies.

In a paediatric study, by Pillen et al. (2007), a highly predictive value between 86- 91% was demonstrated in 150 children, from 1 week to 17.5 years, for the detection of any neuromuscular disorder. This was similar to the sensitivity of 78% and specificity of 91% reported by Zuberi et al. (1999) in detecting any neuromuscular disorder, which increased to a sensitivity of 81% and a specificity of 96% in children over 3 years of age. These studies therefore demonstrate that muscle ultrasound as a suitable as a

screening tool for detecting a neuromuscular condition in children (Pillen et al. 2006a). In specific neuromuscular diseases these sensitivities are different, approaching 100% in clinically affected patients with DMD (Pillen et al. 2007), but only reaching 25-45% in mitochondrial myopathies (Pillen et al. 2006b). The diagnostic value in other specific neuromuscular diseases has not been studied, and only small series have been reported. In children under 3 years, the sensitivity decreases due to structural changes in the muscle are usually very minor at this early age, however if there is an abnormality detected the further more invasive tests would be required.

The pattern of muscle involvement can also be described with ultrasound and this as with MRI and computerised tomography (CT) can help with the differential diagnosis. Muscle ultrasound can also detect suitable muscles to obtain muscle biopsies from, and hence prevent biopsies from too severely affected muscles which have pronounced fibrosis and atrophy and are uninterpretable (Lindequist et al. 1990).

Muscle US changes in muscular dystrophies were first described in DMD (Heckmatt et al. 1980). Preclinical cases of DMD can have a normal US unlike the changes seen in MRI, but once the first clinical signs manifest, then muscle ultrasound is found to be abnormal in almost every patient. The proximal muscles have the highest echo intensities and the within the muscle there is a homogeneous, fine granular appearance (Heckmatt et al. 1982, Fisher et al. 1988). These findings were similar in patients with Becker Muscular dystrophy as well as LGMD, and the intensity increased with age and disease severity (Heckmatt et al. 1988b). This contrasted with the congenital muscular dystrophies (CMD) where all cases were severely affected with increased echo intensity but with no correlation with age (Heckmatt et al. 1982). In CMDs the clinical phenotype can often milder than the muscle biopsy results and imaging. In Bethlem myopathy, a collagen VI disorder, there is an abnormal peripheral signal with relative sparing of the central portion of the muscle, particularly seen in the vasti and the hamstrings. This also appears as a common finding on MRI in Bethlem myopathy (Mercuri et al. 2002, Mercuri et al. 2005a, Mercuri et al. 2005b). There is also a typical ‘central shadow sign’ which can be detected, whereby the middle part of the rectus femoris appears echo dense in the anterior middle part of the muscle and this is also demonstrated on ultrasound images (Bonnemann et al. 2003).

Real-time ultrasound elastography (RTE) is an ultrasound based estimation of strain in living tissues. RTE is increased when the tissue is stiffer, with fibrous or dystrophic tissue, whereas it is reduced in areas of fat. In a case report of a patient with Bethlem myopathy, RTE was used to evaluate the elastic properties of the musculocutaneous tissue. This case report demonstrated that the patient's muscles on RTE correlated with the 'central shadow' findings on MRI and ultrasound. This is the discrete rim of high signal intensity seen at the periphery, in this case, of the vastus lateralis and biceps femoris with central sparing. The RTE correlated with the above images, with increased stiffness detected in the periphery of these muscles. There was also an interesting finding that vastus medialis, not particularly affected on the MRI and ultrasound, demonstrated increased stiffness at the periphery. This finding was not seen on either the US or MRI. This may indicate that RTE may be more sensitive at detecting dystrophic change in this group of patients and therefore further work will be required to determine whether this is a sensitive predictor of early change (Drakonaki and Allen, 2010).

2.1.3 – Discussion

US is therefore a useful imaging tools in experienced hands and has the advantage over MRI and CT as being relatively inexpensive, child friendly and portable. It's main disadvantage however is that it is a subjective measurement when used qualitatively and has poor visualisation of the deeper structures. In paediatric patients, it has high sensitivity and specificity in detecting a neuromuscular disorder from a normal muscle particularly in the older children, and can distinguish certain neuromuscular disorders. This however is not true for the adult population where affected musculature from healthy was discriminated, but distinct disease entities could not.

2.2 - Computerised tomography (CT)

2.2.1 – CT; how it works.

Computerised tomography (CT) was invented by Sir Godfrey Hounsfield and was first installed and used in a patient in 1971 (Richmond, 2004). X-ray CT is a technique which produces two-dimensional and three-dimensional cross sectional images from flat

X-ray images taken around a single axis of rotation (Herman, 2009). Fundamentally CT imaging deals with constructing an image from projections, which are caused by x-ray attenuation. X-ray attenuation is the reduction of intensity of the x-ray beam as it traverses matter, such as muscle, bone and fat. The reduction may be caused by an absorption or deflection of protons from the beam and this can be affected by a number of different factors including, the beam intensity or physical characteristics of the tissues. The characteristics of the tissues being imaged include both the density of that tissue as well as its atomic number. The greater the atomic number or density of the tissue, the greater the attenuation, hence fat appears bright of CT, but not as bright as compact bone. Projection reconstruction is then used to generate a three dimensional image. CT produces a volume of data that can be manipulated through a process known as "windowing", in order to demonstrate various structures within the body by blocking the beam of the X-rays. Whilst historically this was performed in the axial or transverse plane, orthogonal to the long axis of this body, more modern scanners can now reformat the volume of data into various planes or as volumetric (3D) representations of structures (McKetty, 1998).

The radiodensity of the image can be quantitatively graded using the 'Hounsfield unit scale' (Richmond, 2004).

2.2.2 - Clinical application

CT has been used widely in the past to evaluate the presence and the degree of change seen within the skeletal muscles of patients with neuromuscular disorders. It was first used by O'Doherty et al. in 1977 for imaging cases of DMD. With the increasing CT technology, speed splice count and image quality have improved therefore enabling assessment of the muscles with respect to shape and level of dystrophic change, in particular fatty infiltration. However it is not very sensitive at detecting oedema, the early inflammatory change that can be seen before overt dystrophic change in the muscle. This is due to the limited soft tissue contrast. CT imaging is relatively operator-independent, unlike ultrasound, and in comparison is able to evaluate and image deeper muscles. The newer CT techniques have enabled spatial resolution and multi-planar reconstructions. The main disadvantage that CT has over US and MRI is the relatively high dose of ionising radiation; this means that imaging particularly in children using

this modality is difficult to justify ethically in the absence of treatment options. The radiation dose increases with coverage, meaning that whole body scans to delineate the pattern of muscle involvement in neuromuscular conditions are difficult to justify to research ethics boards (Wattjes et al. 2010).

2.2.3 - CT use in neuromuscular disorders

In DMD there have been a number of studies which assess the pattern of infiltration in the muscles of the lower limb in patients with mild, moderate and severe disability. Hawley et al. (1984) observed gross changes in the cases from mild to total disability and reported a sparing of the gracilis muscle and sartorius muscle. The selective involvement in 'mid-stage' DMD were reported by Stern et al. (1984) and Kawai et al. (1985) and both reported the sparing of the gracilis muscle and sartorius muscle as well as the peroneus muscle and tibialis anterior and posterior muscles in the lower leg.

In BMD, the same pattern of sparing was also observed (Termote, 1980, Bulcke, 1981).

Arai et al (1995) studied the use of CT in early DMD patients, aged 6 months to 12 years, who were reported to be preclinical with motor skills still continuing to develop. His group used Hounsfield units and correlated this with the age of the patient. The Hounsfield units refer to the density of the muscle seen on CT imaging. Other authors have used Hounsfield units to assess the degree of muscle involvement (Termote, 1980, Stern, 1984, Bulcke, 1981, Stern, 1985, Nordal, 1988); however unlike Arai et al (1995), they used a method that automatically generated Hounsfield units from an arbitrary region of interest (ROI) within the muscle, rather than the whole muscle as performed by Arai et al (1995). Whilst the other studies on DMD have assessed the muscle damage in the later stages of the disease, Arai et al has assessed the preclinical group and attempted to analyse the scans in an objective, quantitative manner rather than the visual, subjective and qualitative review that have previously been reported. Interestingly the group does show that there are changes in Hounsfield units of the CT in individual muscles and in particular those that visually do not appear to be affected. The Hounsfield units are less affected in the muscles that are generally well preserved, such as the anterior and posterior tibialis muscle, and a very slow and gradual regression rate was reported in the gracilis muscle reflecting the so-called selective pattern of

involvement seen in DMD even in the preclinical stage. This regression rate was analysed cross sectionally correlating the degree of disease severity seen in the boys clinically with the Hounsfield units obtained from the CT in the gracilis muscle. Whilst this study was important from a quantitative perspective and identified that gross visual inspection alone is insufficient, there was no longitudinal data and no aged matched controls.

In LGMD, Vlak et al. (2000) assessed 6 patients, 2 with sarcoglycanopathy (LGMD2D) and 4 with dysferlinopathy (LGMD2B). In these patients there was a possible relationship between those scoring low MRC grades (MRC 1-3) in their physical examination and the grades obtained on the CT images, however the numbers of patients assessed were small (n=6). Those with MRC grades 4 or above either had normal CT grades or severely affected, this was not qualified further in the paper.

2.2.4 - Discussion

CT is an imaging modality which is able to demonstrate the abnormal patterns of muscle involvement seen particularly in muscular dystrophy. It is a quick and relatively readily available imaging tool and has the advantage of being able to visualise deeper muscle structures compared to ultrasound. It is easy to use, non-invasive and can be analysed both qualitatively and quantitatively. It does however have the major disadvantage of ionising radiation, which in the context of research and with a lack of definitive treatment for LGMD2I at present, means that paediatric and longitudinal studies are not ethically justifiable.. The other disadvantage as stated earlier is the low level of soft tissue contrast, compared to MRI, and hence the lack of ability to detect early changes in muscles before the dystrophic process has become established.

2.3 - Magnetic Resonance Imaging (MRI)

In this subchapter I will describe the basics of MRI and how these can be applied to neuromuscular imaging and in particular the LGMDs. I will then conclude with a literature review of the MRI changes seen within this group of disorders, including the dystrophinopathies, the LGMDs and the congenital myopathies, and how the use of MRI can be applied both on a clinical basis and as a research tool.

2.3.1 - Magnetic Resonance imaging – how it works

The initial concept for the medical application of nuclear magnetic resonance (NMR), originated in 1971, however due to the problems of low signal and high sensitivity to motion, body magnetic resonance (MR) was not widely practised until the 1990s (McRobbie, Moore et al. 2003).

MRI is an imaging technique that uses the property of NMR to image nuclei of atoms inside the body. The essential requirement for MR is the magnet and the signals that produce the diagnostic information. These signals are produced from the patient's tissues in response to applied radiofrequency (r.f.) pulses which are generated and received by rf coils. These are usually built into the magnet (McRobbie, Moore et al. 2003). Locally shaped coils, such as a knee coil, are only used as receivers and have better signal reception as they fit closer to the anatomy of interest and are therefore more sensitive (Elster and Burdette, 2001). The signals received are weak and therefore are sensitive to electrical interference. A special cage called the 'Faraday cage', shields against interference from the surroundings and is built into the walls, ceiling, floor and door of the magnet room.

The magnetic field strength is an important factor as it determines the maximum image resolution and scanning speed. Higher magnetic fields amplify the signal/noise ratio (the ratio of signal power to the noise power corrupting the signal; SNR) and hence higher resolution or faster scanning times are possible by comparison with lower field magnets. Higher magnetic field strengths require more expensive magnets. In this study we have used 3.0T scanners.

The homogeneity of the field is also important. This relates to the quality of uniformity of the magnets field. If poor this can lead to degradation and artefacts. The homogeneity of the field determines the maximum special resolution of the image (McRobbie, Moore et al. 2003).

The basic principles

The basic principle underlying NMR and MRI is that all isolated nuclei or particles possess an intrinsic angular momentum known as spin, in addition to any angular momentum caused by rotation of the molecules. The component of the spin angular momentum, **P**, in the z direction, P_z is given by,

$$P_z = \frac{h}{2\pi} M_I$$

Where M_I is the spin quantum number related to *I*, the spin number of the nucleus in question. M_I can take values of –I, -I+1…I-1, I. The hydrogen nucleus and the phosphorus nucleus have a spin number 1/2, meaning that the spin quantum number M_I can only take the values -1/2 or +1/2 The magnetic moment of the nucleus, **μ**, is related to **P** by the following equation;

$$\boldsymbol{\mu} = \gamma \mathbf{P}$$

where γ is the gyromagnetic ratio of the nucleus, and has a distinct value for each nucleus. Only nuclei that possess a magnetic moment can be detected and measured by NMR. When an external magnetic field is applied, B_0, the two spin angular momentum states have different energies, the energy difference, ΔH between the two states are dependent upon the gyromagnetic ratio (γ) of the nucleus and the magnetic field strength (B_0), thus,

$$\Delta H = \gamma \frac{h}{2\pi} B_0 .$$

Nuclei can be made to move between the two energy states by applying electromagnetic radiation of frequency ν_0 (in Hz) or ω_o (in rad s^{-1}) with energy $\Delta H = h\nu_o = h\omega_o/2\pi$ which can be expressed in terms of the Larmor frequency or resonance frequency;

$$\omega_o = \gamma B_0 .$$

$$\nu = \frac{\gamma B_0}{2\pi} .$$

This means that for a 3.0T scanner, with γ= 42.6 MHz T^{-1}, the gyromagnetic ratio for ^{1}H, the resonance frequency is 127.8MHz: for phosphorus with γ= 17.3 MHz T^{-1}, the resonance frequency is 51.8MHz. A radiofrequency (r.f.) pulse therefore set at the relevant resonance frequency results in sinusoidal MR signals, which will be detected by the receiver coil at the same frequency. The proportionality of field and frequency underlies the process of image acquisition. After the excitation pulse, the r.f. pulse is discontinued and the r.f. coil is used to detect the signal from the sample as it loses energy and returns to the equilibrium state. The decay of these oscillatory signals is determined by relaxation processes which are the basis of the T_1weighted scans and T_2 weighted scans which will be described below (McRobbie, Moore et al. 2003).

In order to image objects, we must distinguish the spatial origin of spin signals. We can use magnetic field gradients to do this. Gradient coils produce an small additional gradient in the main magnetic field, such that the total magnetic field strength varies linearly by position along the applied axis. These gradients produce images from the localisation of the MR signals in the body, by creating short-term spatial alterations in magnetic field strength across the patient. A stronger gradient enables more detailed and specific anatomical characteristics to be visualised and allows faster scanning (McRobbie, Moore et al. 2003).

2.3.2 - MRI – Basic scans

T_1 weighted (T_1w) scans are one of the most common image types utilised and have been used in this study. They differentiate fat from water, using a gradient echo sequence with a short echo time (T_E) to minimse T_2 weighting (T_2w) and a short repetition time (T_R). After nuclei have been excited by an r.f. pulse, they have excess energy. As the system returns to equilibrium, with recovery of the longitudinal part of the magnetisation (M_z,) this energy is redistributed to the surrounding environment, or 'lattice', and this process is known as spin-lattice relaxation. Between each signal acquisition and the next excitation, there must be a delay (the recycle time, TR). When the recycle time is shorter, longitudinal magnetisation does not fully recover and therefore the signal becomes saturated. Therefore on T_1w images the fat containing tissues appears bright, due to the short relaxation time whereas the fluid containing tissues are dark. Due to the short T_R this scan can be run very fast and can collect high

resolution 3D datasets. It therefore can highlight fat deposition well (McRobbie, Moore et al. 2003).

The other method of relaxation is spin-spin relaxation (T_2 relaxation), where the phase coherence of the spins is lost across time. In T_2w scans, fat again is differentiated from water, but in this case the water appears bright due to the long T_2 relaxation time. Damaged tissue tends to initially develop oedema, the unbound fluid having a long T_2 relaxation time appears bright and therefore makes T_2w scans useful for identifying pathological tissue. T_2 weighting is created with long T_E and avoiding T_1 weighting with long T_R. (McRobbie, Moore et al. 2003).

2.3.3 - Quantitative MRI.

Quantitative MRI methods such as T_2 relaxation time, magnetic resonance spectroscopy (MRS), perfusion imaging and muscle fat quantification using the 3 point Dixon technique are all tools that can be used to analyse and quantify the degree of pathology in the striated muscle. The 3 point Dixon technique, used in this study on LGMD2I, is a powerful way to quantify the individual contributions of fat and water in each voxel of tissue, from which the fat fraction is calculated for detection of signal intensity from small numbers of fat protons (Dixon, 1984). In this approach, the chemical shift difference between water and fat is encoded into images with different echo shifts (Kovanlikaya A et al. 2005a, Kovanlikaya A et al. 2005b). This technique, in part developed to overcome sensitivity to magnetic field inhomogeneity, has been found highly reproducible, accurate, and useful for in vivo quantification of fat in lean tissues, such as skeletal muscle (Kovanlikaya A et al. 2005a, Kovanlikaya A et al. 2005b).

2.3.4 – Clinical application of MRI in neuromuscular disorders

The interest in using MRI in neuromuscular conditions has been increasing over the last decade, however most studies in the literature have been limited by small numbers, are qualitatively scored and cross sectional rather than longitudinal. During this time substantial progress has also been made in the genetic diagnosis of many neuromuscular conditions, defining and redefining dystrophic and non dystrophic conditions of skeletal muscle. Imaging has also contributed to this process of diagnosis, providing images of

the lower limb muscles predominantly and defining the differential involvement of different muscle groups. This pattern of muscle involvement can help to distinguish between certain muscle disorders and if particular patterns are seen, such as the 'central shadow' in Bethlem (Chapter 2.1.2) can be almost pathognomonic, hence narrowing the differential diagnosis (Mercuri et al. 2005a).

Assessing skeletal muscle by MRI can provide information on both the shape and volume of the muscles and in particular whether a muscle is hypertrophied or atrophied, and also the architecture (Mercuri et al. 2005b, Mercuri et al. 2007). MRI is a relatively safe modality and attractive as an imaging method due to lack of ionising radiation, however in some circumstances, such as with very young patients, sedation may be required. MRIs are typically performed using a multi-sequence approach obtaining T_1w and T_2w images as well as fat suppressed images. The images are typically acquired in the axial plane and are approximately 5-7mm thick however other anatomical planes have been used, such as coronal or sagittal (Wattjes et al 2010).

Using these approaches previous MRI studies have detected changes in dystrophic muscles and been able to identify patterns of involvement in certain conditions. These changes include initial inflammation and oedematous changes, picked up best on T_2w fat suppressed images and fatty degeneration and atrophy on T_1w images. As commented in the earlier chapter, chapter 2.2, MRI has been shown to have a higher degree of sensitivity and specificity than CT for detecting this early inflammatory stage. (Ozsarlak et al 2001, Schedel et al 1992).

There is a number of rating scales used to access T_1w images of muscle, with many assessments being made based on the scales derived by Mercuri et al (2002a/2002b), Kornblum et al (2006) and Fischer et al (2008). All these scales are based on a visual judgement on the degree of fat infiltration from normal-appearing muscle to severely affected, with only a fascial rim and neurovascular structures distinguishable (**table 2.1**).

Table 2.1: Summary of the three well established rating scales on MRI concerning the visual rating of dystrophic change in striated muscle.

Grade	Mercuri et al. 2002a/b	Kornblum et al. 2006	Fischer et al. 2008
0		Normal appearance	Normal appearance
1	Normal appearance	Discrete moth-eaten appearance with sporadic T_1 hyperintense areas.	Mild: traces of increased signal intensity on the T_1 weighted MR sequences.
2a/2	Mild involvement; Early moth-eaten appearance with scattered small areas of increased signal or with numerous discrete areas of increased signal with beginning confluence, comprising less than 30% of the volume of the individual muscle.	Moderate moth-eaten appearance with numerous scattered T_1 hyperintense areas.	Moderate: increased T_1 weighted signal intensity with beginning confluence in less than 50% of the muscle.
2b		Late moth-eaten appearance with numerous confluent T_1 hyperintense areas.	
3	Moderate involvement; Late moth-eaten appearance with numerous discrete areas of increased signal with beginning confluence, comprising 30-60% of the volume of the individual muscle.	Complete fatty degeneration, replacement of muscle by connective tissue and fat.	Severe: increased T_1 weighted signal intensity with beginning confluence in more than 50% of the muscle
4	Severe involvement; Washed out appearance, fuzzy appearance due to confluent areas of increased signal or end stage appearance with muscle replaced by increased density connective tissue and fat, and only a rim of fascia and neurovascular structures distinguishable.		End stage appearance, entire muscle replaced by increased density of connective tissue and fat.

2.3.5 - MRI imaging in neuromuscular disorders

(i) The Dystrophinopathies

The dystrophinopathies (DMD/BMD) are the most common muscular dystrophies worldwide, with DMD affecting one in 3,500 boys (Hoffman et al 1987). The patients present with progressive muscle weakness by the age of 5 years and if untreated will lose ambulation in their early teens, with death in their late teens/early twenties.

Due to the progressive nature of DMD, there has been a need to develop an objective and non invasive measurement of disease progression. Muscle MRI allows evaluation of the muscle over time, and whilst standard T_1w images may be normal in the early stages of the DMD, after six to seven years there is progressive involvement evident.

The abnormal signals are initially confined to the gluteus maximus and adductor magnus, followed by involvement of the quadripceps, rectus femoris and biceps femoris. There is relative sparing of the sartorius, gracilis, semimembranosus and semitendinosus (Liu et al 1993). In the lower leg the gastrocnemius muscles are affected earlier than the other muscle groups, however on T_2w and STIR images, oedematous changes and signs of inflammation can be seen in the muscles not thought to be affected on the standard T_1w, where normal signal intensity is seen. These findings are interesting as they do suggest an inflammatory element, or phase of necrosis associated with oedema, pre-dating the fibrotic /dystrophic change seen later (Mercuri et al, 2007).

Whilst there have been many studies assessing the pattern of fatty infiltration and temporal change, Wren et al (2008) demonstrated, by using the 3 point Dixon technique, that quantification of fat infiltration was possible. They correlated these findings with both histopathology results and functional testing. They demonstrated that whilst there was a strong correlation with disease progression, as indicated on the functional testing, manual muscle testing and myometry were not as strong. The limitation of this study was its small size, nine patients, and no control data. It did however demonstrate that quantitative MRI by 3 point Dixon is a useful non invasive tool, which correlates strongly with disease progression and in particular functional testing. It was not reliant on patient effort and in a paediatric condition this is vitally important.

(ii) Early onset Limb girdle muscular dystrophies

Clinically the limb girdle muscular dystrophies (LGMD) represent a heterogeneous group of conditions and late onset BMD patients and manifesting female carriers of the DMD mutation may often fall into the differential diagnosis of LGMD. This overlap is particularly seen with LGMD2I as respiratory and cardiac involvement is common to both BMD and manifesting carriers of DMD, as well as LGMD2I.

The MRI changes, however, are very different. With respect to the pattern of infiltration in the thigh, dystrophinopathies have pronounced signal changes in the anterior compartment rather than the posterior compartment, as seen in LGMD2I.

In Northern European countries, LGMD2A, LGMD2I and LGMD2B are probably the most common LGMD forms. Previously a muscle biopsy has been required to distinguish between the different sub-types; however MRI can now help to direct the clinician towards the genetic analysis.

As covered in chapter 1.1.3, a novel gene encoding FKRP was described and discovered to be responsible for an early onset congenital muscular dystrophy (MDC1C) and also a less severe phenotype, LGMD2I (Mercuri et al 2003, Poppe et al 2003). It has been reported that LGMD2I patients have a characteristic phenotype on muscle MRI, with involvement of the adductor muscles, and the posterior compartments of both the thigh and lower leg.

In previous studies, specific temporal patterns have also been highlighted, with the initial stages of the disease associated with gluteus maximus muscle involvement more severely and earlier than the gluteus medius muscle in the pelvic region. In the thigh the adductor magnus muscle and biceps femoris muscle were often involved first, and with further progression of the disease it has been reported that the rest of the hamstrings become involved and eventually the anterior thigh muscles, vastus lateralis and vastus intermedius muscles. Vastus medialis muscle and rectus femoris muscle, to date, have only been involved in patients that are advanced in disease severity. Sartorius and gracilis muscles are relatively spared and often hypertrophied (Fisher et al. 2005).

In the lower leg in LGMD2I it has been documented that again there is a posterior pattern of involvement and that the infiltration of the gastrocnemius muscles are diffuse and uniform. The anterior compartment of the lower leg was often spared until late on in the disease process. The tibialis anterior muscle was usually spared and often hypertrophied (Wattjes et al. 2010).

In LGMD2A, Calpainopathy, caused by a mutation in the Calpain-3 gene, there appears to be very similar involvement on the muscle MRI in comparison with LGMD2I. There are however a few differences in the MRI which allows some distinction. In the thigh there is the similar pattern of posterior involvement, with infiltration of the gluteus muscles, biceps femoris muscle, adductor muscles and the semimembranosus muscle with relative sparing of the vastus lateralis muscle, sartorius muscle and gracilis muscle. (Wattjes et al. 2010, Mercuri et al. 2005c) There does appear to be more sparing of the vastus lateralis muscle in the thigh.

In the lower leg, whilst there is again posterior preference in infiltration, there are more selective changes seen in the medial gastrocnemius muscle and soleus muscle than in LGMD2I, where the pattern of infiltration appears more diffuse. The tibialis anterior muscle is also less likely to be hypertrophied compared to LGMD2I.

LGMD2B, dysferlinopathy, is caused by a mutation in the dysferlin gene. This mutation produces a predominantly distal Miyoshi myopathy and also a form of a distal anterior compartment myopathy (Liu et al. 1998, Illa et al. 2001). The MRI in this LGMD is different to LGMD2A and LGMD 2I, as both posterior and anterior compartments are affected in the thigh, with sparing of sartorius muscle and gracilis muscle. The pattern of muscle involvement can be more variable compared to other LGMDs. This is predominantly due to the variable clinical presentation of distal anterior weakness, distal posterior weakness and proximal lower limb weakness (Illa et al. 2001). It has been reported that changes in the posterior thigh exceeded the changes in the anterior thigh muscles (Cupler et al. 1998, Linssen et al. 1997, Mahjneh et al. 2001, Miyoshi et al. 1986).

In the lower leg there is posterior involvement but with selective sparing of the medial head of the gastrocnemius muscle (Illa et al. 2007, Kesper et al. 2009). This is distinct from the other LGMDs described earlier (Fisher et al. 2005).

Clinically LGMD2D can be differentiated from the other LGMDs, and in particular LGMD2I, as the calf muscles are often the earliest and most severely affected, leading to weakness of plantarflexion. This remains strong in LGMD2I patients even in advanced stages. There is also the absence of scapular winging and cardiac involvement, commonly seen in LGMD2I (Linsenn et al. 1997, Miyoshi et al. 1986, Weiler et al. 1999).

(iii) Congenital Myopathies

The congenital myopathies are generally characterised usually by non-progressive muscle weakness, hypotonia, and presentation at birth or shortly after with delayed milestones, plus or minus contractures.

Collagen VI disorders primarily cause two forms of muscle disease. Bethlem myopathy is an autosomal dominant myopathy, characterised by early onset, with a slow progressive course and contractures, mainly the elbows, long finger flexors and ankles. The other condition is Ullrich congenital muscular dystrophy which is autosomal recessive and is characterised by generalised muscle weakness, hyper laxity of the distal joints with contractures of the more proximal joints. It is associated with early respiratory involvement and normal intelligence.

The MRI images of these two conditions have characteristic findings. In the thigh of BM patients there is a consistent finding of an abnormal signal around the periphery of the vasti, with the central part spared (Mercuri et al. 2005a), another frequent finding is that of a ‘central shadow’ seen in the rectus femoris. This had previously been observed in a family with BM using muscle ultrasound (Bonnemann et al. 2003).

In Ullrich congenital muscular dystrophy, there was a more diffuse involvement at the thigh level with relative sparing of the gracilis, sartorius muscle and adductor longus muscles. The vastus lateralis muscle also exhibited the same concentric involvement as

seen in BM. The ‘central shadow’ seen in BM was observed in the Ullrich congenital muscular dystrophy patients, however not as obvious (Mercuri et al. 2005a).

In the lower leg level, there was a rim of peripheral involvement between the gastrocnemii muscles and the soleus. This change was more striking in the less affected BM patients.

Mutations in the skeletal muscle ryanodine receptor RYR1 gene are associated with a wide variety of phenotypes including central core disease (CCD) (McCarthy et al. 2000), CCD with nemaline rods (Monnier et al. 2000), Multi-minicore disease (MmD) (Ferreiro et al. 2002, Jungbluth et al. 2002) and the malignant hyperthermia susceptibility trait without muscle biopsy abnormalities (McCarthy et al. 2000).

The MRI shows a characteristic pattern with main involvement at the pelvic level in the gluteus maximus muscle, at the thigh level in the medial compartment (the adductor magnus muscle) and the anterior compartment (the vastus lateralis muscle and vastus intermedius muscle). Sparing of the adductor longus muscle, gracilis muscle and biceps femoris muscle is common. In the lower leg the soleus muscle and lateral head of gastrocnemius muscle are the most severely affected (Jungbluth et al. 2004).

Congenital muscular dystrophy with early rigidity of the spine (RSMD1), due to recessive mutations in the selenoprotein N (SEPN1) gene (Mercuri et al. 2002a) often show a selective infiltrative pattern of the posterior thigh muscles and sartorius muscle with sparing of the quadriceps muscles and the gracilis muscle.

2.3.6 - Discussion

The importance of clinical muscular imaging is becoming increasingly evident when assessing patients with a suspected or known inherited muscle disease. Whilst muscle US is safe and easily transportable it is operator dependent and visualisation of deeper structures can be difficult. CT scanning on the other hand is quick, easy and operator independent but has limitations dues to its poor soft tissue contrast and high ionising radiation dose, therefore making it unsuitable for paediatric cases and longitudinal studies. MRI, whilst relatively expensive does not have these side effects.

Cross sectional data is available on numerous neuromuscular conditions and has defined the pattern of muscle involvement in many of the muscular dystrophies and congenital myopathies. This has provided guidance to clinicians for further investigations such as muscle biopsy, further imaging and genetic testing.

Whilst in the past we have concentrated on the lower limbs and the pattern of muscle involvement seen in the thigh and lower leg, full body MRIs allow the imaging of organs and tissues other than striated muscle to be viewed. This would demonstrate further characteristics in these rare neuromuscular diseases which, as we become increasingly aware of the complexity of them, highlight the interplay they have with many vital structures such as the heart.

More recently there has been a shift to using more quantitative methods to demonstrate pathological damage in striated muscle. Quantitative MRI techniques such as T_2 relaxation times, muscle fat quantification using the 3-point Dixon technique, magnetic resonance spectroscopy and perfusion images are all modes that can be employed to analyse the pathological changes seen in muscle.

These techniques are extremely useful in longitudinal studies to accurately chart the change in muscle infiltration in individual muscle diseases. If therapeutics are to advance there needs to be a robust, sensitive and objective tool for monitoring improvement as well as disease progression. Further work is required to assess whether these methods provide a viable monitoring tool in the individual muscle diseases. In this study we used both the 3 point Dixon technique and magnetic resonance spectroscopy in assessing the LGMD2I patient cohort both on cross sectional basis and longitudinally.

2.4 – Skeletal Magnetic Resonance Spectroscopy

Magnetic Resonance Spectroscopy (MRS) is a non-invasive technique that measures the concentration of specific chemical compounds within a tissue using radiofrequency pulses and strong magnetic fields. MRS produces a spectrum which is a plot of signal intensity versus frequency that shows the chemical shift or frequency difference between different elements. The chemical shift is measured in parts per million (ppm).

The chemical compounds that can be detected depend upon the nuclei being studied (^{1}H, ^{13}C, ^{31}P, ^{23}Na). For these chemical compounds to be detected they also need to be of sufficient concentration and in the case of ^{31}P (phosphorus-31), which we are using in our study, a concentration of approximately 1mM. It also needs to have a unique spectral line that has a chemical shift that distinguishes it from other chemical species containing ^{31}P (Argov et al. 2000).

2.4.1 - ^{31}P MRS

^{31}P (phosphorus-31) magnetic resonance spectroscopy has been used in the study of muscle disease since the early 1980's (Gadian et al. 1981, Ross et al. 1981). Phosphorus MRS records signals from phosphate-containing metabolites involved in energy metabolism in vivo. It is useful for studying muscle metabolism in the resting, exercising and recovery phase, both the oxidative and glycolytic pathways (Argov et al. 2000). The phosphorus spectra consists of a number of peaks, 3 from adenosine triphosphate (ATP), one from phosphocreatine (PCr), one from inorganic phosphates (Pi) and two other peaks detected are those from the phosphomonoesters (PME) and the phosphodiesters (PDE). PDE has a role membrane in catabolism as the peak is seen to increase in membrane breakdown (Younkin et al. 1987). The area under each peak is proportional to the concentration of the metabolite (in the absence of T_1 or T_2 weighting) and the horizontal distance between Pi and PCr that yields pH.

Due to the non-invasive nature of MRS it can be repeated in a longitudinal study. It can also be used with the patient performing exercises as well as at rest; this will therefore provide information on muscle under stress as well as at rest and in the recovery phase.

2.4.2 - The MRS at rest;

Muscle homeostasis is maintained by the mitochondrial oxidative phosphorylation at a low metabolic rate. The ratio of muscle PCr to Pi is a measure of the energy state in muscle cells in vivo. Adenosine diphosphate (ADP) does not produce a peak that is visible on the spectra, as the unbound ADP concentration is too low at 10-25μM (Zohar et al. 2000). It does however regulate the rates of mitochondrial ATP production. The free ADP can be calculated using the creatine kinase (CK) equilibrium equation; [ADP]

= [ATP][Cr]/[PCr][H+]$K_{eq,}$ where K_{eq} is the Creatine kinase equilibrium constant (1.7 x 10^6 L.mM, [TCr] is the total creatine concentration and is taken to be 42.5mM and [ATP] is adenosine triphosphate concentration and is taken to be 8.2mM (Arnold et al. 1984, McGilvery et al. 1974).

The Pi peak observed in the spectra is actually the combined peak of HPO_4^{2-} and H_2PO_4. As the basic and acidic forms are in fast chemical exchange they are represented as one peak (Argov et al. 2000). The position of this peak is dependent on the concentration of protons in the chemical environment of the Pi i.e. the pH. The pH determined from this method is the cytoplasmic pH as the vast majority of the Pi peak originates from the cytoplasmic Pi, which is typically lower than the extracellular pH (pHi~7.0). This is due to the fact that the intracellular water space is approximately five times larger than extracellular water space and the intracellular phosphate concentration is approximately twice the extracellular concentration of phosphate in normal muscle (Bore et al. 1981).

Inorganic phosphate is removed from the cytoplasm by transport into the mitochondria and by the ATPase reaction (Argov et al. 2000). Patients with mitochondrial disorders therefore have high Pi concentrations at rest, due to the dysfunctioning mitochondria. A raised Pi concentration can also been seen in conditions such as DMD and BMD as well as in patients with an inflammatory myopathy who do not have a primary mitochondrial disorder (Kemp et al. 1993, Younkin et al. 1987).

2.4.3 - MRS during exercise:

During exercise both glycolysis and oxidative metabolism generate ATP, which is used for muscle contraction, in mitochondria stimulated by raised concentrations of ADP. ATP is thus maintained at a steady state via the CK reaction which shuttles phosphoryl groups from PCr to ADP to generate ATP. This therefore maintains a constant concentration of ATP, however results in a decrease in PCr concentration and a corresponding increase in Pi concentration (Argov et al. 2000).

During exercise the early changes in intracellular pH reflect the early proton consumption by PCr hydrolysis reaction and also the rising lactate levels from

glycolysis. As exercise continues this may produce a more severe intracellular acidosis (pH<6.5) (Ryschon et al. 1997).

2.4.4 - MRS during the Recovery phase;

During the recovery phase, glycolysis ceases and oxidative phosphorlylation continues, therefore replenishing the phosphate stores used during exercise and restoring the pH to its resting value. The PCr concentration recovers post-exercise, because oxidative metabolism, stimulated by ADP continues to run at a high level, this produces ATP at a raised rate. A subsequent decrease in Pi and ADP is seen, as the pH returns to normal. The rate of recovery of these various metabolites provides information about the mitochondrial function (Argov et al. 1997).

The PCr recovery rate is significantly affected by the pH generated during exercise (Arnold et al. 1984, Argov et al. 1996, Bendahan et al. 1990). The initial PCr resynthesis seems less affected by the pH in the first few seconds of recovery (Walter et al. 1997). As the type and intensity of exercise will affect the pH, this therefore is reflected in the PCr half time for recovery. ADP recovery is less affected by the pH after exercise compared to PCr (Iotti et al. 1993). ADP has been shown to be relatively unaffected by end-exercise conditions, such as pH and ADP concentrations. Initially the ADP rapidly falls and, depending on the degree of acidosis achieved with exercise, may fall below the resting ADP level. This 'undershoot' reflects the degree of muscle acidosis (Argov et al. 1996, Wackerhage et al. 1998). The initial ADP decline and subsequent half life measurement appears to be an accurate reflection of mitochondrial function, both in healthy and disease states (Argov et al. 1997).

The Pi recovery phase is 50% faster than that of PCr, this may be due to redistribution of the Pi into compartments that is not reflected in the MRS, such as the mitochondria (Iotti et al. 1991, Iotti et al. 1993, Miller et al. 1987). The observation is that activation of different muscle fibres might lead to the multiple Pi resonances and in our LGMD2I cohort may reflect the degree of fat fraction and the inhomogeneous nature that is seen (Yoshida et al. 1996).

The recovery of the pH after acidifying exercise is dependent upon the proton efflux, the removal of protons from the cells, which requires active transport across the cell

membrane and removal from the extracellular space by perfusion. PCr resynthesis also continues to produce protons, which adds to the acid load and means that pH may continue to fall for a time after cessation of exercise (Kemp et al. 1997).

2.4.5 - Influencing factors

There are a number of factors that need to be borne in mind when analysing results of MRS. These include gender and age, level of fitness, neuromuscular conditions including those with mitochondrial cytopathies, cardiopulmonary and respiratory status. In our study cohort of LGMD2I patients the age, level of fitness and the dystrophic process are the most likely contributors to the abnormal spectra.

Generally women have a higher energy cost per muscle work and a larger consumption of PCr (Mattei et al. 1999) .Advancing age is associated with reduced oxidative capacity and hence response of pH and PCr will be different. The PDE peak increases with age (Taylor et al. 1997), and therefore it is important to have age matched controls in patients with muscle diseases, particularly those commencing in the older population, as in our cohort.

The affects of physical fitness on MRS results have been extensively studied. Phosphorus MRS has been used to monitor skeletal muscle metabolic adaptations to aerobic training (McCully et al. 1988, Minotti et al. 1989, Takashashi et al. 1995). The type of exercise the individual does can influence the results and should be compared with a similar control individual. With deconditioning, such as in a chronic muscular disorder, there is a reduction in mitochondrial oxidative phosphorlylation, a lower pH during exercise and conversion to more anaerobic muscle fibre types evident. This is modest compared to patients with gross mitochondrial dysfunction(Argov et al. 2000).

Cardiovascular and pulmonary reserve is obviously important in the oxidative metabolism and hence decreased oxygen delivery to the muscles will have a detrimental effect. This was evident both during exercise, with lower PCr and pH, and the prolonged recovery phase observed in normal healthy subjects who were exposed to reduced inspired oxygen (Haseler et al. 1999). Whilst primary lung disorders and peripheral vascular disease are not common in muscular dystrophies, cardiomyopathies

and respiratory failure (type II) are. These lead to a reduced oxygen tension and might cause impaired oxidative metabolism.

In muscular dystrophy the main contributors to a change in the MRS metabolites could be aging, deconditioning and level of fitness. Cardiopulmonary impairment may have some influence on the spectra, however in our cohort neither the cardiac involvement or respiratory failure was severe.

2.4.6 - MRS studies in Muscular Dystrophy

There have been a number of studies examining patients with either DMD or BMD. These studies have demonstrated that in BMD and DMD there is a high intracellular Pi in association with a loss of PCr at rest (Kemp et al. 1993, Younkin et al. 1987, Griffiths et al. 1985, Newman et al. 1982). This therefore resulted in a high cytosolic ADP. It has been suggested that these changes could be the result of secondary mitochondrial dysfunction. There is also evidence of a progressive increase in the PDE peak possibly secondary to membrane breakdown (Younkin et al. 1987). These changes have also been demonstrated in the female carrier patients (Kemp et al. 1993, Barbiroli et al. 1992a). Membrane breakdown and sodium accumulation in the cells with associated "compensatory" proton extrusion has been postulated as leading to the intracellular alkalosis associated with the many neuromuscular patients. Lodi et al. (1997) demonstrated that patients with a sacoglycanopathy, there was a direct relationship to muscle fatty infiltration detected on MRI and the cytosolic pH. The fatty fraction inversely correlated to the pH whilst directly correlating to the PCr/ATP ratio.

During exercise there is an early reduction in PCr and a reduced acidosis is observed in BMD patients (Barbiroli et al. 1992b, Lodi et al. 1999). The reduced acidosis is not well explained to date. A reduced level of glycolytic or glycogenolytic activity has been assumed as there appears to be an abnormality in glucose metabolism that is common to dystrophinopathies.

In DMD it has been shown with proton NMR studies that there are reduced levels of glucose, alanine and glutamine, a major gluconeogenic precursor. Further metabolic investigations have also demonstrated that there may be a reduction in the glycogenic pathway as reflected by a decrease in lactate and a relatively predominant oxidative

metabolism with an increase in α-ketoglutyrate. Loss of membrane integrity has been suggested due to a lowered choline and glycerophosphoryl choline (Sharma et al. 2003).

Deconditioning cannot fully explain the changes either as you would expect an increased acidosis rather than a reduction (Argov et al. 2000). Normal mitochondrial function has been reported in BMD (Lodi et al. 1999), DMD (Kemp et al. 1993), sacoglycanopathy-deficient limb girdle muscular dystrophy patients (Lodi et al. 1997) and in oculopharyngeal muscular dystrophy (Zochodne et al. 1992) as demonstrated by the rate of ATP production during recovery.

2.4.7 – Discussion

^{31}P - MRS therefore represents a non invasive tool that can be quantified and longitudinally assessed in patients with neuromuscular disorders. These disorders studied to date do not include LGMD2I; rather concentrate on BMD and DMD. The only limb girdle muscular dystrophy studied with MRS has been LGMD2D, a sarcoglycan deficient LGMD. MRS can assess the level of muscle membrane stability and integrity reflected in the intracellular pH as well as in the PDE peak, and hence provide further insight into the muscles' handling of acidosis under stress such as exercise. This raises an interesting point that deconditioning can lead to a decreased pH and there is much debate at present as to the role of exercise in this group of patients and indeed whether programmed exercise can help. Further longitudinal studies would be needed to assess this in this cohort of patients.

2.5 -Cardiac muscle imaging and spectroscopy

2.5.1 - Conventional screening methods in detecting cardiomyopathy in muscular dystrophies

The conventional tools for screening for cardiomyopathies are the electrocardiogram (ECGs) and transthoracic 2D echocardiogram (ECHO) used in combination. Muscular dystrophies are a heterogeneous group of conditions with variable phenotypes and prognosis. Cardiac complications occur in many muscular dystrophies, but the prevalence, precise nature and progression are specific to the genotype of the dystrophy.

Significant morbidity and mortality occurs due to the cardiomyopathy or conduction defects. Improvements in diagnostics, including molecular, histopathological and imaging have led to a greater understanding in the mutation-specific cardiac complications. This has subsequently led to improved surveillance and treatment.

The dystrophinopathies, Duchenne muscular dystrophy and Becker muscular dystrophy, have cardiac involvement as do some of the limb girdle muscular dystrophies (LGMDs). Cardiomyopathies in particular occur in the sarcoglycanopathies (LGMD2D-G) and in LGMD2I. Emery-Dreifuss muscular dystrophy (EDMD), an X linked recessive condition and the less common autosomal dominant EDMD, a LGMD caused by a mutation in a gene encoding for lamin A and C, (LGMD1B), are also at risk of either cardiomyopathies or conduction defects (Beynon and Ray, 2008).

In ***Duchenne muscular dystrophy***, cardiac involvement is common and a considerable cause or morbidity and mortality with approximately 10-15% of all patients with DMD dying from the consequences of left ventricular dysfunction (Bushby et al 2003). There appears to be no correlation between the degree of skeletal involvement and heart involvement, but there appears to be a similar mechanism (Melacini et al. 1996). At autopsy the cardiac myocytes demonstrate hypertrophy, atrophy and fibrosis (Moriuchi et al. 1993). This is thought to be secondary to a loss of sarcolemmnal integrity leading to fibre necrosis and replacement with fat and connective tissue. The initial changes are usually seen after the age of 10 years; however there are reports of younger patients with cardiomyopathy (Vita et al. 2008). The initial change tends to be asymptomatic regional wall motion abnormalities with the posterobasal and lateral walls of the left ventricle affected. Conduction defects then become apparent as fibrosis becomes widespread. 50% of patients will show evidence of cardiomyopathy on ECHO at age 14 years and this increases to almost 100% over 18 years of age with 75% showing cardiac symptoms (Beynon and Ray, 2008).

Following an ENMC international workshop, guidelines for the screening of DMD patients and carriers were agreed (Bushby et al. 2003). This involves cardiac screening, in the form of an ECG and ECHO at diagnosis or before the age of 6 years, with subsequent ECHO and ECG monitoring on a 2 yearly basis until 10 years then annually thereafter, unless an abnormality is detected earlier and requires intervention. For the

carriers, they are screened with an ECG and ECHO at diagnosis and then every 5 years, unless an abnormality is detected earlier and requires intervention. More recently the use of cardiac magnetic resonance imaging (CMR) has been used in these patients, particularly when borderline results are obtained.

In ***Becker muscular dystrophy***, as in DMD, the cardiomyopathy also does not correlate with the skeletal involvement: in particular, the cardiac involvement can predate the skeletal problems in BMD (Saito et al. 1996). In a study by Nigro et al. of 134 BMD patients, 50% had evidence of ECG changes by the age of 20 years and 40% had evidence of dilated cardiomyopathy on ECHO by 30 years. In the cohort of 134 patients, 7 died over an average 8 year follow up period, 5 of those were from cardiac failure (Nigro et al. 1995). Guidelines for the monitoring of these patients were drawn up after the ENMC workshop (Bushby et al. 2003), similar to those of DMD. This involves cardiac screening, in the form of an ECG and ECHO at diagnosis, with subsequent ECHO and ECG monitoring on a 5 yearly, unless an abnormality is detected earlier and requires intervention. For the carriers, they are screened with an ECG and ECHO at diagnosis and then every 5 years, unless an abnormality is detected earlier and requires intervention.

LGMD1B occurs both as an uncommon autosomal dominant and a rare autosomal recessive LGMD, both due to a mutation in the gene encoding for the nuclear proteins lamin A and C (Bonne et al. 1999). In LGMD1B left ventricular involvement is more common and increases with age independent of the skeletal involvement (Bonne et al. 2000). Cardiac conduction defects are an ongoing problem, as in X-linked Emery Dreiffus Muscular Dystrophy (EDMD), and there is a high risk of sudden cardiac death. This risk of sudden death is not reduced by permanent pacemakers like in X-linked EDMD, suggesting a ventricular origin (Becane et al. 2000). Consideration therefore should be given to the placement of an implantable cardio defibrillator (ICD) if a pacemaker is required.

In LGMD1B annual screening, as with X linked EDMD, with an ECG and holter monitoring should be undertaken, however in LGMD1B more frequent ECHOs are required as they do develop left ventricular involvement.

In **LGMDs**, cardiac disease is disease specific and hence accurate diagnosis is paramount. In the sarcoglycanopathies, LGMD2C-2F, the cardiomyopathy course can follow that of DMD and therefore screening as per DMD needs to be employed with annual assessments in the form of an ECG and ECHO. In LGMD2I, as referred to in chapter 1.1.2, previous studies have indicated variable degrees of cardiac involvement in LGMD2I patients with prevalence ranging from 10-50% in the literature using conventional screening tools such as the ECG and echocardiography. Reduced left ventricular ejection fraction (LVEF), increased end-diastolic volume (EDV) and dilated cardiomyopathy have all been reported in the literature (Wahbi et al. 2008, Gaul et al. 2006, Sveen et al. 2006, Poppe et al. 2003, Poppe et al. 2004, Boito et al 2005, Mercuri et al. 2003, Walter et al. 2004).

The DGC is expressed in cardiac muscle as well as skeletal muscle and links the sub-sarcolemmal cytoskeleton to the extracellular matrix. As already described in chapter 1.1.1, dystroglycan is a key component of the DGC, consisting of the transmembrane protein, β-dystroglycan and the peripheral membrane protein, α-dystroglycan. It is suggested that abnormal FKRP function in LGMD2I leads to the dissociation of the tight bond between the DGC and the extracellular matrix and consequently to increase susceptibility to shear forces along the longitudinal axis of muscle fibres during cycles of contraction and relaxation. Loss of membrane integrity due to contraction-induced sarcolemmal damage will eventually lead to cardiomyopathy (Michele et al. 2009).

In the normal mammalian heart there is a complex fibre architecture that varies fromsubepicardium through to subendocardium. In the human heart, the subepicardium fibres are oriented obliquely in a left-handed spiral at 75^{o} from the circumference of the heart and subendocardial fibres in the opposite direction at -70^{o} (MacGowan et al. 1997, Rademakers et al. 1994). Midwall fibres are oriented circumferentially. Important functional consequences occur as a result of these fibre oritentations (MacGowan et al. 1997, Rademakers et al. 1994). At the subepicardium, maximal fibre shortening occurs along the fibre length, whereas at the subendocardium maximal shortening occurs at almost right angles to the fibre direction. This subendocardial ‘cross-fibre’ shortening is thought to be due to the greater radius and mechanical advantage of the subepicardium. Additionally due to its own contraction the subendocardium shortens in both the fibre and cross fibre directions. As there is shortening in 2 planes, there must be enhanced

thickening in a third plane to preserve volume. This results in marked radial thickening, which is an essential part of normal ejection. Torsion of the left ventricle occurs through shortening of the obliquely oriented subepicardial fibres partially counteracted by subendocardial fibre shortening (Arts et al.1979).

Thus, normal left ventricular ejection is caused by a balance of subepicardial andsubendocardial strains (being the percentage shortening of the tissue). With respect toLGMD2I associated cardiomyopathy, impaired binding of the extracellular matrix components to α-dystroglycan could cause injury along the fibre bundles leading to a selective loss of myocardial strains in the left ventricle, previously described in animal data (Michele et al. 2009).

2.5.2 - Cardiac MRI (CMR) use in muscular dystrophy

Information about wall mass, thickness and blood pool size in the diastolic and systolic phase can be obtained by analysing 25 cine images taken which cover the cardiac cycle in perpendicular orientations. This will be covered further in the methods chapter, 3.4.2. More recently CMR has been used in the detection of abnormalities in muscular dystrophy. In general these have mainly concentrated on DMD, including both mouse models of DMD and human studies with DMD. CMR is more sensitive, in detecting cardiomyopathies compared to conventional screening methods, such as echocardiograms (Russel et al. 2008, Gaul et al. 2006, Grothues et al. 2002). Gaul et al. in 2006 evaluated nine patients, all with LGMD2I and homozygous for the common *FKRP* mutation, 826C>A. Cardiac involvement was detected in eight, however by conventional methods Gaul et al. (2006) only diagnosed cardiac involvement in 4 of the patients. The main pathological finding was left ventricular dysfunction (Russel et al. 2008). As in the patients described by Poppe et al (2004) cardiac involvement is highly variable, even within the same family and may occur early in the disease (Muller et al 2005). Wahbi et al. (2008) also detected a high prevalence of cardiac involvement in a group of 23 LGMD2I patients (22 of which were homozygous for the c.826A mutation). Of those that had CMR, 11 out of the 13 patients investigated had abnormalities, 2 of which had been normal on echocardiography.

CMR tagging works by nulling signal from the myocardium in a rectangular grid pattern in diastole and tracking the deformation of these tags through the rest of the cardiac cycle. It can be used to detect and calculate abnormalities in torsion. This will be covered further in the methods chapter, 3.4.2. Torsion is the wringing motion induced by the contraction of the left ventricular myofibres, in order to eject blood from the ventricle. During early diastole the rapid uncoiling is a major indicator of the restoring forces that contribute to the rapid filling. The myofibers orientation, structure and function reflect the health of the heart and abnormal torsion has been found to be a sensitive marker of systolic and diastolic dysfunction (Gotte et al. 2006). Subendo- and subepicardial myofibers are obliquely orientated in opposite directions, with a smooth transmural transition between fibres. In the healthy heart, torsion occurs such that there is homogeneity of fibre shortening across the myocardial wall and is a marker of the dominance of epicardial fibres over endocardial fibres as a consequence of the greater radius in the epicardium. The relationship between torsion and strain can be approximated by a ratio of the peak epicardial torsion and the peak circumferential strain in the endocardial third of the myocardium and we refer to this as the torsion to endocardial strain ratio (TSR) (Lumens et al. 2006, van der Toorn et al. 2002). This ratio has been shown to be near constant amongst healthy subjects of the same age, and to increase with both healthy ageing and disease. Thus, both torsion and the torsion to strain ratio are measures of epicardial – endocardial interactions

The question as to whether regional torsion is useful clinically still remains unanswered. In comparing the circumferential segments and regional torsion, there appears to be a wide variation at only small variations in the axis of rotation (Russel et al. 2008). Torsion would appear to be a more global measurement, considering the orientation of fibres and its relation to torsion. Due to the connection of the myofibrils one would expect torsion of the whole heart to be affected even if only a regional circumferential location was detected as abnormal, for example with ischemia. Transmural variations, resulting from abnormalities between the subendo- and epicardial oblique fibres, may be a suitable early tool for picking up subclinical heart failure (Russel et al. 2008).

2.5.3 - Cardiac spectroscopy

Cardiac phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) is still in the realms of research, rather than in clinical practice, however with future developments cardiac ^{31}P-MRS may become a useful adjunct in the clinical setting. This would rely on improvements in spatial and temporal resolution, and would only be useful in the setting on an integrated cardiac MR examination. Cardiac ^{31}P-MRS enables determination of the phosphocreatine to adenosine triphosphate ratio (PCr/ATP), which is an indicator of the energetic state of the cardiac muscle, ATP is needed to drive the myofibrils and that ATP is generated in the mitochdonria. The ATP is not directly transferred between these two sites, but the high phosphate bond is moved via PCr through two creatine kinase reactions. (Radda, 1986) This would allow for further evaluation of the morphology of the heart, global and regional function, perfusion, coronary anatomy and metabolism. As MR machines are now more widely available this may be the next development, but would need to be subject to thorough multicentre long-term clinical studies before routine use in the workup of patients with heart failure, valve disease and cardiac transplantation (Neubauer et al. 1998).

The patients are positioned prone, as this produces fewer movement artefacts and a smaller distance to the 31-P coil. Cardiac muscle is also located behind a layer of chest wall skeletal muscle that gives rise to a strong 31-P signals that need to be suppressed. Due to the need to use additional localisation techniques, there is considerable signal loss and required voxel sizes need to be quite large (Neubauer et al. 1998).

In a study of patients with dilated cardiomyopathy (Neubauer et al. 1992), the PCr/ATP ratio correlated inversely with the severity of the heart failure after grouping according to their clinical severity, the PDE/ATP however did not change. In an extended group of patients (Neubauer et al. 1995), correlations were also demonstrated between the PCr/ATP ratio and the left ventricular wall thickness and left ventricular ejection fraction. Patients with the thinnest left ventricular walls had the lowest PCr/ATP ratios. There was no other correlation with any other haemodynamic indices either on left or right heart catheterization. This reduction in PCr/ATP ratios with an increasing severity in heart failure, either ischaemic (Hardy et al. 1991) or non-ischaemic (Neubauer et al. 1995, Hardy et al. 1991) has been documented in other studies.

Crilley et al. (2000) assessed DMD patients and carriers using cardiac ^{31}P-MRS and demonstrated a significant reduction in the PCr/ATP ratio as compared to controls. The magnitude of the reduction was similar in both patients and carriers, whether manifesting or non-manifesting. This reduction was in the absence of any left ventricular (LV) systolic or diastolic dysfunction or an increase in LV wall thickness, implying that metabolic dysfunction in DMD precedes the deterioration of LV function, and not a result of it.

2.5.4 Discussion

In LGMD2I cardiac imaging is therefore a vitally important tool, due to the high prevalence of cardiomyopathy seen in this population. Whilst the conventional screening techniques are widely available, newer techniques such as cardiac MRI are emerging as useful and complementary imaging modality. It has better sensitivity and can detect ventricular hypokinesis, dilatation and hypertrophy, as demonstrated by Gaul et al. (2006) as well as fat infiltration and late enhancement in patients with normal echocardiography.

Cardiac tagging can detect torsion and both torsion and the torsion to strain ratio are measures of epicardial – endocardial interactions. Abnormalities between the subendo- and epicardial oblique fibres may be a suitable early tool for picking up subclinical heart failure.

And finally cardiac ^{31}P-MRS may be useful in assessing early and preclinical change in the hearts of patients with muscular dystrophy and in particular LGMD2I as in the studies in DMD, Crilley et al. (2000) demonstrated the reduction the PCr/ATP ratio reflecting the myocardial energetic state pre-structural damage. As treatment of these cardiomyopathies associated with muscular dystrophy respond to early and possibly pre-clinical treatment with ACE inhibitors, the earlier that cardiac abnormalities are detected and treated the better.

Chapter 3 - Method

3.1 – Participants – demographics and ethical approval

Participants in the LGMD2I study were recruited from four main neuromuscular centres, Newcastle upon Tyne (NCL) and London (LON) in the UK, Copenhagen in Denmark and Paris in France.

Participants were recruited from their local centre, using local patient databases for their area. The patients were then screened for suitability for entry into the study. Only after inclusion and exclusion criteria were met were the patients approached. The study and all it involved was explained by the local researchers and information leaflets and consent forms given to the patients (Appendix A and B). The patients were given 3-5 days to consider the study before being contacted again and asked whether they would like to participate.

All participants underwent informed consent locally. Ethical approval was obtained in Newcastle upon Tyne for the NCL and LON patients, and locally from the other two centres, using the protocols and manuals devised for the study. Data were acquired locally at all of the four centres at baseline between June 2009 and April 2010, and between June 2010 and April 2011 for the 12 month follow up assessment. Each centre used pre-agreed standardised MRI, clinical and functional assessments with a standardised reporting method.

In NCL, 11 of the 13 patients underwent a total of four assessments, whilst the remaining two had the two assessments at baseline and 12 months later. The assessments at baseline and 12 months included the standardised physical assessments and MRI. On visits 2 and 3, the 11 patients underwent the physical assessment only, one for validation within four weeks of baseline and one 6 months later. The NCL cohort, in addition to the standardised physical assessments, underwent manual muscle testing and an adapted North Star Ambulatory Assessment (NSAA) for Limb Girdle Muscular Dystrophy. This was also completed at the Follow up visit after 12 months in all 13 patients. Visits 2 and 3 were not completed by two of the patients as they were not local to Newcastle upon Tyne and travel would have been difficult.

The other three centres had the two assessments as outlined above, one at baseline and 12 months with a standardised physical assessment and MRI.

Accelerometry was also completed in two of the centres at baseline (NCL and Copenhagen) and results were retrievable from 14 patients.

MRS analysis was completed in 2 centres (NCL and Paris) in 20 patients.

Cardiac MR imaging was completed in one centre (NCL) in 11 patients.

Inclusion and exclusion criteria

Thirty eight patients fulfilled the inclusion criteria for entry into the study and 34 completed the follow-up imaging. The inclusion criteria included identical genetic diagnosis with homozygous c.826C>A *FKRP* mutations, ambulant for more than 50 metres, no ventilator requirements and able to lie flat with no contraindications for MRI scanning. All participants were asked to fill out a questionnaire by the MRI team to establish if magnetic resonance scanning would be safe and a further safety briefing was given to the patient prior to the scan.

Two were unable to undergo the follow up MRI scan as they were in the early stages of pregnancy; one of these did complete the standardised physical testing as she was part of the NCL cohort and agreed to complete the fourth assessment. One participant was undergoing treatment for a recently diagnosed malignancy and one did not feel he could participate any further.

Patient details

The 38 participants at baseline (19 male and 19 female) were aged between 18 years and 64 years, mean ages of 41.7 years in the males and 39.5 years in the female group (**table 3.1**). Disease duration was 0-49 years with disease onset in childhood (less than 16 years) recorded in 47.4%, 26.3% in childhood aged 10 years or younger.

The first symptoms that predominate are difficulty in climbing stairs (28%), difficulty running (21.1%) and myalgia and cramps (21.1%). 10.5% of participants reported that falling and recurrent falls were a presenting complaint and 10.5% reported difficulty rising from a chair. 7.9% reported fatigue and one participant reported weight loss as an

initial complaint in his early 20's when he was attending a gym regularly to try and 'bulk' up his muscles. One participant also reported that getting up from the floor was the initial symptom and one participant still remains asymptomatic. One patient was diagnosed following a routine blood test and had raised liver enzymes; his case is reviewed in **chapter 4**.

The creatine kinase was recorded in 32 of the 38 participants and ranged from 222units/l to 23,858 units/l, with a mean of 5,142units/l. Cardiomyopathy was present in 16 of the 38 patients (42%) as documented on echocardiography results. 77% of those with cardiomyopathy were male and 23% female, the age range was 21-64 years, with a mean age of 44.4 years and 25% of those with a cardiomyopathy were in their 20's and all male.

Respiratory involvement as reported by the patients was present in 21% (n=8) of the cohort, however 33.3% had a forced vital capacity (FVC) </= 75% predicted value for their height. 16.6% had a >/= 20% decrease in their FVC in lying and 33.3% had a 10 – 19% decrease in their FVC. 31% of the cohort that had a cardiomyopathy also had respiratory involvement (n=5).

These figures are similar to previous studies of patients with LGMD2I, where cardiac involvement ranges between 10% and 55% (Walter et al. 2003, Poppe et al. 2004). 29% of LGMD2I patients were documented to have a cardiomyopathy by Sveen et al. (2008) with a higher prevalence in the male group (38%) compared to the female group (18%). Poppe et al. (2004) also reported similar findings with 83% males and 42% of females having either a confirmed or possible cardiac involvement.

Table 3.1: Summary table of the clinical characteristics of the LGMD2I cohort (n=38).

Patient	Sex	Age (years)	First symptom	Age First Symptom	Serum CK	Cardiomyopathy Y/N	Respiratory involvement Y/N
1	M	62	Flat feet, difficulty climbing stairs	45	2335	Yes	Yes
2	M	64	Poor running and falls	15	2471	Yes	Yes
3	F	58	Difficulty getting out of a chair and climbing stairs	43	n/a	Yes	No
4	M	46	Weight loss	23	1212	Yes	No
5	F	55	Difficulty climbing stairs and falls	41	n/a	No	No
6	M	21	Toe walker and slow at running	9	8050	Yes	No
7	F	25	Slow at running and odd gait when climbing stairs	9	2600	No	Yes
8	M	33	Raised CK detected when giving blood.	Early 20's	12000	No	No
9	M	28	Recurrent falls	24	3302	Yes	No
10	M	39	Poor at sport in school, unable to climb ropes.	10	n/a	Yes	No
11	M	54	Poor running and myoglobinuria	Late 40's	1000	Yes	No
12	M	37	Recurrent falls	Early teens	n/a	No	No
13	F	41	Slow at running and unable to climb school wall	10	222	No	No
14	F	46	Difficulty climbing stairs	22	856	No	Yes
15	F	46	Difficulty climbing stairs after first pregnancy	31	n/a	No	No
16	F	29	Pain in thighs and difficulty climbing stairs	12	13414	No	No
17	F	45	Difficulty climbing stairs	23	421	No	No
18	M	50	Difficulty climbing stairs	37	4950	Yes	No
19	M	30	Pain in buttocks and thighs	15	7545	No	No
20	F	27	Difficulty descending stairs	17	23858	No	No
21	F	30	Pain and cramps in legs	10	n/a	No	No
22	F	31	Cramps in calf muscles	10	2446	No	No
23	M	36	Difficulty getting out of a chair	20	11421	No	No
24	F	47	Poor at running	10	1940	No	Yes
25	M	18	No symptoms	Nil	3000	No	No
26	M	28	Cramps, myalgia	17	7000	Yes	No
27	M	51	Difficulty in running	14	1535	Yes	Yes
28	F	43	Cramps, myalgia	13	7260	No	No
29	F	42	Fatigability and difficulty climbing stairs	33	1528	Yes	No
30	F	40	Cramps, myalgia	6	3170	No	No
31	F	26	Cramps, myalgia	7	5000	No	No
32	F	38	Fatigability and weakness in lower limbs	16	5155	Yes	No
33	M	51	Fatigue with physical activities	37	n/a	Yes	No
34	F	58	Falling over	38	n/a	Yes	Yes
35	F	40	Difficulty getting out of a chair	32	n/a	No	No
36	M	64	Difficulty getting up from the floor	43	n/a		
37	M	45	Difficulty climbing stairs	34	n/a	No	No
38	M	21	Difficulty running and rising from a chair	10	n/a	Yes	Yes

Follow up assessments and analysis

At follow up, 35 patients completed the physical assessment, 34 patients completed the MRI as part of the follow up and 32 participants were included in the final analysis at follow up (17 male and 15 female). They were aged between 19 years and 65 years, mean ages of 40.9 years in the males and 42.3 years in the female group.

8 healthy adult controls (5M: 3F age range 23-61 years, mean 40 years) were recruited. These were recruited locally in NCL using advertisements in and around the university and the Newcastle Magnetic Resonance Centre (NMRC). The healthy controls matched the age group and gender that we had in our patient groups.

3.2 – Tools and Protocols

A detailed study protocol was distributed to all centres including the medical form to be completed by the assessor, a reference manual for performing the standardised physical examination including clinical photographs for positioning, a record sheet and the consent form (Appendix B, C, D, E).

A detailed technical MRI protocol was sent to all the participating radiology departments outlining the technical data and pre-study quality assurance on water/fat phantoms and healthy subjects ensured that resulting images were quantitatively equivalent. This will be covered later in the chapter (section 3.4).

Validation and reliability

In NCL, the patients had two further visits in addition to baseline and twelve months. In order to validate the tests and assess test-retest reliability the patients were seen within 4 weeks of the first assessment and a further assessment was performed at 6 months from baseline. These assessments in NCL included the repeat respiratory function, myometry and timed tests, but also included additional tests including manual muscle testing (MMT) and an adapted North Star Ambulatory Assessment (NSAA for LGMD). The MMT included both upper and lower body on the dominant side.

Medical History

All the patients were reviewed and had a medical history taken including presenting symptoms and age at which they presented, best motor ability and age when this was achieved. Data was also collected on the current status of the patient including current motor ability, respiratory involvement, cardiac involvement, medications and any other concurrent medical conditions (Appendix E).

Physical Examination

All patients underwent a standardised physical examination. This was performed locally at each of the centres before the MRI examination.

The assessment procedure was set out in a full manual with a standard recording sheet in the order that the tests should be performed. The manual and assessments were discussed with the other centres taking part via email and a teleconference call in order to standardise the study. All centres were familiar with the testing procedures and step by step instructions were laid out in the manual with photographic, as well as descriptive text, to describe the assessments and the position that the patient and examiner has to be in to perform them (Appendix C).

All the patients performed respiratory function tests with hand held spirometry measuring the forced vital capacity (FVC) in sitting and lying.

The strength assessments included ***myometry*** testing using a hand held device. This was used to test hip flexion, hip adduction and abduction, knee flexion and extension and ankle dorsiflexion. The best of three measurements were taken and recorded in pounds of force.

The Newcastle upon Tyne (NCL) cohort was also tested with ***manual muscle testing*** (MMT) on visits 2,3 and 4, a familiar test with clinicians and neurologists, but in clinical trials it is considered a rather subjective measure compared to the myometry measurements (Merlini et al. 2002). The NCL cohort also completed the ***Adapted North Star Ambulatory Assessment*** (NSAA) for LGMD (Appendix C), not tested in the other centres, during assessments 1, 2, 3 and 4. The NSAA is regularly used in patients with DMD in the clinic setting and addresses many functional elements including some of

the timed tests that we had included in the original protocol. The NSAA was adapted for this adult cohort by taking out the item 11 ‘rise from the floor’ and supplemented (S1) with a ‘squat’. The ‘rise from floor’ is functionally important in school age boys with DMD who need to be able to sit on the floor for school based activities. This becomes less important in an adult population and could also pose a health a safety risk with adult patients. A ‘squat’ is more functionally appropriate for an adult population as this manoeuvre is used daily for retrieving low-lying items. This gives a score out of 34 that can be easily compared visit by visit.

The timed tests included a ***10 metre walk/run*** if able. This needed to be performed in a quiet corridor with a course set out with a 10 metre measuring tape and when the patient was ready the time taken to reach the 10 metre mark was recorded. As well as timing the assessment, the patient was also graded as to how well he performed the assessment. This included whether the patient needed any aids to walk with, picked up speed or not, was nearly running with double stance or could manage to run. These forms are included in the appendix (D).

This grading method of recording function was applied to the other timed tests including the stair ascend and descend. The patient was timed on a standard set of four stairs both ascending and then descending. They were graded according to whether they climbed using the handrails either one handrail or both and whether they marked time or used alternate feet to climb the stairs.

The ***chair rise*** and ***‘Timed up and Go’ (TUG) test*** was performed using a standard chair with arm rests and the test was performed timing the patient in getting from the seated position to standing with arms by their sides and graded depending on whether they required additional help in getting out of the chair either by using their own legs to push up on, or other furniture or the arm rests (Appendix D). The TUG was a combined chair rise with a 3 metre walk from the chair and back again and the test was completed once the patient was seated again in the chair. This was a timed test only.

The ***6MWD*** was completed in a quiet corridor that was long enough to mark out a 25 metre course and included a tape measure marking out the course, two traffic cones at both ends and a stop watch. The patient was required to walk from end to end

completing each lap which was timed and turning at either end at the cones to walk back down along the tape measure. The patient's blood pressure and pulse were taken both pre and post test. A chaser followed the patient in case they needed to stop or they fell. When the six minutes were completed, the patient stopped. The distance completed from either end of the cones was noted. This was then combined with the number of laps completed; each lap is equivalent to 25 metres, to give a total distance. The LON patients performed the 6MWD over a 10 metre course as there was no available space to set out a 6MWD course as above. This was recorded however the number of turns is therefore increased. This highlights the difficulty of performing this test particularly in a busy clinic setting.

Data collection

The data was recorded on excel spread sheets with centre codes and patient codes. The spread sheets were electronically transferred to NCL once completed from the other three centres. The four excel spread sheets were then combined for both the examination findings and medical history at baseline and 12 months. These were then recorded on the Statistical Package for the Social Sciences (SPSS) (version 17.0) data sheet for further analysis.

The MRI scans were all sent either electronically or by compact disc (CD) to NCL. The CDs and scans were all coded for patient confidentiality and the same codes used throughout the study. The MRI analysis was performed at Newcastle upon Tyne and all the values recorded in log books at the time of analysis and then transcribed onto the excel spread sheet and SPSS data sheets for further analysis and statistical testing. The cardiac MR imaging was performed and analysed in NCL and the MRS data was sent from Paris and analysed with the NCL data. Further details on the technical aspects and analysis of the MRI scans, MRS data and cardiac data will be covered in the later section 3.4.

Statistical Methods

Non-parametric statistical tests were used for analysis using the SPSS version 17.0 software (IBM, USA). Spearman's rank correlation (coefficient reported as r_s) was used to compare the Dixon quantitative fat analysis and the following factors: age, disease

duration (time from symptom onset) and appropriate functional measurements. Comparisons of fat infiltration between muscles were analysed using the Wilcoxon signed-rank test and between genders using the Mann-Whitney U test. Median fat fraction and change over time was analysed using the paired Wilcoxon signed-rank test for both MRI fat infiltration and muscle strength and function.

Rasch Analysis

Item response theory (IRT) and the Rasch measurement are two new forms of psychometric methods which can be used to analyse the scores obtained from rating scales and how these relate to variable measurements. They are both based on a mathematical background and can therefore be rigorously tested to enable verification of the data. Both methods can be used for analysing data from rating scales as well as use for constructing appropriate rating scales. Mathematical models are extremely useful in formally expressing relationships between variables, and this can be used to determine how observations satisfy the 'fit' of the predictions of the model, that is the extent to which the data satisfies the theory, and secondly can be used to predict future outcomes. The development of a mathematical model to predict how close a 'fit' to a set of items in a group of individuals using the Rasch method was developed by Georg Rasch (Rasch. 1960, Wright. 1982). This model determines if a set of items forms a reliable and valid rating scale on which people can be located, preferably in interval-level units. The IRT and the Rasch model focus on the relationship between a person's measurement and the probability of them responding to an item, rather than the relationship between a person's measurement and their observed total score on the scale. This is exemplified by Rasch's measurement theory. This postulates that the probability of a person's response to each of the categories of a rating scale is governed by the difference between where the person is on the scale and where the item is on the continuum measured by the item set. In essence, it measures the extent to which responses of the observed item accord with the responses of the mathematical model (Hobart et al. 2007).

The benefits of a Rasch analysis are firstly, that it offers the ability to construct interval-level measurements from ordinal-level data (Thurstone, 1925), which is essential from applying rating scales to clinical trials. Secondly it can be used for individual person

analyses rather than just group comparison studies (Hobart et al. 2007). Thirdly subsets can be used rather than all the items in the scale, and fourthly, it computes the missing data mathematically and uses an estimate based on the available data (Hobart and Cano, 2009).

The rating scale of the NSAA and the functional grades from the timed tests for the NCL patients has been subjected to the Rasch analysis. This method was applied to the scales used in order to define whether the items being used were appropriate for this group of patients and are reported in the results section (chapter 4.1).

3.3 - Physiotherapy tools

In the assessments of patients with neuromuscular disorders strength and functional ability are vitally important. It is important that the tests used for each patient group are appropriate for the particular disease and will detect both a 'floor' and a 'ceiling' in each participant's abilities. The tests also need to be standardised and validated, especially if using across centres and countries, and that agreement is sought before the study begins to enable consistency and comparisons to be made.

The most fundamental approach in analysing muscle function is the measurement of force, whether this is by clinical scales or more quantitatively by dynamometry. Measurements involving functional scales reflect the activities of daily living and tend to have a more direct impact on a patients level of functioning and their perception of their strength. These scales both involve timed tests and a grading to demonstrate the ease of difficulty that they are performed. Task specific functions, such as hopping or balancing on one leg are recorded. In this following chapter the various methods of assessments used will be outlined.

3.3.1 - Myometry

Hand held myometry has gained popularity over the last few years with the need for more quantitative measures of strength in neuromuscular conditions (Cook and Glass, 1987, Beck et al. 1999, Escolar et al. 2001). It has obvious advantages over manual muscle testing and other clinical scales, and due to its portability is useful in the clinic

setting. Reproducibility and interobserver agreement level is high (Wiles and Karni, 1983, Bohannon and Williams 1987, Hosking et al. 1976, Beck et al. 1999, Escolar et al. 2001).

Standard approaches need to be adhered to in order for the values to be comparable and measuring techniques using virtually isometric testing are used. Standard reference figures have been published for both adults and children. In some cases the normal male adult exceeds the dynamometer scale, however it was felt that expanding the scale would be of little use as these forces may be too difficult for the average examiner to measure.

It has been demonstrated that the mean ratio of female to male adults is about two thirds in both the 5^{th} and 50^{th} centile values. There does not appear to be a significant influence over strength and measures obtained from height, weight or age and in particular little or no decline in strength before 60 years of age (Sepic et al. 1986, Vandervoort and McCormas, 1986).

Variation between dominant sides does not appear significantly different. In a study by van der Ploeg et al. (1991) the 95% reference limits for the ratio left to right is on average 0.82 to 1.22, which means that the stronger side did not exceed the weaker by more than 22%. This is also reported in a paediatric cohort where a difference of less than 15% between the right and the left was seen in 80% (Hosking et al. 1976).

Repeatability is tested by comparing either the 'best of three' value or mean value within a set time, between a few days to a month, to test both repeatability and also intra and interobserver error. In van der Ploeg's study a good repeatability was demonstrated after one week, using the same myometer (van der Ploeg et al 1991). There were only small differences seen between muscles tested, which have also been confirmed in other studies (Wiles and Karni, 1983). It has been reported that measurements seldom differ by more than 20% from the first measurement. Wiles and Karni (1983) found that in measurements taken up to four days apart differed less than 20%, 80% of the time.

In patients with neuromuscular conditions, dynamometry, whether fixed or hand held, appears to be more reliable than grading using the MRC scale (Beck et al. 1999, Escolar et al. 2001, Kilmer et al, 1997). It also is useful in non-ambulant patients in assessing

arm strength and small changes in these more severely affected patients. Quantitative muscle testing (QMT) has been used as an outcome measure in a number of neuromuscular studies including amyotrophic lateral sclerosis, DMD and spinal muscular atrophy (SMA) (Beck et al. 1999, Cook et al. 1990, Escolar et al. 2001, Stuberg and Metcalf, 1988, Merlini et al. 2002).

Intra and interrater reliability has been previously published and is good, with intrarater results better than interrater and good results obtained (Intraclass Correlation Coefficient (ICCo) >0.85) in both upper and lower limbs except in ankle dorsiflexion (Merlini et al. 2002). This poor result for ankle dorsiflexion has been demonstrated in a number of other studies involving neuromuscular patients (Escolar et al. 2001, Kilmer et al, 1997, McMahon et al. 1992, Phillips et al. 2000). A number of reasons have been suggested, however, one of the main factors is the short lever arm used and the difficulty in getting the myometer in the correct position for measuring the line of force accurately. The position that ankle dorsiflexion is measured in also does not account for gravity as it is measured in the sitting position. In neuromuscular patients myometer positioning is often difficult and the results inconsistent if they have ankle contractures (Escolar et al. 2001).

3.3.2 - Manual Muscle testing

Manual muscle testing is the most common method used to evaluate muscle strength. It measures the strength of individual muscles using the Medical Research Council (MRC) scale (Medical Research Council, 1976, Haige et al. 2001).

This scale is an ordinal scale from 0 to 5, where 0 is no contraction/flicker of movement in the muscle and 5 is normal power against gravity. Whilst this scale is easy to administer in a clinical setting, it is subjective, dependent of adequate training and difficult if the patient has contractures, as in many neuromuscular patients, and therefore limits its ability to demonstrate true weakness (Escolar et al. 2001). As this scale is only 0-5 it is not particularly sensitive, especially when a patient is weak but cannot overcome gravity and also in patients who can overcome gravity but are still weak. The scale is also not sensitive enough to pick up small changes over time and hence the use

in longitudinal clinical trials is limited. Finally it has been demonstrated that there is a high rate of intra- and interrater variability which could compromise clinical studies

3.3.3 – Functional testing and timed tests

(i)Adapted North Star Ambulatory Assessment (NSAA for LGMD)

The NSAA is a functional scale specifically designed for boys who are ambulant with DMD and recently this scale has been used as one of the outcome measures in the clinical trials for DMD. With continued clinical trials, outcome measures and scales are constantly developed and hence the previous studies in patients with neuromuscular disorders primarily focused on strength, however more recently functional scales have been proposed. These scales have included the Gross Motor Function Measure (Russell et al. 1989), the Vignos scale (Vignos et al. 1963), the Hammersmith Functional motor scale in non ambulant patients with Spinal Muscular Atrophy (Main et al. 2003) or the Hammersmith Motor Ability Scale for DMD boys (Scott et al. 1982).

The NSAA is a functional scale that has been designed specifically for ambulant DMD boys and was an adaption from the Hammersmith Motor ability scale, however included functional items such as hopping and running, not previously included and defined the scoring for each item. These items were included in order to detect improvement in the treated DMD cohort. The scale was developed and piloted in the United Kingdom by the North Star Clinical Network for Neuromuscular Disease Management. It demonstrated good intra and interobserver reliability and has been applied to a large multicentre study with positive outcomes. (Mazzone et al. 2009, Scott et al. 2006, Mercuri et al. 2008, Eagle et al. 2007)

The NSAA consists of a scale of 17 items, ranging from standing, with or without compensation (item 1), to more difficult items such as standing on one leg, hopping and running. The scale includes items that are necessary to remain ambulant and functionally independent such as the ability to get from lying to sitting and from sitting to standing. These items, as in DMD, progressively deteriorate in patients with LGMD2I over time. Each item is scored on a 3 point scale using a simple criteria; 2-normal and able to execute task without any assistance, 1 –modified but does not require any assistance from another person and 0 – unable to achieve independently.

The total score therefore achievable with 17 items is 34 and can range from 0 to the maximum score (Mazzone et al. 2010).

The NSAA was adapted in this study for the adult patients with LGMD2I and is based on an appropriate construct for this group. 'Rise from the floor' was replaced with a 'squat'. This was perceived as a more functional activity required of adults in their daily lives compared to the 'rise from the floor' in children, who often spend considerable time on the floor either playing or at school and therefore is a very functional task for them. As adults we do not necessarily need to get up from the floor, whereas squatting to lift or pick up items from the floor, is more functional. Adults, with LGMD2I, have great difficulty in rising from the floor and many would object to performing this, however the 'squat' item was designed that if a patient could squat with at least 90 degrees of hip and knee flexion without using the arms for assistance and get back up from that position, they would score maximal points, 2, if they could initiate a squat to more than 10 degrees flexion of hips and knees, they scored 1 and if they were unable to initiate a squat then they scored 0.

In a recent study, the NSAA correlated well with the 6MWD in DMD boys, -.589 and the 10 metre walk/run correlated well with the 6MWD, -.601 (Mazzone et al. 2010). Both these were significant at $p<0.01$. It appeared that the boys that scored </=16 on the NSAA whilst able to complete the 6MWD, majority of them walked less than 300m (66%). 75% took more than 8 seconds to perform the 10 metre walk/run and 70% took more than 10 seconds to rise from the floor. In comparison, the boys who scored >/= 30 on the NSAA, generally walked more than 400m (69%), took less than 8 seconds to perform the 10 metre walk/run (60%) and less than 5 seconds to rise from the floor (78%) (Mazzone et al. 2010).

(ii) 10 metre walk/run

The gait speed spontaneously adopted by the patient was reported to be a reliable index of locomotor impairment in the patients with various pathologies of the lower limbs (Bernardi et al. 1999). Extensive work has been done in the elderly population on gait speeds and the correlation with self-reported physical function as well as the need for referral to physical therapy, and the 10m walk to assess gait speed has been reported as

having a sensitivity of 80% and specificity of 89% in screening elderly patients as to the need for referral to physical therapy (Harada et al. 1995). The 10m test was considered as a reliable and valid measure in patients with amyotrophic lateral sclerosis (Goldfarb and Simon, 1984), immune mediated polyneuropathies (Merkies et al. 2003) and in patients with Charcot-Marie-Tooth neuropathy (Solari et al. 2008). Gait speeds in patients with DMD/BMD/LGMD was moderately correlated with activity limitations in those patients (Vandervelde et al. 2009)This item is included in the original NSAA for DMD but is equally applicable to ambulant LGMD patients. The grading is based on score 2 for 'able to run' (no double stance phase), 1 for 'can go faster than a walk' and 0 as 'unable to go faster than a walk'.

(iii)Timed up and Go test (TUG).

The TUG test was introduced in 1991 by Podsiadlo and Richardson as a modification of the Get up and Go test of Mathias et al. (1986). Podsiadlo and Richardson (1991) described the TUG test as the time in seconds taken for an individual to - "rise from a standard armchair, walk to a line on the floor 3 metres away, turn, return, and sit down again".

It has been well documented that this test can be performed reliably with inter-rater agreement on the time scoring and is reproducible. Most of the work has been carried out in the elderly population who are prone to falls to assess their functional ability when rehabilitating them to get back home. Shumway-Cook et al. (2000) reported that TUG time scores greater than 13.5 seconds identify people who are likely to fall in the community; this test has a sensitivity and specificity of 87%. This test, however, also has implications for other disorders, such as vestibular disturbances with balance disorders as well as those with weakness that may affect their function. The TUG test includes basic mobility tasks encountered in daily life such as standing up, walking, turning and sitting down, which not only require strength but balance and coordination. The TUG has been shown to correlate with other rating scales such as the Berg Balance scale, gait speed/time, stair climbing and other functional indexes (Steffen et al. 2002). In our group of patients with LGMD2I, the stair climb, hill/slope climbing and getting out of a chair are the most difficult and many patients who are severely affected will opt to stand rather than sit as it is too exhausting to contemplate the chair rise. This is also

coupled with the fear of falling and then not being able to get up. This fear and lack of confidence has been documented in the elderly population for which this test is predominantly used for (Murphy and Issacs, 1982). The TUG test along with the stair climb is therefore a good test in the LGMD2I population. A chair height standardised at 44-47cm with armrests should be used. The TUG test in the LGMD2I study was split into a timed and graded chair rise as well as the complete TUG test. The chair rise element seemed to pose the most difficulty as well as taking considerable time in some patients. The grading documented the use of the armrests, other furniture or their own thighs to assist on the chair rise.

(iv) Six minute walk distance (6MWD)

The six minute walk distance (6MWD) is an accurate, reproducible, simple to administer and well tolerated test. It has been validated for use in adults (ATS statement, 2002) and further studies have more recently been published in children as an outcome measure particularly in DMD (McDonald et al. 2010). The American Thoracic Society (ATS) guidelines use the 6MWD for evaluating the functional capacity in patients with heart- and lung-related problems (ATS statement, 2002). Functional capacity, including endurance is an important aspect of everyday living and has it has also been established that increases in the 6MWD by 10% -15% in patients with chronic lung disease are consistent with a considerable improvement in well-being (Redelmeier et al.1997). It has already been used in patients with neuromuscular conditions as a primary outcome measure in registration-directed studies of laronidase in mucopolysaccharidosis type I (MPS I) (Wraith et al. 2004), idursulfase in mucopolysaccharidosis type II (MPS II) (Muenzer et al. 2006) and alglucosidase-α in Pompe disease (Wokke et al. 2008). The 6MWD has also been used as a primary outcome measure in myotonic dystrophy (Takeuchi et al. 2008), spinal muscular atrophy (Kierkegaadr et al. 2007, Takeuchi et al. 2008, Montes et al. 2010), and more recently in DMD (Mazzone et al. 2010) and in a large international therapeutic trial with Ataluren (PTC 124) (McDonald et al. 2010). The test has required some modification for the paediatric population with neuromuscular conditions and for control values. In DMD it was noted by McDonald et al. (2010) that there was a strong correlation with age, height and weight, however the 6MWD subsequently decreased with increased age and height as the disease process of DMD progressed. The patients with DMD had a

progressive decrease in their stride, a widening of the base of their support, stride width and decreased cadence. It was also noted that there was consistency of walking velocity suggesting shorter distances or timings could be used as a measure of ambulation, minimising the effort and potential for falls. This particularly would be applicable to the adult LGMD2I group who are also prone to falls but unlike children avoid any activity that may precipitate a fall due to the difficulty of getting up again afterwards.

3.4 - MRI specifics

3.4.1 – Skeletal MRI – technical data

The study consisted of (i) standard T_1w imaging, allowing for whole muscle qualitative evaluation and (ii) quantitative Dixon imaging optimised for localised analysis of fat fraction. These protocols were defined for use on 3.0T scanners (Philips Intera Achieva, Siemens TIM Trio) with surface arrays for signal detection. While the use of different scanners and vendors required the use of slightly different protocols, pre-trial quality assurance on water/fat phantoms and healthy subjects ensured that resulting images were quantitatively equivalent.

(i) T_1w imaging

T_1w imaging was performed with a turbo spin echo sequence (TR/TE = 671/10ms, (Newcastle/Paris) or 16ms (London), 12ms (Copenhagen), number of averages 2, acceleration factor 3, slice thickness 5mm, interslice gap 10mm, 256x192 matrix interpolated to 512x384). FOV 410mm using multiple stacks to cover both legs from the ankle to the pelvic crest.

(ii) Quantitative Dixon imaging

Spoiled gradient echo sequences which removes transverse coherences were used. Protocol details varied slightly between sites: Newcastle/London: 3 point Dixon images acquired in 2D with TR/TE=100/3.45, 4.6, 5.75ms, flip angle = 10 degrees, 10 slices of 10mm slice thickness, 5mm gap; Paris: 3D acquisition of 2 point Dixon with correction

for B_0 inhomogeneity (Coombs et al. 1997), 64 slices of 5mm slice thickness with TR/TE = 100/2.45, 3.675ms, flip angle = 10 degrees; Copenhagen: as per Paris but with 36 slices per acquisition. In and out of phase echo times were determined locally due to variation in actual B_o magnitude between scanners (2.9-3.1T). Images were collected at mid-calf and mid-thigh: the central plane of acquisition was defined with respect to bone landmarks as follows: legs were positioned with the patella anterior; the calf images were centred by finding the broadest part of the calf muscle, and recording the distance from lower border of patella; thigh images were centred by locating superior border of patella, and the anterior superior iliac spine, with centreing 1/3 distance superior to the patella; the distance was recorded for follow up scans. Each leg was imaged individually using 160 x 160 matrix interpolated to 256x256, FOV 200x200mm: in Paris, it was possible to scan both legs together at the same resolution using FOV 448x244mm. The data was analyzed to produce separate fat and water images (Coombs et al. 1997, Glover et al. 1991). The fat content of the image was expressed as a percentage of the total signal per voxel.

(iii) Interpretation

The T_1w images were assessed on an individual muscle basis and graded according to the scale published by Mercuri et al. (2002a, 2002b) (**table 3.2**). Each individual muscle was assessed at all levels analysed from the proximal to the distal end of the muscle and a grade assigned to the muscle as a whole by averaging all the individual grades at all levels. The quantitative fat images were analysed by defining regions of interest (ROIs) in individual muscles on the separated water image at the midpoint of both the lower leg and thigh. The cross sectional midpoint was then analysed using 'image J' software and a fat fraction obtained for each individual muscle defined by its ROI. Each ROI was delineated and drawn by hand. This ROI and slice number were recorded and used in the re-analysis of the follow up scan to give the second fat fraction. The muscle groups analysed are shown later in **Chapter 5**. The fat percentages in the 14 muscle groups analysed were averaged to give an 'average fat percentage' for each patient, the hamstring muscles, the biceps femoris muscle, semitendinosus muscle and the semimembanosis muscle were averaged for each patient to give an 'average hamstring fa t%' and the quadriceps muscles, vastus medialis muscle, vastus lateralis muscle and

the rectus femoris muscle were averaged for each patient to give an ‘average quadriceps fat %’. The vastus intermedius muscle was not quantitatively analysed.

Table 3.2: Description of the qualitative muscle grading scale from Mercuri *et al.* (2002a)

Grade	Description
0	Normal appearance
1	Early moth-eaten appearance with scattered small areas of increased signal
2a	Late moth-eaten appearance with numerous discrete areas of increased signal with beginning confluence, comprising less than 30% of the volume of the individual muscle
2b	Late moth-eaten appearance with numerous discrete areas of increased signal with beginning confluence, comprising 30–60% of the volume of the individual muscle
3	Washed-out appearance, fuzzy appearance due to confluent areas of increased signal
4	End stage appearance, muscle replaced by increased density of connective tissue and fat, with only a rim of fascia and neurovascular tissue distinguishable.

3.4.2 – Cardiac MRI – technical data

(i) Cardiac Magnetic Resonance Cine Imaging

Cardiac examinations were performed on the NCL cohort (n=11), using a 3T Philips Intera Achieva scanner (Best, NL). A dedicated 6-channel cardiac coil (Philips, Best, NL) was used with the subjects in a supine position and electrocardiogram (ECG) gating (Philips vectorcardiogram). Cardiac magnetic resonance cine imaging was acquired to assess cardiac morphology, and systolic and diastolic function. A stack of balanced steady-state free precession images was obtained in the short axis view during breath holding covering the entire left ventricle (FOV = 350mm, TR/TE = 3.7/1.9ms, turbo factor 17, flip angle 40^{o}, slice thickness 8mm, 0mm gap, 14 slices, 25 phases, resolution 1.37mm, temporal duration approx. 40ms per phase, dependent on heart rate). Image analysis was performed using the cardiac analysis package of the ViewForum workstation (Philips, Best, NL). Manual tracing of the epicardial and endocardial borders was performed on the short axis slices at end-systole and end-diastole. Details of the algorithm for contour selection and the methods for subsequently calculating left ventricular mass, systolic and diastolic parameters have been described elsewhere (Jones et al. 2010). The ratio of the left ventricular (LV) mass to the end-diastolic volume was calculated as this parameter is often quoted as a measure of concentric remodelling (Cheng et al. 2009).

(ii) Cardiac tagging

Tagged images of the myocardium in the short axis were obtained at the same session as the morphological imaging using the same cardiac coil. Cardiac tagging works by nulling signal from the myocardium in diastole in a rectangular grid pattern and tracking the deformation of these tags through the rest of the cardiac cycle (**figure 3.1**). By tagging two parallel planes it is possible to calculate myocardial torsion (**figure 3.2**) and in-plane analysis allows circumferential strains to be calculated across the myocardial wall. A multishot turbo-field echo sequence with TFE factor 9 was used (TR/TE/FA/NEX = 4.9/3.1/10^{o}/1, SENSE factor 2, FOV 350x350mm, voxel size 1.37x 1.37mm, orthogonal complementary spatial modulation and magnetization (CSPAMM) grid (Fischer et al. 1993) with tag spacing of 7mm). Two adjacent short-axis slices of 10mm thickness were acquired at mid-ventricle with a 2mm gap. The Cardiac Image Modelling package (University of Auckland) was used to analyse the tagging data by aligning a mesh on the tags between the endo- and epi-cardial contours. Circumferential strain and the rotation of the two planes were calculated throughout the cardiac cycle. Circumferential strain is quoted for both the whole myocardial wall and the endocardial third of the wall thickness. The epicardial torsion between the two planes (taken as the circumferential-longitudinal shear angle defined on the epicardial surface) was calculated as previously described (Buchalter et al. 1990) to account for the radius of the ventricle (**figure 3.2**).

Figure 3.1: Cardiac cine-imaging (*top*) and cardiac tagging (*bottom*) at diastole (*left*) and systole (*right)*, showing how a rectangular grid of nulled signal applied at diastole remains with the tissue through the cardiac cycle, allowing calculation of strain and torsion.

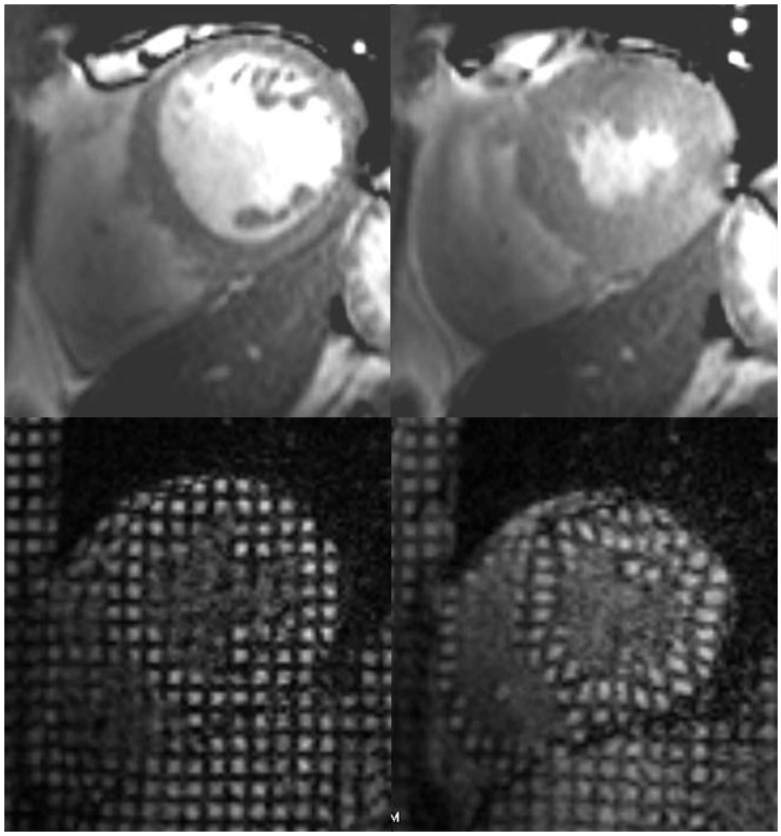
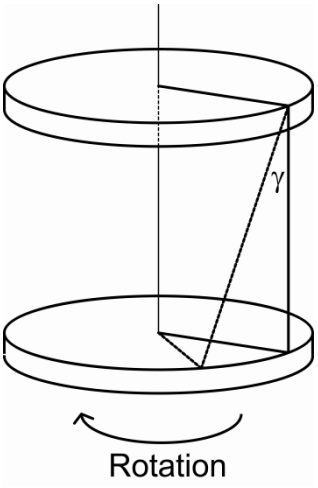


Figure 3.2: Tagging in two parallel sections allows the calculation of the torsion (the longitudinal-circumferential shear angle γ) between the two planes.



Longitudinal shortening was determined from cine-MRI in the 4-chamber view by determining the perpendicular distance from the plane of the mitral valve to the apex in systole and diastole, and expressing the difference in the measures as a percentage of the diastolic value. The myocardial wall thickness at systole and diastole were determined from the standard imaging at the same mid-ventricular level as the cardiac tagging by averaging the distance between the epicardial and endocardial countours around the left ventricle, and the percentage increase in wall thickness (radial thickening) from diastole to systole was calculated.

(iii) Cardiac spectroscopy

Cardiac high-energy phosphate metabolism was assessed using ^{31}P MRS on the same occasion as the other assessments. Data were collected using a 3T Intera Achieva scanner (Philips, Best, NL) with a 10cm diameter ^{31}P surface coil (Pulseteq, UK) for transmission/reception of signal. Subjects were placed in a prone position and moved into the magnet so their heart was at magnet isocentre **(figure 3.3** and **figure 3.4**). Localising images were collected using the in-built body coil to confirm location of the heart. Shimming was performed using a cardiac triggered, breath-held field map (Schar et al. 2002). A slice-selective, cardiac gated 1-dimensional chemical shift imaging (1D-

CSI) sequence was used with a 7cm slice selective pulse applied foot-head to eliminate contamination from the liver, with spatial pre-saturation of lateral skeletal muscle to avoid spectral contamination. 16 coronal phase-encoding steps were used, yielding spectra from 10mm slices (TR = heart rate, 192 averages at the centre of k-space with cosine-squared acquisition weighting, approx. 20 mins acquisition time). Spectral locations were overlaid onto an anatomical image and the first spectrum arising entirely beyond the chest wall was selected. Quantification of phosphocreatine (PCr), the γ resonance of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (DPG) was performed using the AMARES time domain fit routine in the jMRUI processing software (Vanhamme et al. 1999). After fitting, the ATP peak area was corrected for blood contamination by 1/6 of the amplitude of the combined 2,3-DPG peak (Conway et al. 1998), and the PCr/ATP ratios were calculated and corrected for saturation, with T_1 values of cardiac phosphocreatine and ATP taken from the literature (Tyler et al. 2006). Flip angle correction was made using a gadolinium-doped 20mM phenyl phosphonic acid phantom at the centre of the coil and a calibration dataset (Buchli et al. 1993, Haase et al. 1984).

Figure 3.3: prone position for the cardiac MRS

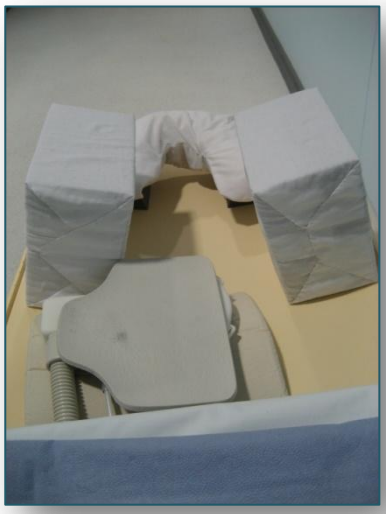


Figure 3.4: surface coil for cardiac MRS

(iv)Ultrasound examination

Routine clinical cardiac ultrasonography reports, performed for screening purposes, were sought for the LGMD2I subjects, for qualitative comparison with MRI findings.

(v) Statistical Analysis

Statistical analysis was performed using SPSS version 17. Data are presented as mean and standard deviation and comparisons were drawn between LGMD2I patients and control subjects using Student's t-test with Bonferroni correction for multiple comparisons. Where the LGMD2I group was divided, comparisons were made between group means using ANOVA with post-hoc comparison with Bonferroni correction. Correlations were performed between measures of cardiac energetics wall motion from cardiac tagging, morphological and functional measures. Correlations were executed as a two-tailed test using the Pearson correlation method. Statistical significance level was set at $p<0.05$.

3.4.3 – Skeletal MRS acquisition - technical data

Phosphorus MRS records signals from phosphate-containing metabolites involved in energy metabolism in vivo. It is useful for studying muscle metabolism in the resting, exercising and recovery phase, both the oxidative and glycolytic pathways (Argov et al. 2000). The objective was to assess whether there was any abnormalities in the metabolism of the muscles studied that predated any MRI (both qualitative and quantitative) or clinical changes. In this chapter I will describe the two methods used to obtain the results in the two centres that were able to perform the MRS, NCL and Paris.

(i) NCL data

MRS data were acquired using a 3T Intera Achieva scanner (Philips, Best, NL) with a 14cm diameter ^{31}P surface coil for transmission/reception of signal and the in-built body coil for anatomical imaging. A purpose-built exercise apparatus was developed for operation within the MRI scanner (**figure 3.5**).

Figure 3.5: Exercise apparatus in the MRI.

This apparatus permitted a controlled plantar flexion to exercise the soleus and gastrocnemius muscles with the patient lying supine: restraining straps prevented the recruitment of other muscle groups (e.g. quadriceps). Subjects performed two periods of exercise, consisting of three minutes rest, three minutes of plantar flexion at 0.5 Hz and 3 minutes of rest to measure recovery to equilibrium. The first period used a fixed load of 25% of the Maximum Voluntary Contraction (MVC, determined prior to spectroscopy) to accurately measure oxidative metabolism in recovery whilst changing pH levels as little as possible (Kemp et al. 1994). The second 3 minute period was carried out after a total of ten minutes rest, it used a higher fixed load (35% of MVC) to produce greater anaerobic metabolism and allow measurement of pH handling. Phosphorus spectra were collected at 10s intervals throughout the exercises using a fully adiabatic 1D-ISIS sequence to localise signal to gastrocnemius and soleus muscles. Quantification of phosphocreatine (PCr), inorganic phosphate and pH was performed using the AMARES time domain fit routine in the jMRUI processing software (Vanhamme et al. 1999), assuming single Lorentzian resonances for Pi, PDE and PCr, with the α, and γ moieties of ATP modelled as doublets of equal area with 17Hz separation, and the β-ATP moieties modelled as a triplet with 17Hz separation and area ratio 1:2:1. We assume a concentration of 8.2 mM ATP at rest and correct for relative saturation by comparison with a fully relaxed spectrum (TR = 25s, 4 averages) (Kemp et al. 1997). pH was calculated by measuring the difference in chemical shift between the fitted Pi and PCr resonances and applying the formula (Kemp et al. 1994):

$$pH = 6.75 + \log_{10}\left(\frac{\delta - 3.27}{5.63 - \delta}\right).$$

A mono-exponential fit to the PCr data in recovery was made to estimate the half-time for recovery of PCr to equilibrium, with correction for end-exercise pH value (Iotti et al. 1993). The ratio of Pi/PCr is quoted as this is often quoted in other muscular dystrophy studies and may be taken to represent an alteration of the set-point of the creatine kinase equilibrium, perhaps due to damaged cell membranes.

(ii) Paris data

MRS data were acquired using a 4T Bruker scanner using an elliptical 6 x 8 cm surface coil. Muscle acidication during aerobic plantar flexion exercise and efficiency of oxidative phosphorylation at recovery were measured by ^{31}P MRS (TR = 1.5s, 4 averages). Exercise (1 plantar flexion/1.5s) was performed in the magnet using a computer driven pneumatic ergometer. The initial load was determined from the cross-sectional area of the widest part of the calf and adjusted for level of muscle degradation: the load was adjusted incrementally and the ^{31}P spectra were monitored until the subject depleted the PCr resonance by approximately 40%. Data of PCr recovery were measured for 15 minutes following cessation of exercise – in the Paris protocol this extended period allows for the simultaneous measurement of leg perfusion.

Metabolite concentrations were evaluated by baseline correcting the spectra, and integrating the resonances of the phosphorus metabolites using fixed chemical shift boundaries (Pi: 5.6-3.8ppm, PDE: 3.5-1.9ppm, PCr: 1.5-(-1.5)ppm, γ-ATP: (-1.5)-(-3.5)ppm, α-ATP: (-6.2)-(-9.2)ppm, β-ATP (-14.2)-(-17.1)ppm). Again the concentration of ATP is assumed to be 8.2mM as per the Newcastle method. pH values were calculated by picking the peak of the Pi resonance and PCr resonance and using the formula detailed above. Phosphocreatine recovery was modelled as per the method outlined for NCL above.

Of the 7 patients and controls studied, 1 patient had good resting spectra, but exercise spectra which were unsuitable for analysis. The data from a further LGMD2I patient was poor shimmed, and this data point was discarded.

Chapter 4 – Strength and Functional measure Results

One objective of this study was to examine whether standardised physical tests, including strength and functional measurements would be firstly, the appropriate measures to use in a cohort of adult patients with LGMD2I, secondly, how well would these measures correlate with the fat infiltration seen on the quantitative MRI, and thirdly would these measures detect change in the follow up time period of 12 months.

4.1 – Assessments scales

The assessment scales drawn up to address this cohort of LGMD2I patients were, as stated in chapter 2, largely decided from a combination of previous experience in the trials with DMD, prior research into scales that have proved useful in patients with proximal muscle weakness and the knowledge of the difficulties encountered by patients with LGMD2I, notably climbing stairs and slopes and getting out of low chairs.

The initial assessments that were performed at all the centres on the 38 patients included respiratory function, with forced vital capacity (FVC) in both sitting and lying, strength testing, using hand held myometry, of the dominant side of the lower limbs, goniometry of the ankles, and timed tests. These timed tests included a stair climb and descend of 4 standard stairs, time to rise from a chair, the TUG test, 10 metre walk/run and the 6MWD.

4.2 - Cross sectional results of assessments

(i)Respiratory function

FVC recordings, in both sitting and lying, were obtained in a total of 30 patients. The results show that the median values of the FVC in sitting and lying were 78% and 71% respectively. The maximum values were 107% sitting and 105% lying, and the minimum values were 51% sitting and 36% lying.

At baseline; 33.3% had an FVC </= 75% predicted value for their height in sitting. 16.6% had a >/= 20% decrease in their FVC in lying and 33.3% had a 10 – 19% decrease in their FVC.

(ii)Myometry

All 38 patients attempted myometry; at baseline all patients registered a recording with hip abduction, hip adduction, ankle dorsiflexion, knee extension and knee extension. One patient was not able to generate enough force to produce a reading at baseline. The hip abductors were significantly stronger than the hip adductors ($p<0.01$), and stronger than hip flexion (ns). The knee extensors were significantly stronger than knee flexors ($p<0.01$). The ankle dorsiflexors were the strongest on myometry with a range of 5.6 to 86.3 pounds, median 38.5 pounds of force (**table 4.1).**

Table 4.1: Minimum, maximum and median values of the myometry measurements at baseline.

Muscle	Baseline min	Baseline max	Baseline median
Hip flexion (pounds)	2.2	81.1	15.7
Hip Abd (pounds)	1.4	86.1	18.2
Hip Add (pounds)	1.6	58.9	14.0
Knee Flex (pounds)	1.9	66.1	18.6
Knee Ext (pounds)	4.3	156.6	24.9
Ankle DF (pounds)	5.6	86.3	38.5
Abd = abduction, Add =adduction, Flex=flexion, Ext=extension, DF=dorsiflexion.			

There was strong correlation between the ‘hamstrings average fat percentage’ and knee flexion ($r = -.73$, $p<0.01$). The ‘Hamstrings’ average fat percentage, was calculated by averaging the fat fraction of the hamstring muscles including the semimemebranosus muscle, the semitendinosis muscle and the biceps femoris muscle for each individual.

There was strong correlation between the ‘quadriceps average fat percentage’ and knee extension ($r = -.655$, $p<0.01$) and a strong correlation with hip flexion ($r = -.494$,

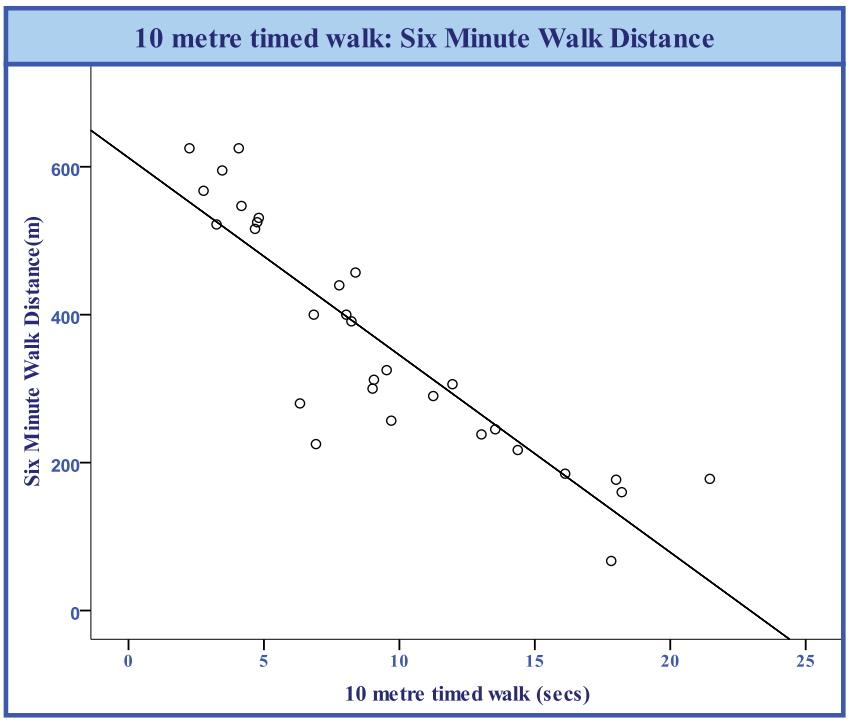
p<0.01). In the same way the 'quadriceps' average fat percentage was calculated, by averaging for each individual the fat fraction of the quadriceps muscles including the vastus lateralis muscle, the vastus medialis muscle and rectus femoris muscle.

(iii) 10 metre walk/run

The 10 metre walk/run was completed by all but one patient. This patient did not feel confident to perform the 10 metre walk/run without her stick. The times for the 10 metres were very variable from 2.25 seconds to 21.5 seconds. Whilst some able bodied and more confident were able to run the course (n=9; minimum time; 2.25 seconds, maximum time; 4.8 seconds, median time; 3.46 seconds), others were less confident and fearful of falling, hence took the 10 metre walk at a slower pace.

The 6MWD is currently a primary outcome measure in several clinical trials, however in a busy clinic setting and in the spaces allocated for clinics, a clear corridor of 25 metres is often unavailable. As can be seen from **figure 4.1** there is strong correlation (r = -.88, p<0.01) between the 10 metre walk and the 6MWD, suggesting that this can be as valid a test in LGMD2I, where speed and stride length are constant.

Figure 4.1: Correlation between the 10m timed walk and the 6MWD.



(iv) 6MWD

Only one patient who was ill with a flu like illness was unable to perform the 6 MWT. The results varied from a minimal value of 67 metres to values within normal limits at 625 metres. Three of the patients were reported as using the walls at times for balance and support, although none of them needed these for full support often their confidence and fear of falling would be appeased by touching or having the wall to hand. One patient found turning at the cones slightly difficult, and he appreciably slowed at the cones, as he felt unbalanced. In general the speed and step numbers were consistent, with neither quickening nor slowing of pace. In LON there was no available 25 metre walkway, so 6 of the patients did their 6MWD using a 10 metre course with more frequent turns.

The 6MWD strongly correlated with the 10 metre walk time, $r = .88$ ($p<0.01$) (**figure 4.1**), suggesting that the 10 metre walk /run could be used as an alternative to the 6MWD in this group of patients. This is particularly so in this group of adult patients as their speed and stride length varied very little in the 6MWD and appeared constant, compared to children who at times, speed up and slow down.

(v) TUG and timed chair rise

At baseline six of the patients were unable to perform the TUG test and this was mirrored in the chair rise test as 5 were unable to perform this, 4 of who had been in the group unable to do the TUG test. The times taken for patients to do this varied greatly with mildly affected patients achieving times less than 5 seconds (minimum 4.3 seconds) for the TUG and less than one second (minimum 043 seconds) for the chair rise. More severely affected patients found this test extremely challenging, with some patients taking up to 50.5 seconds to achieve the TUG and 48.5 seconds to achieve the chair rise (not in the same person). In order to achieve this more severely affected patients would adopt a wide based gait and would even need other furniture, such as another chair in front of them, to pull themselves up. Other patients would propel themselves forward and lock out their legs with their head dropped to the ground and gradually crawl up their legs or use the wall to climb up in order to become erect (**figure 4.2**). This obviously uses up a lot of energy as well as being time consuming.

Figure 4.2: One of the patients more severely affected with LGMD2I, attempting the TUG.

(vi) Timed stair climb and descend

33 completed the stair climb and descend, 5 were unable to attempt it. The median time to climb the stairs was 5.4 seconds (minimum; 1.43 seconds, maximum 46.2 seconds) and the median time to descend was 3.8 seconds (minimum; 1.07 seconds, maximum 39 seconds). Majority of patients (n=28) descended the stairs on average 31.5% quicker. Four maintained the same speed and one increased in time required. Four patients improved on their grading coming down the stairs as they were able to mark time and use alternate feet when descending, one came down backwards, and one sideways.

Figure 4.3: (a) This figure shows one of our more able patients with LGMD2I, who was able to ascend the stairs marking time. (b) This figure illustrates a more severely affected patient using the rails for support and needing to nudge his foot gradually further onto the step as unable to fully lift his leg in hip flexion.

(a)  (b) 

(vii) Manual Muscle Testing

Manual muscle testing (MMT) was performed on the 13 NCL patients on visits 2, 3 and 4 (**table 4.2**). The MMT was conducted on the dominant side, right sided in 12 patients and left in one. The MMT in hip abduction, hip adduction and knee extension correlated with the results obtained with myometry ($r = 593$, $p<0.05$; $r = .757$, $p<0.01$; $r = .607$, $p<0.05$ respectively). The other measurements did not correlate and this may be due to the subjective nature of the test, although in NCL this was usually performed by the one physiotherapist experienced in MMT. It may also be due to the patient's ability to compensate with other muscle groups particularly in hip flexion and knee flexion, which may go unchecked with the MMT manoeuvre compared to using the myometer.

Table 4.2: Minimum, maximum and median values of the manual muscle test scores at baseline.

Muscle	Baseline minimum	Baseline maximum	Baseline median
Hip Flex	2	5-	2+
Hip Ext	1	5-	2
Hip Abd	1	5-	3-
Hip Add	1	2	4
Knee Flex	0	5	3+
Knee Ext	2	5	4-
Ankle DF	5	5	5
Ankle PF	5	5	5
Abd = abduction, Add =adduction, Flex=flexion, Ext=extension, DF=dorsiflexion, PF=plantar flexion			

(viii) Accelerometry

Accelerometers were worn by the patients for seven days following their assessments to monitor their activity. Activity was graded according to how strenuous it was and how long it lasted. Length of time in supine and sleep time was also recorded. Average steps taken per day were correlated with average fat percentage and the 6MWD. Accelerometry readings were obtainable in 14 of the subjects and out of this small group a strong correlation was seen with average fat percentage ($r = -.68$, $p<0.01$) and the 6MDW ($r =.62$, $p< 0.05$).

(ix) The adapted North Star Ambulatory Assessment for LGMD

In the NCL cohort (n=13), the patients were seen within 4 weeks to establish reliability of the testing, between the two assessments. The adapted North Star Ambulatory Assessment (NSAA) was also completed at visit 1, 2, 3 and 4 in 11 of the patients and twice in two of them. The NSAA is used widely in the DMD population. As mentioned in **chapter 3** this scale is useful for assessing a patient's ability, using the activities needed for independent daily living. Whilst all these patients were ambulant, the spread of ability was wide, to include those that could still run and were very active with no apparent limitations to those that only just fulfilled the inclusion criteria of being ambulant for more than 50m, however, not able to get up from a standard height and or climb the stairs. Timed walking tests, such as the 10m and 6MWD were therefore manageable, albeit slow but the TUG, chair rise and stairs were not possible. The NSAA is more detailed and grades in a standardised way several functional activities important for daily living such as standing, standing on one leg, and sitting up from a lying position (See Appendix C)

Whilst the NSAA is widely used in the DMD community, such a scale has not been employed in an adult population with a limb girdle muscular dystrophy before or in the clinical setting - routine assessments often comprise of manual muscle testing and comparing these scores with the previous ones. It is imperative, that as clinical trials continue to expand to include the rarer neuromuscular disorders, there needs to be robust, validated, sensitive and reliable outcome measures. In order to assess whether the adapted NSAA and TUG would be suitable for this patient cohort, Rasch analysis was applied to analyse the spread of the items used and whether the tests showed a good 'fit' for this cohort.

4.3 - Rasch analysis of the North Star Ambulatory Assessment (NSAA)

The adapted NSAA was tested on the 13 adult patients from NCL. The NSAA was adapted from the original design to measure ambulatory function in DMD, by replacing item 11 on the form, rise from the floor, with S1, 'squats'. The analysis also included a functionally graded run, rise from chair and stair climb and stair descend.

The full testing schedule was carried out twice on two individuals and four times on eleven individuals giving a total of 48 assessments spanning one year.

These data were examined using Rasch analysis techniques (Hobart et al. 2007) for item fit, targeting, clinical cohesiveness, independence, reliability and stability of items to better understand the clinical utility of these items to measure change in this specific group. Mayhew et al. (2011) has demonstrated that the NSAA is an effective measurement tool, using Rasch analysis. The numbers however were small and therefore this has to be borne in mind when reviewing this. The patients used were just the NCL cohort, and comprised of 11 patients assessed on 4 occasions and 2 patients assessed on 2 occasions.

(i)Cohort description

48 assessments were included in the dataset for the 12 month period. Data were entered onto the software program Rasch Unidimensional Measurement Model（RUMM2030). No invalid records were noted. No extreme individual scores were noted. **Table 4.3**, illustrates the fit statistics, the item fit addresses whether the 'items' or tests used for this group are appropriate and the Fit Residual Mean (FRM) should ideally be close to zero and the standard deviation (SD) close to one, which suggests a good item fit. The person fit, refers to the fact that the entire group had a similar pattern of involvement and predictably fitting the pattern, again the FRM is close to zero and the SD is close to one, suggesting a good person fit.

Table 4.3: Summary Test of Fit Statistics; this illustrates that the item fit is good as the FRM is close to zero and the SD is close to one. The person fit is also good suggesting that the involvement fits the pattern expected.

	Fit Residual Mean (FRM)	**SD**	
Item Fit	0.081	1.226	Suggesting good item fit
Person Fit	0.108	0.680	Suggesting good person fit
Chi Squared Probability	0.000		
Degrees of freedom	42		
Pearson Separation Index (PSI)	0.97		The power of your construct to discriminate between the respondents. Showing the test is able to discriminate between four groups or more. High reliability.
SD=standard deviation, PSI=person separation index			

Table 4.4 illustrates that the items had a progression of difficulty to them. The item person fit residual for each item summarises the fit of the observed data to the statistical model from the perspective of the items. A residual is the difference between the observed response (score) of a person to an item and the expected value of that person to that item as predicted by the model. For each item, residuals are generated for every person in the sample who responds to that item. These residuals are then combined across persons to give a summary value which is then standardised and transformed so that perfect fit has a mean of 0 and standard deviation of 1. Larger fit residuals mean worse fit of observed data to the measurement model. Values in the range −2.5 to +2.5 are considered within statistically acceptable limits (Andrich et al. 2004). Thus, the size of the fit residual indicates the degree to which observed responses to items are inconsistent with predictions based on the mathematical model. The accompanying positive or negative sign gives information as to the nature of this misfit. All but two

items fit the construct, with an FRM between -2.5 and + 2.5. The highlighted items are are a slight source of misfit within the scale but significantly. There are no hugely misfitting items. The negative items, such as walk and stand, may suggest a redundancy of the item within a scale. All patients were able to do this and did not help differentiate degrees of severity within the ambulant group. The scale reflects a sensible clinical order of difficulty with rise from chair and standing up from a seated position as the most difficult to achieve items. Hop was also graded as being particularly difficult for this group, however many of the adults were surprised at the request as not many adults tend to 'hop' on a regular basis. The group also found balancing on either leg, an activity not usually attempted, and many surprised themselves that they could do this.

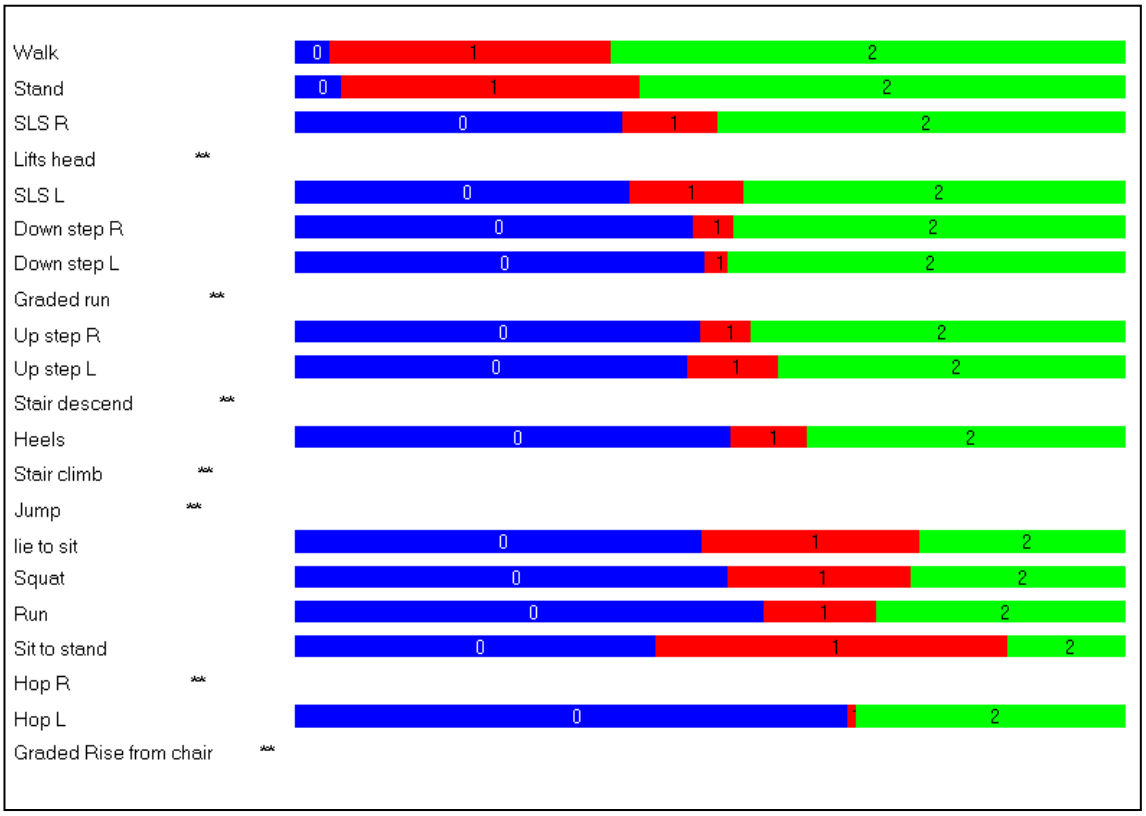
Table 4.4: Individual Item Fit for the 17 Items in item location (i.e. order of difficulty, easiest to most difficult). The FRM should be between +2.5 and -2.5 if the items fit the construct. Two items highlighted below do fall outside of the FRM, but only just and are assessed to be clinically relevant.

Item	Item description	Location	SE	FRM	ChiSq	Prob
I0002	Walk	-8.506	0.64	-0.065	1.937	0.379721
I0001	Stand	-7.875	0.56	-0.071	1.386	0.500117
I0004	SLS R	-2.243	0.381	0.089	8.618	0.013449
I0011	Lifts head	-2.1	0.336	0.169	64.661	0
I0005	SLS L	-1.701	0.372	0.458	4.782	0.091517
I0008	Down step R	-0.88	0.349	-0.469	2.624	0.2693
I0009	Down step L	-0.78	0.349	-0.439	2.832	0.242673
I0018	Graded Run	-0.688	0.284	-1.114	1.282	0.526826
I0006	Up step R	-0.484	0.347	-0.381	2.801	0.246423
I0007	Up step L	-0.282	0.343	-0.517	2.966	0.226913
I0020	Stairs down	0.658	0.205	1.713	5.22	0.073534
I0012	Heels	0.868	0.34	0.01	4.376	0.11215
I0019	Stairs up	1.614	0.194	3.333	9.258	0.009764
I0013	Jump	1.856	0.282	-0.031	0.614	0.735698
I0010	Lie to sit	2.179	0.38	2.835	12.039	0.002433
I0017	Squat	2.441	0.377	-2.183	4.347	0.113806
I0016	Run	2.455	0.366	-0.325	1.386	0.500003
I0003	Stand up	2.812	0.459	-0.335	2.259	0.323247
I0014	Hop R	3.244	0.407	-0.62	3.255	0.196381
I0015	Hop L	3.471	0.403	-0.594	0.724	0.696169
I0021	Graded rise from chair	3.94	0.28	0.249	7.371	0.025083
SLR= single leg stance, R=right, L=left, FRM= Fit Residual Mean						

(ii)Threshold ordering

The ordering of item threshold statistics indicates the extent to which the item response categories are working as intended, to define a progression from 'less' to 'more' functioning. Thresholds are transition points for adjacent categories. They mark the points on the continuum at which a person is equally likely to respond to one or other of two adjacent categories.

Figure 4.4: Threshold Map for the Adapted NSAA showing 3/17 disordered thresholds for the original NSAA scale (lifts head, jump and hop (right) and 4/4 disordered thresholds for the graded items (graded run, stair climb, stair descend and rise from chair). SLS=single leg stance, R=right, L=left.

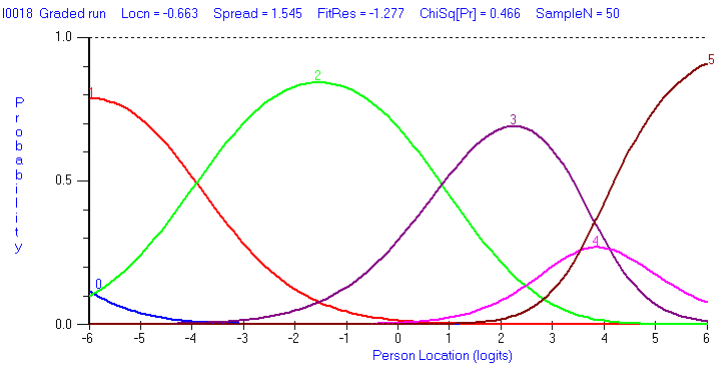


(iii)Category Probability Curves

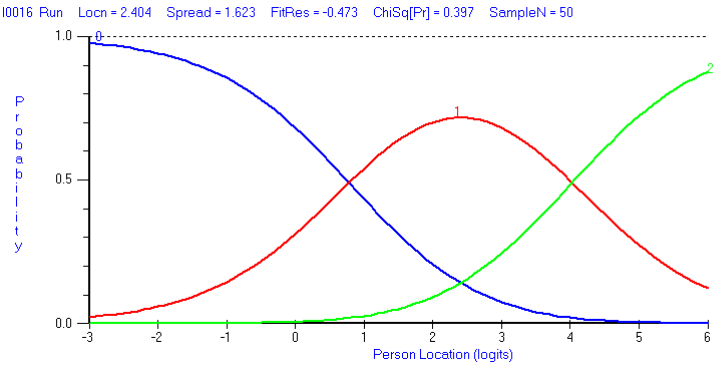
The Item characteristic curves (ICC) for each item is a graphic indicator of fit which provides complementary qualitative information about the fit of the observed data to the model, from the perspective of the trait measured by the items. The ICC is the plot of the expected value for an item (*y*-axis) against the latent variable measured by the set of items (in this case, physical or psychological functioning). That plot includes the observed mean scores for the people in each class interval defined by their level of functioning. The better the fit of the data to the model, the closer the proximity of the observed scores to expected values. As a person's ability increases (from left to right on the graphs, negative to positive logits) there should be distinct points where they are more likely to score from 0 to 2 or from 0 to 5 (graded items). The Category Probability Curves (CPC) below maps the grading issues with the four graded items.

Figure 4.5: (a) Category Probability Curves for Graded Run (a) compared to original Run Item (b). In 4.5a, there is clear progression from grade 1 to 2, 2 to 3 and 3 to 5, with limited progression in 4 indicating that this could be combined with grade 3. In 4.5b, whilst there are less grades, 0-2, there is a clear progression seen.

(a)



(b)



In **figure 4.5** it can be seen that there is not a clear progression illustrating grade 4 (defined as nearly running) could be combined with grade 3, for a better progression. It can be seen from some of the other categories that increasing the number of categories has added sensitivity although not all the new categories are working correctly.

(iv)Differential Item Functioning(DIF).

This DIF outlined in **Table 4.5** is difficult to explain with such a small data set, however the item, descend step right and left, is graded higher in all levels of severity in the women compared to the men in the group. This is seen more visually in the graph, **figure 4.6**, where the female patients score higher compared to the male patients. This is an important finding given the gender differences demonstated on the quantitative MRI and the pattern on involvement of the vastus medialis in the med compared to the sparing of this muscle seen in the female group (Chapter 5.2.1).

Table 4.5: DIF by Gender; This shows that the female patients perform better than the male patients independent of the severity in the items I0008 and I0009 (I0008, steps down box right, I0009, steps down box left,).

Item	MS	F	Prob		MS	F	Prob
I0001	0.14176	0.43161	0.514704		-0.11644	-0.35453	0.999999
I0002	0.10295	0.49802	0.484176		-0.09264	-0.44818	0.999999
I0003	0.22931	0.32384	0.572267		-0.16892	-0.23856	0.999999
I0004	2.63787	2.90301	0.095632		12.65412	13.92603	0.000553
I0005	0.96164	0.31141	0.57971		18.80671	6.09024	0.017649
I0006	1.19312	3.90855	0.054475		-0.80087	-2.62359	0.999999
I0007	0.03956	0.1153	0.735845		0.42341	1.23393	0.272818
I0008	2.18754	19.90332	0.000058		-1.00825	-9.17352	0.999999
I0009	1.99042	18.47603	0.000097		-0.97535	-9.05364	0.999999
I0010	0.68887	0.22532	0.637419		1.27743	0.41783	0.521457
I0011	349.2626	13.89507	0.00056		-90.8856	-3.61579	0.999999
I0012	0.03549	0.04109	0.840319		5.14167	5.95215	0.018897
I0013	1.14361	6.18128	0.016874		1.65653	8.95362	0.004573
I0014	0.05765	2.00366	0.164123		0.03218	1.11843	0.296163
I0015	0.26433	1.65901	0.204627		0.13186	0.82757	0.368048
I0016	0.06909	0.09472	0.759751		0.40413	0.55407	0.460709
I0017	0.10937	0.52682	0.471879		2.36546	11.39402	0.001572
I0018	0.7132	1.34415	0.252701		0.24682	0.46518	0.498869
I0019	13.12267	3.62902	0.063482		42.9942	11.88988	0.001275
I0020	1.34541	0.5135	0.477502		17.66956	6.74393	0.012825
I0021	5.50319	7.21033	0.010256		-1.38819	-1.81882	0.999999

Figure 4.6: DIF for descends box step right. The females consistently score higher (red) compared to male patients.

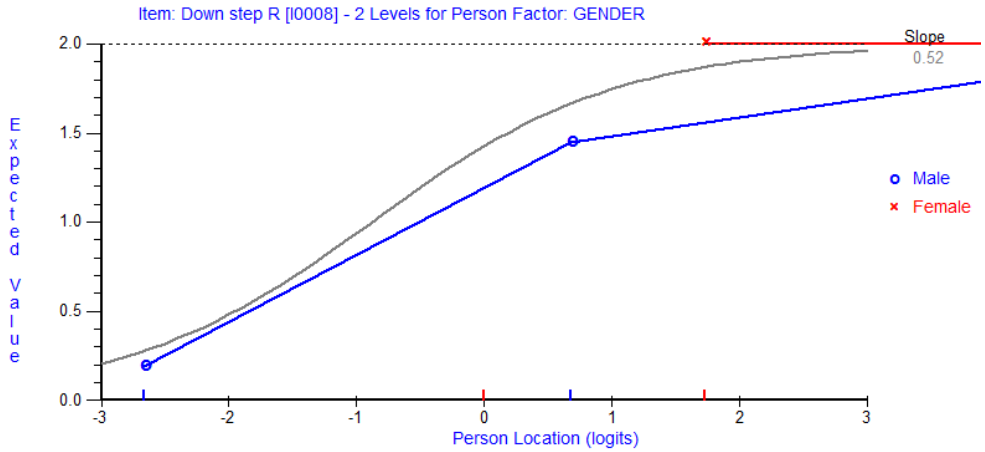
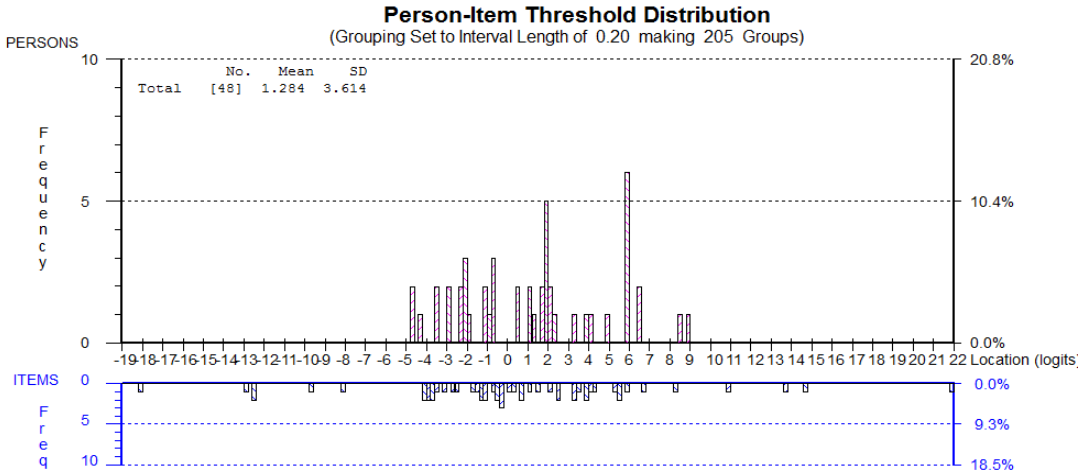


Figure 4.7: Person Item Threshold Distribution; The columns in the top half are the person locations; the columns in the lower half are the item locations. The locations of both items and persons are on the same, equal-interval metric. The metric is unbounded and, therefore, runs (theoretically) from –∞ to + ∞ and is centred around 0 because the analysis always centres the mean of the item locations at zero. Clearly, people located at different places on the continuum are assumed to have different levels of mobility. Likewise, the adapted NSAA items (functional tests) located at different places on the continuum need different amounts of mobility.



In **figure 4.7** the spread of the items is wide and the spread equal for the population (blue blocks underneath) being measured but there is a number of items at either end which are not necessary to measure ability in this population, those items such as stand, which is too easy for the cohort. There are also some areas where some of the population are not being tested, and hence a true representation of their ability may not be reflected. The cohort studied however was small, and therefore may not be a true reflection of this group of patients and will need to be extended to include the other LGMD2I patients form the other centres.

Conclusion of the assessment scales

All the tests employed were acceptable to the patients and although some patients felt less confident due to the risk of falls, most were able to complete the assessment. There were no falls or adverse events. All the patients were aware of their limitations and in some cases were surprised at the ability that they still had for certain tests, such as; standing on one leg. This for many was a task that had not been attempted for a long time. Others in the cohort whilst weak proximally on myometry, still had excellent functional ability, being able to run, squat and perform normal values in the 6MWD.

Using the limited Rasch analysis to assess the goodness of 'fit' and suitability of the adapted NSAA to use for individuals with LGMD 2I has proved reasonably successful in this cohort. Many of the items have ordered thresholds, which are clinically relevant, and the targeting of the scale is appropriate. The limitations in this study were the size of the cohort, using the adapted NSAA, and therefore further research needs to be completed using this scale in a larger cohort of LGMD2I. This could also be applied to assessing other LGMDs to assess motor performance and functional ability.

4.4 – Correlations with MRI changes

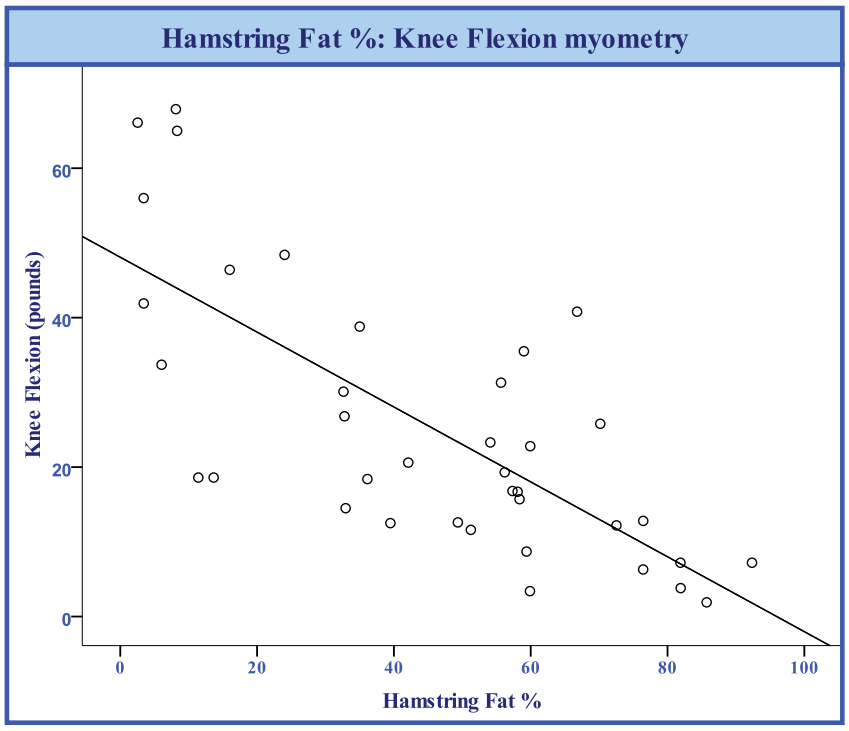
Whilst the patients in this study were well motivated and keen to achieve the best results with the strength and functional testing, this is a subjective measurement and hence may not always reflect the true status, extent and indeed progression of the muscle disease.

MRI is an objective measurement and as will be seen in the results chapters 5 and 6 can accurately delineate the muscles affected as well as detect change longitudinally. Cross sectional analysis was made on the 38 patients at baseline, correlating the degree of fat infiltration with the standardised physical testing.

Strong correlations were demonstrated between myometry measures and fat infiltration in appropriate muscle groups. The 'hamstring average fat %' correlated strongly with knee flexion r = -.73 (p<0.01) (**figure 4.8**) and the TUG test (r = .580, P<0.02). There was also strong correlation between 'hamstring average fat %' and the stair climb (r = .52, p<0.01), the stair descent (r = .46, p<0.01) and the 6MWD (r = -.79, p<0.01).

The vastus lateralis muscle (r = -0.75), vastus medialis muscle (r = -0.68) and the rectus femoris muscle (r = -0.79) correlated strongly with knee extension. All were significant; p=0.01. The 'quadriceps average fat %' strongly correlated with the stair climb (r = .718, p<0.01), the 6MWD (r = -.832, p<0.01), the timed chair rise (r = .743, p<0.01) and the TUG test (r = .753, p<0.01).

Figure 4.8: Hamstring average fat% correlated with knee flexion myometry (pounds). This shows a strong correlation r =-.73 (p<0.01).



The 6MWD, used as an outcome measure in many clinical trials, was also correlated against average fat infiltration and the hamstrings. The 'average fat percentage' is the mean across the 14 muscles quantitatively analysed and calculated per person. This therefore gives 38 average fat infiltration measurements. This measurement was strongly correlated with the 6MWD, r = -.73 ($p<0.01$) (**figure 4.9**). The 'hamstring average fat %' also strongly correlated with the 6MWD at r = -.79 ($p<0.01$) (**figure 4.10**).

Figure 4.9: Average fat% correlated with the 6MWD. This shows a strong correlation r = -.73 ($p<0.01$).

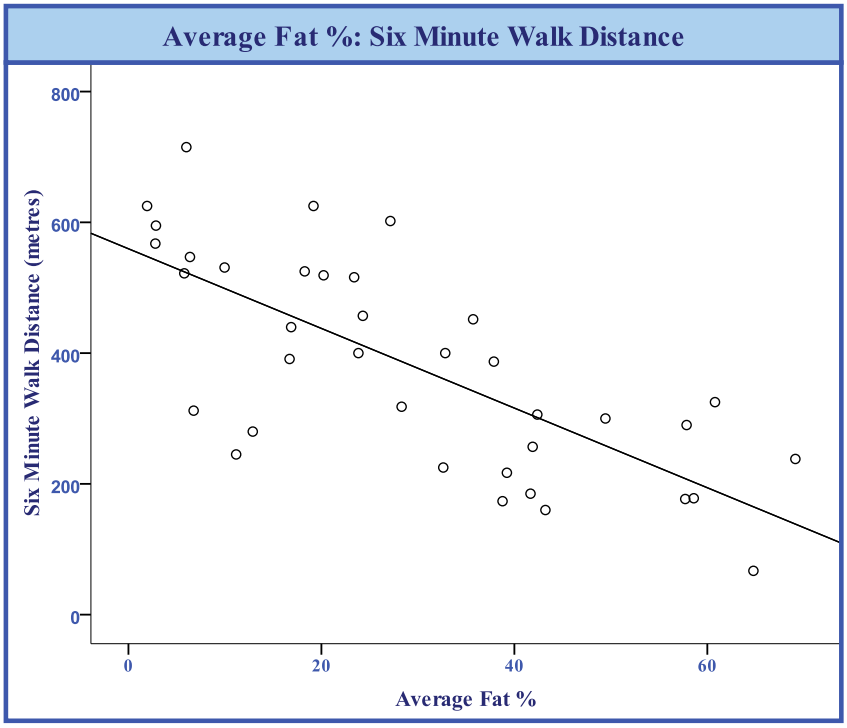
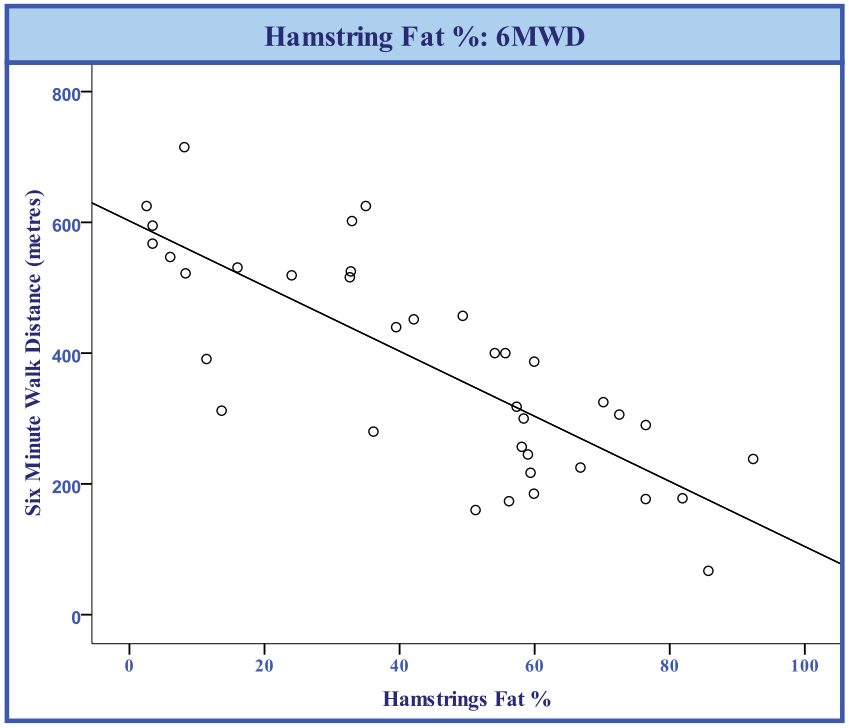


Figure 4.10: Hamstring average fat % correlated with the 6MWD. This shows a strong correlation r = -.79 (p<0.01).



4.5 – Longitudinal analysis of the assessments

In this subchapter I will discuss the findings of the longitudinal analysis of the physical and functional assessments performed.

Table 4.6 illustrates that over the 12 month period there was no significant changes in the patients standardised physical test measurements. The analysis was performed on 35 paired individuals (3 did not complete the assessment at follow up, one due to early pregnancy, one had recently received a diagnosis of prostate cancer and undergoing treatment and one, did not want to participate any further).

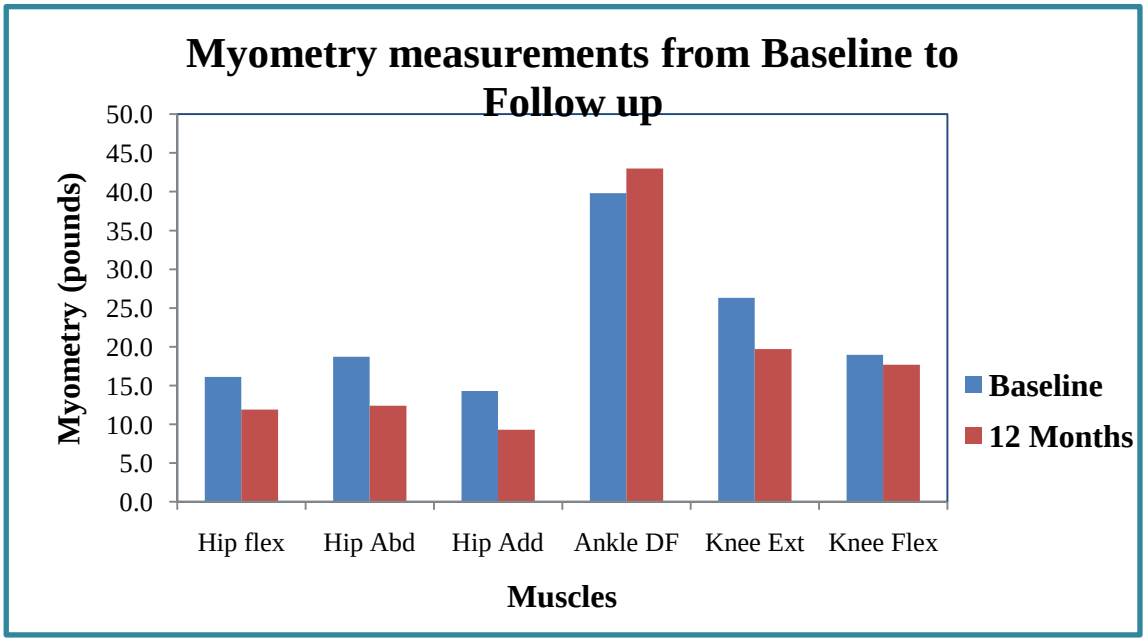
Table 4.6: Minimum, maximum and median values of myometry and the timed tests at baseline and follow-up in 35 paired individuals (Non-parametric paired Wilcoxon signed rank test). There is no significant difference found between the results at baseline and at follow up.

Muscle	Baseline min	Baseline max	Baseline median	12 months min	12 months max	12 months median	Difference (sig)
Hip Flex (pounds)	2.2	81.1	15.7	3	64	13.2	0.313
Hip Abd (pounds)	1.4	86.1	18.2	5.2	78.5	12.4	0.442
Hip Add (pounds)	1.6	58.9	14.0	4.0	74.8	9.10	0.120
Knee Flex (pounds)	1.9	66.1	18.6	0	70.5	17.9	0.2
Knee Ext (pounds)	4.3	156.6	24.9	4.4	142.3	21	0.74
Ankle DF (pounds)	5.6	86.3	38.5	4.0	93.6	45.0	0.249
Stair climb time (secs)	1.43	46.16	5.32	1.69	∞	5.47	0.149
Stair descend time (secs)	1.80	39.9	3.23	1.25	∞	3.9	0.501
Chair rise time (secs)	0.43	27.61	2.60	0.62	∞	2.94	0.882
TUG (secs)	4.3	50.5	11.1	4.1	∞	9.8	0.64
10 metre time (secs)	2.25	21.46	8.30	2.45	25.0	8.8	0.221
6MWD (metres)	67	625	318.5	50	717.5	354	0.992

∞ - the maximum time used for the analysis was infinity, as some of the patients were no longer able to do this test. Abd = abduction, Add =adduction, Flex=flexion, Ext=extension, DF=dorsiflexion, TUG=timed up and Go, 6MWD=six minute walk distance.

All 35 patients attempted the myometry; at baseline all patients registered a recording with hip abduction, hip adduction, ankle dorsiflexion, and knee extension. 1 patient was not able to generate enough force to produce a reading at both baseline and follow up for hip flexion and knee flexion. At follow up there was one further patient that was not able to produce enough force to register a recording in both hip flexion and knee flexion.

Figure 4.11: Myometry measurements from baseline to 12 months. This figure demonstrates that there is a general trend of decreasing strength apart from ankle DF which remains strong. (Abd = abduction, Add =adduction, Flex=flexion, Ext=extension, DF=dorsiflexion)



Whilst the time required to perform the 10 metre walk/run and stair climb increased in 60% of the cohort (n=21), this was not statistically significant. There was no change in the time required to descend the stairs in the cohort.

At baseline six of the patients were unable to perform the TUG test and this was mirrored in the chair rise test as five were unable to perform this, four of who had been in the group unable to do the TUG test. At follow up the number achieving the TUG test had remained the same as baseline; however one of the patients that could not do it at

baseline managed to at follow up and one of the other patients had deteriorated over the year and could no longer achieve this task.

All patients were able to perform the 6MWD at follow up, however one patient at baseline did not feel able to as he had flu like symptoms and did not feel he had enough energy to sustain his walking for that length of time. This illustrates the subjective nature of these assessments; if the patient has not had adequate sleep, or is unwell with a viral type illness or does not feel motivated at the time of their assessment, this can lead to some misleading values.

Figure 4.12: Functional Assessments from baseline to 12 months. This figure shows no major changes in the timed tests. (TUG=Timed up and Go, 10m=10 metre walk/run)

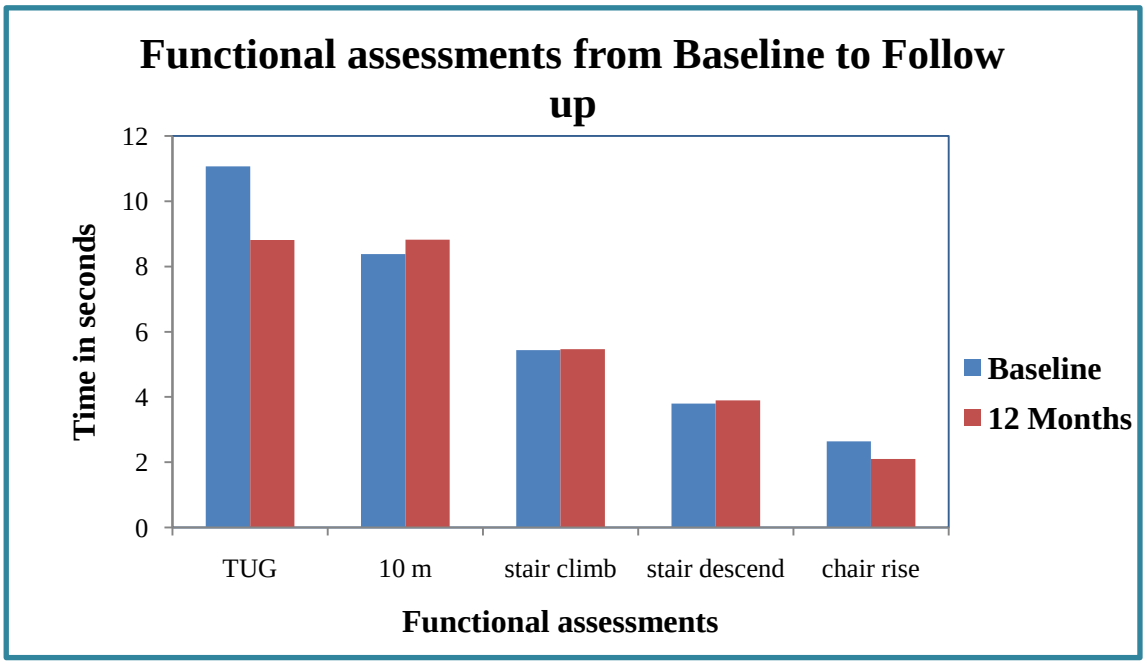
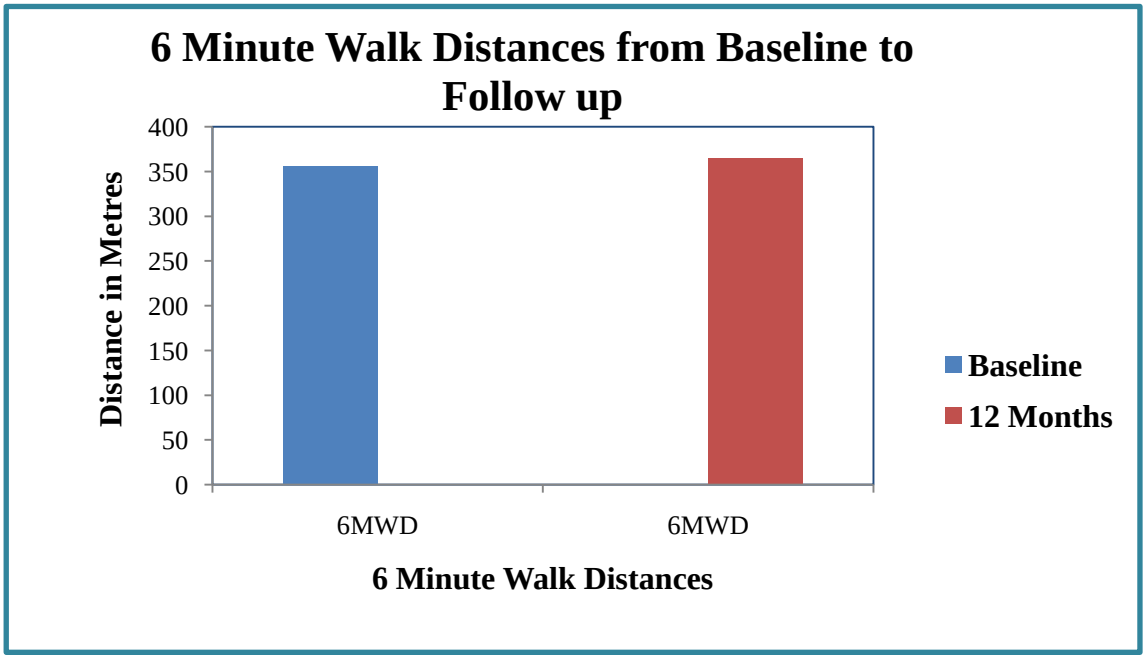


Figure 4.13: Six Minute Walk Distance (6MWD) changes from baseline to 12 months. This figure demonstrates no change in the median value of the 6MWD at baseline (318.5 metres) and at follow up (354 metres).



The functional tests were also graded in relation to their difficulty; 1; unable to do, whilst a grade 6; is able to do task normally, i.e.: run in the 10metre walk and climb the stairs alternate feet with no need for rails. These grades enable scoring of a patient who has deteriorated. Whilst they may still able to do the activity, the timing of the activity may not reflect the functional loss or compensation that the patient has developed. Therefore a patient may take longer to rise from the chair at baseline, but not use their hands to push up on the arms of the chair, therefore achieving a better grade (grade 4), whereas at follow up they may have deteriorated but have a faster time on the test, as they are now compensating and using their arms to push up from the chair, thereby scoring a lower grade (grade 2). The timings if taken in isolation may therefore present a false result.

Analysis of the grades for stair climb, 10 metre walk/run and rise from chair at baseline and follow up was undertaken (**table 4.7**). This showed no significant change over the 12 month period. The majority of the cohort scored the same grade at both baseline and follow up, there were a small number who decreased in grades with the stair climb and

rise from the chair, however in the 10 metre walk/ run, there were more patients who had improved (8/35) compared to those that had deteriorated (4/35).

Table 4.7: The grading of the timed tests, stair climb, 10 metre walk/run and rise from the chair, at baseline and follow-up in 35 Paired individuals (Non-parametric paired Wilcoxon signed rank test).

Timed test	Number decreased grade	Number increased grade	Number same grade	P value
Stair climb	7	2	26	0.150
10 metre walk/run	4	8	23	0.153
Rise from chair	6	4	25	0.834

FVC changes longitudinally

As previously stated in this chapter, respiratory involvement as reported by the patients was present in 21% (n=8) of the cohort, however when formally tested 33.3% had an FVC </= 75% predicted value for their height in sitting. 16.6% had a >/= 20% decrease in their FVC in lying and 33.3% had a 10 – 19% decrease in their FVC.

At follow up FVC recordings, in both sitting and lying, were obtained in a total of 27 patients (**table 4.8**). The results show that the median values of the FVC in sitting and lying were 75% and 67% respectively. The maximum values were 100% sitting and 100% lying, but the minimum values were 48% sitting and 28% lying.

Table 4.8: The minimum, maximum and median FVC measurements in % predicted for height in both sitting and lying, at baseline and follow-up in 28 patients and 27 patients respectively.

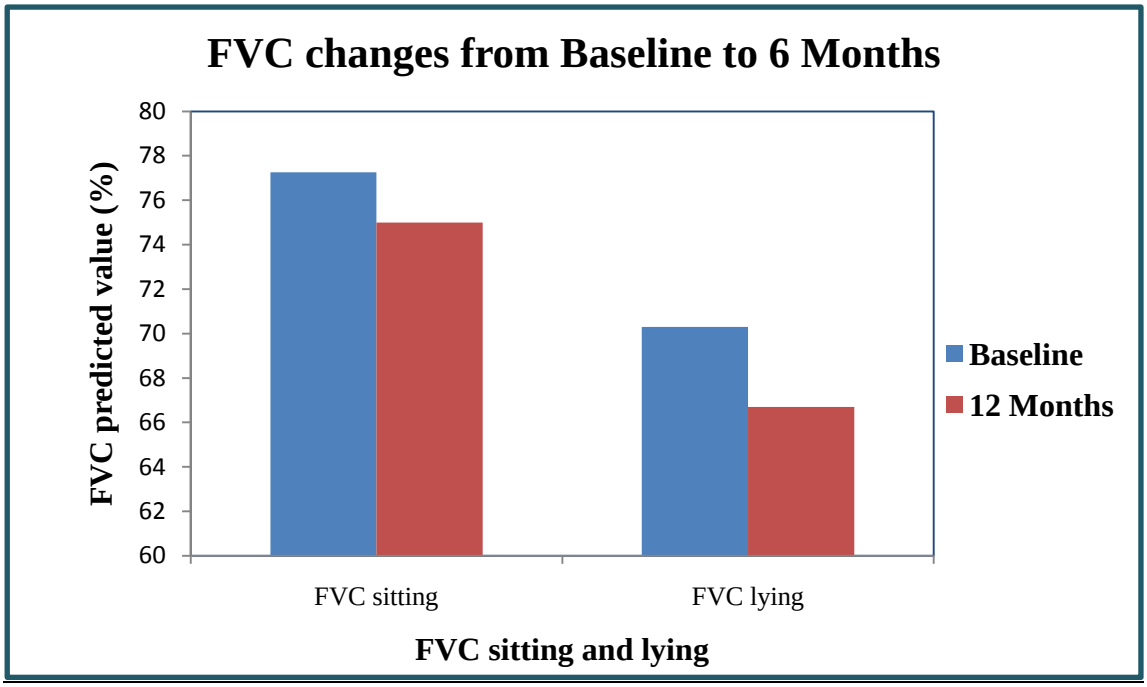
	FVC sitting (baseline) (n=28)	FVC lying (baseline) (n=28)	FVC sitting (follow up) (n=27)	FVC lying (follow up) (n=27)
Minimum	51%	36%	48%	28%
Maximum	107%	105%	100%	100%
Median	78%	71%	75%	67%

52% had an FVC </= 75% predicted value for their height in sitting. 19% had a >/= 20% decrease in their FVC in lying and 19% had a 10 – 19% decrease in their FVC.

One patient in the cohort now receives nocturnal continuous positive airway pressure (CPAP). This did not prevent her from completing the MRI.

As can be seen in **figure 4.14**, the FVC changes are apparent in this cohort of patients. The FVC measurements were obtained from 27 paired patients paired at baseline and follow up. This showed that the majority of patients had a decrease in both their sitting and lying FVC, with the decrease in the FVC sitting significant at $p=0.001$ and lying at $p<0.05$.

Figure 4.14: Forced Vital capacity (FVC) changes from baseline to 12 months, with a significant decrease in both sitting (p=0.001) and lying FVC (p<0.05).



4.6 – Discussion

The assessments performed in this cohort of ambulatory patients with LGMD2I were all acceptable to the patients taking part, and no patient fell or injured themselves during the assessments. The assessments themselves were labour intensive and required at least 2 physiotherapists/clinicians to perform them safely and accurately. The 38 patients that took part in the initial baseline assessment showed great variability in their disease severity, illustrating the heterogeneous nature of this condition and were equally matched in both age group and gender. As in previous reports, our cohort had respiratory involvement in about a third of patients with 50% of them demonstrating a postural drop in their FVC on lying.

The 6MWD was attempted by all except one at baseline, due to a flu like illness, and again this demonstrated great variability in the distances covered. Some patients were able to walk at a normal pace, whilst others had to use the wall for reassurance and support if they felt as if they were falling. Of interest is the fact that these patients generally did not alter their speed and hence the pace and number of laps completed were stable. This was also reflected in the 10 metre walk/run, where the majority did not pick up speed or alter their pace. The time for the 10 metre walk/run strongly correlated with the distance attained on the 6 MWD ($r = -.88$, $p<0.01$), therefore the 10 metre walk/run maybe an alternative to the 6MWD in this group of patients. This may not however be ideal for other conditions, and in particular in children where there is often great variability in pace. This would therefore mean that this would be a more acceptable and user friendly measurement that could be regularly used in a clinical setting due to the space and time required to perform it.

The Rasch analysis, performed on 13 of the patients, 48 assessments in total, proved that the adapted NSAA test items were a good fit for this cohort of LGMD2I, although this was in a very small cohort and repeated measurements in the same individuals. It did highlight items that did not particularly test the patients abilities, such as walking and standing, whereas at the other extreme, some items, in particular the rise from a chair and hopping, proved difficult. The Rasch analysis does however show a good spread of items and clear progression in order of the tests, with the majority testing the group. There was an interesting gender difference, illustrating that the women

irrespective of their disease severity, performed the descend step in both right and left leg differently to the men. The results are limited due to the analysis being performed on a small cohort of the larger group. In order to fully assess the 'goodness of fit' of these items for assessing LGMD2I, one would need to expand the numbers tested. As LGMD2I is a relatively rare disorder, this would need to be coordinated on an international basis and hence the use of the FKRP registry to do this may be the key.

The correlations between the quantitative MRI were strong including the functional assessments, particularly the 6MWD and 10 metre walk. There was strong correlation between the hamstrings and stair climb, stair descend as well as the myometry measurements of knee flexion and extension. Knee extension and hip flexion correlate strongly with the quadriceps muscles, which in turn correlate with the 10 metre walk/run and 6MWD.

The detection of change over time with standardised physical tests did not show any statistical significant change apart from the FVC in both the sitting and lying positions. Whilst these functional and strength tests were not significantly different over the twelve months, many of the patients did feel that there had been a change in their abilities especially in those that were moderate to severely affected. This was not reflected in the testing results at 12 months in the group. There are many reasons why this may occur. From the literature we know that LGMD2I, whilst a heterogeneous condition, does tend to progress slowly with time compared to the more rapid progression of DMD and therefore one reason that change may not be appreciated, is that a longer interval between examinations may be required. Adult patients also adapt very well to a slowly progressive condition and devise ways and means of compensating for their weakness, such as employing other muscles to do a manoeuvre when the principle muscle is affected by the disease. There is also a threshold as to when a muscle becomes so infiltrated with fat and connective tissue that it affects function. The patient may then experience a sudden loss of function, such as ambulant to non-ambulant, although the process has been slowly ongoing for many years.

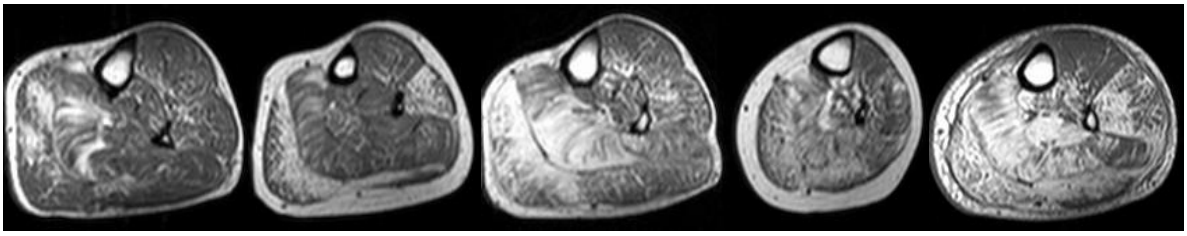
Chapter 5 – The cross sectional MRI results.

The aims and objectives in this study were to examine whether quantitative MRI could be applied to a cohort of LGMD2I patients and to compare this data with the qualitative MRI grading technique. In this chapter I have reported the MRI scoring using the qualitative grading technique in 38 LGMD2I patients and the quantitative fat fraction percentage as analysed using the 3 point Dixon technique. These have then been compared against each other as well as with some of the functional parameters reported in Chapter 4.

5.1 Semi-quantitative analysis – T_1w images

Pelvis, thigh and lower leg T_1w images were analysed for the patient group (n=38), with only 22 muscles (1.9%) assessed to be normal (grade 0) and 103 (9.1%) having early changes (grade 1), compared to 474 (41.7%) scoring grade 3 and 4. (**table 5.1**). Although the gluteus maximus muscle was not acquired quantitatively, the analysis of the T_1w images revealed that 80% of the patients scored grade 3 or 4. In the lower leg, involvement of the gastrocnemii and soleus muscles was most noticeable with relative sparing of the tibialis anterior until a late stage (**figure 5.1a-e**). This was most striking in patients who had relatively severe involvement of the gastrocnemii with little change in the tibialis anterior muscles. The fat fraction in the calf muscles was specific with a variegated, striped appearance of the soleus (**figure 5.1a-c**) and a lacy, reticular pattern in both lateral and medial gastrocnemius, commencing initially from the internal borders (**figure 5.1b & d**). The peroneus longus muscle demonstrated 'salt and pepper' speckling (**figure 5.1b & e**).

Figure 5.1: T_1weighted images of the lower leg in LGMD2I patients with increasing fat fraction (a-e). In image (a) there is involvement of MG and sol compared to image (e) where all but TA is severely infiltrated.



In the literature it has been reported that there is initial posterior involvement in the thigh with gradual progression anteriorly as the disease progresses (Wattjes et al. 2010, Fischer et al. 2005). In this cohort there appeared to be the general trend of fewer changes seen on MRI in those with milder symptoms compared to those with more severe symptoms. The biceps femoris (long head) muscle was most severely affected with the semimembranosus and the semitendinosus muscles next (figure 5.2b- e). These muscles had a reticular pattern of involvement from the more internal borders out to the periphery (figure 5.2c). The vastus lateralis muscle was generally spared until late in the disease process. The vastus lateralis muscle demonstrated peripheral sparing, (figure 5.2d), a reverse of the pattern in Bethlem and Ullrich congenital myopathy when central sparing is seen (Mercuri et al. 2005a). The sartorius and gracilis muscles were relatively spared and had a stippled appearance with hypertrophy when initially affected (figure 5.2a, b, c & e), however become atrophied as the fat fraction increases. The rectus femoris was also relatively spared with gross hypertrophy in less severe participants (21.1% of study cohort) (figure 5.2d). Atrophied recti were seen in patients with more extensive changes, as with the sartorius muscle and the gracilis muscle.

Figure 5.2: T_1weighted images of the thigh in LGMD2I patients with increasing degrees of fat fraction (a-e). In (a) this is almost normal, however in (e) only sparing of gracilis and sartorius is seen.

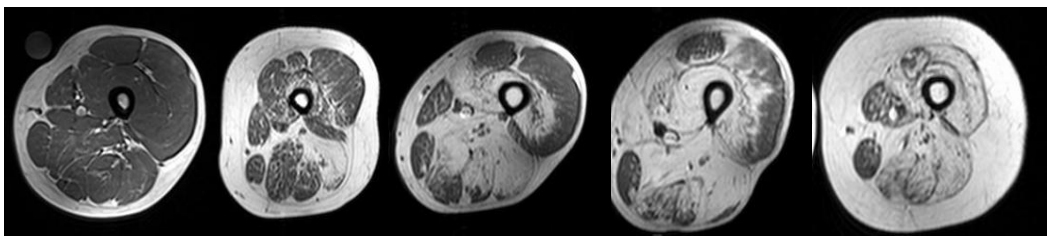
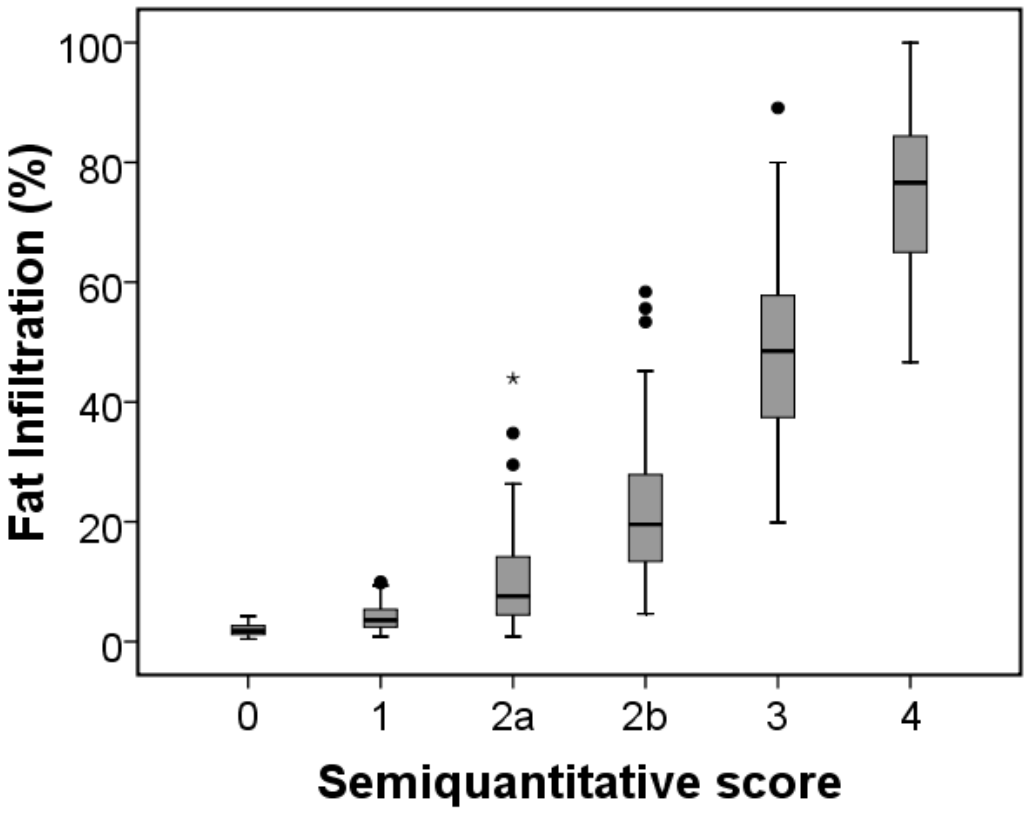


Table 5.1: Percentage of LGMD2I patients in each category of each qualitative grade for individual muscle groups using the Mercuri et al. (2002a) scale, with the median grade for each muscle.

Semi quantitative grade	0	1	2a	2b	3	4	Median grade
Muscle							
Gluteus Maximus (GM)	0	0	10.5	10.5	26.3	52.6	4
Biceps Femoris long head (BFLH)	0	2.6	10.5	10.5	13.2	63.2	4
Semitendinosus (ST)	0	5.3	13.2	10.5	31.6	39.5	3
Semimembranosus (SM)	0	0	18.4	26.3	18.4	36.8	3
Biceps Femoris short head (BFSH)	0	8.6	31.4	25.7	25.7	8.6	2b
Sartorius (SAR)	2.6	7.9	34.2	28.9	21.1	5.3	2b
Vastus Medialis (VM)	0	15.8	26.3	13.2	26.3	18.4	2b
Gracilis (GRAC)	2.6	10.5	42.1	13.2	21.1	10.5	2a
Vastus Lateralis (VL)	5.3	5.3	21.1	28.9	28.9	10.5	2b
Rectus Femoris (RF)	10.5	15.8	26.3	21.1	15.8	10.5	2a
Medial Gastrocnemius (MG)	5.3	7.9	18.4	23.7	21.1	23.7	2b
Lateral Gastrocnemius (LG)	2.6	18.4	18.4	23.7	28.9	7.9	2b
Peroneus Longus (PL)	0	18.4	26.3	34.2	18.4	2.6	2b
Soleus (SOL)	2.6	5.3	42.1	26.3	18.4	5.3	2a
Tibialis Anterior (TA)	7.9	34.2	47.4	10.5	0	0	2a

Comparison of the qualitative grading and the quantitative Dixon imaging across all the analysed muscles in the LGMD2I patients ($r_s = 0.87$, $p < 0.01$) correlated strongly (**figure 5.3**). The grey bars represent the middle 50% of the distribution between the upper and lower quartile and their corresponding fat fraction. The dots and stars represent points which lie more than 1.5 times but less than 3 times the interquartile range (dots) or beyond (stars) 3 times the interquartile range from the upper quartile. Whilst this demonstrates that the techniques of grading correlate with the quantitative fat fraction percentage values, there is still considerable spread within each grade and a high degree of overlap between the grades, suggesting poor specificity.

Figure 5.3: This figure illustrates the variability seen in patients with LGMD2I; a comparison between the semi-quantitative scores and the 3 point Dixon analysis. $r_s = 0.87$, $p < 0.01$.



5.2 - Quantitative analysis –3 point Dixon technique

Nine muscles mid thigh and five muscles mid lower leg, a total of 14 muscles per patient, were analysed in all 38 subjects. **Figure 5.4** summarises the quantitative results. The degree of muscle pathology varied significantly from severe, as in the biceps femoris long head muscle (median fat percentage 69.7%), to very mild involvement in the tibialis anterior muscle (median fat percentage 5.9%). **Table 5.2** highlights that all muscles in the LGMD2I cohort were affected compared to the control group according to the measured quantitative Dixon fat fractions.

Figure 5.4: The grey bars illustrate the quantitative fat percentages for LGMD2I patients with median qualitative score at the left of the bars. Box indicates lower and upper quartiles with median bar; stems show range with outliers more than 1.5 Interquartile ranges marked separately).

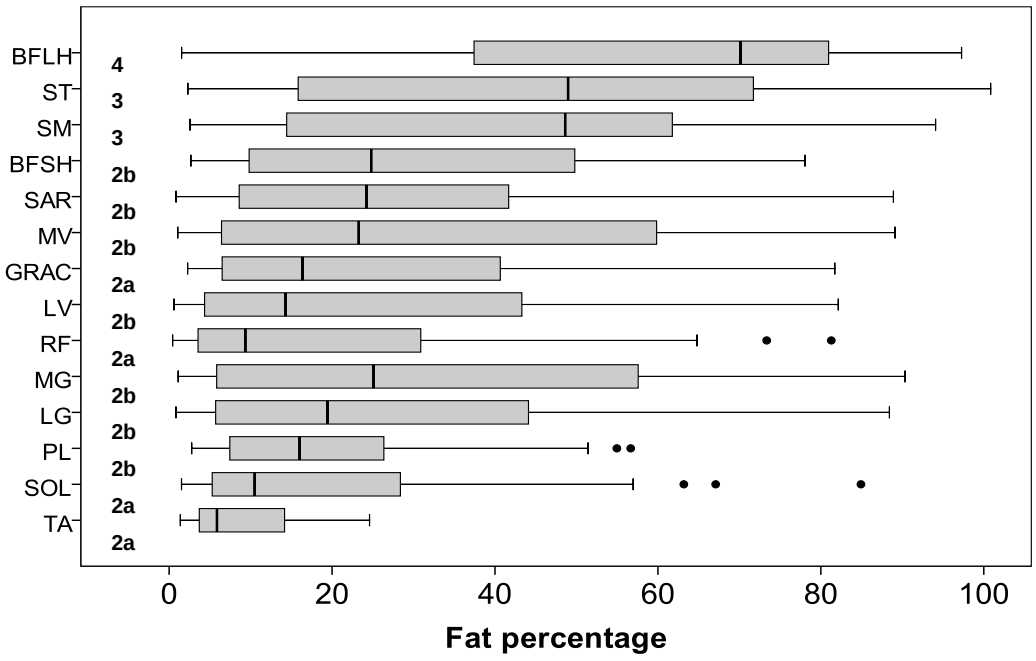
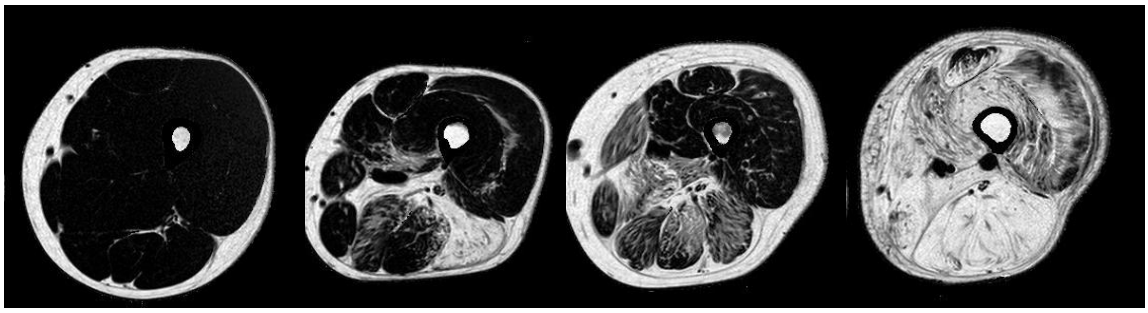


Table 5.2: The median values of fat fraction (%) in the patient group and the control group. The p values represent Mann-Whitney U test between patients and controls

Muscle	Median LGMD2I	Median Control	P value
Biceps Femoris long head (BFLH)	69.7	3.9	0.0001
Semitendinosus (ST)	49.0	2.3	0.00001
Semimembranosus (SM)	48.6	2.9	0.0001
Biceps Femoris short head (BFSH)	25.5	3.2	0.001
Sartorius (SAR)	24.2	3.9	0.001
Vastus Medialis (VM)	23.3	3.0	0.01
Gracilis (GRAC)	16.4	3.0	0.0001
Vastus Lateralis (VL)	14.3	5.5	0.04
Rectus Femoris (RF)	9.4	3.3	0.03
Medial Gastrocnemius (MG)	25.1	2.2	0.001
Lateral Gastrocnemius (LG)	19.4	2.1	0.001
Peroneus Longus (PL)	16.0	4.9	0.01
Soleus (SOL)	10.5	3.0	0.001
Tibialis Anterior (TA)	5.9	2.8	0.02

The pattern of involvement is illustrated in **figure 5.5**, with increasing severity in the thigh from normal (in a control) (a) to severe (d). These figures illustrate the gradual loss of muscle and replacement with fat as seen in the T_1w images. The biceps femoris long head muscle is particularly affected in **figure 5.5b** and as can be seen in **figure 5.5c** there is increased loss of muscle particularly in the posterior thigh muscles affecting the biceps femoris (long head) muscle, semimemebranosus muscle and semitendinosus muscle, adductor magnus muscle (not quantitatively measured) and the gracilis muscle and sartorius muscle. The anterior thigh muscles are spared. **Figure 5.5d** represents the late stage of this disease with minimal muscle seen with mostly fat and fascia visible. This patient, although severe was still ambulant.

Figure 5.5: Quantitative Dixon fat fraction from a control thigh (a) and of LGMD2I patients with increasing fat fraction (b)-(d).



5.2.1 - Gender differences

There were some striking gender differences in muscle pathology in our LGMD2I cohort. In **table 5.3** it can be seen that in the female group there was diffuse involvement of both the gastrocnemii muscles; however in the male group the lateral gastrocnemius muscle had a lower fat fraction. This contrasts with previous LGMD2I studies (Wattjes et al. 2010, Fischer et al. 2005), which had reported diffuse and equal involvement of medial gastrocnemius and lateral gastrocnemius compared to LGMD2A, where medial gastrocnemius is more involved than lateral gastrocnemius.

The hamstrings have previously been reported as more severely affected compared to the anterior thigh muscles (Wattjes et al. 2010, Fischer et al. 2005). In the female subjects there was a trend towards semimembranosus being spared relative to semitendinosus (median 28.2% compared with 47.3%). In males these muscles were similarly affected, 56.2% and 51.8% respectively.

The gracilis and sartorius muscles have previously been reported as being relatively and equally spared (Wattjes et al. 2010, Fischer et al. 2005). In our male cohort the gracilis muscle is more preserved compared to sartorius ($p = 0.01$). In the female group the gracilis and sartorius muscle were equally affected.

The anterior thigh muscles also demonstrated gender differences; in the female subjects the vastus lateralis and vastus medialis were affected similarly (18.2% and 18.9%,) whilst in the male subjects there was a greater fat fraction, 45.7% in vastus medialis compared to 11.2% in vastus lateralis ($p<0.005$) (**figure 5.6**). This result is particularly interesting given the results of the Rasch analysis on the adapted NSAA reported in chapter 4 (**table 4.5**), where the women regardless of their disease severity, completed the task of descend box step, right and left, consistently better than the male patients and when visualising their walking appeared to walk straighter without a compensatory turn out of the feet and side to side swing of the legs as seen in many of the male patients. This however was carried out on a small cohort (n=13) of the larger group and would therefore need to be extended to the other centres to examine whether this finding is replicated in the larger group.

Table 5.3: Median values of the fat fraction for the study cohort (19 males and 19 females). It illustrates significant differences between the anterior thigh muscles in the male group and preferential sparing of the gracilis compared to sartorius. These features are not found in the female group.

Muscle	Male	Female
Semitendinosus (ST)	51.8	45.3
Semimembranosus (SM)	56.2*	28.2
Sartorius (SAR)	24.0	25.1
Gracilis (GRAC)	13.7**	25.0
Vastus Lateralis (VL)	11.2	18.2
Vastus Medialis (VM)	45.7§	18.9
Medial Gastrocnemius (MG)	22.2	28.0
Lateral Gastrocnemius (LG)	15.1†	33.7

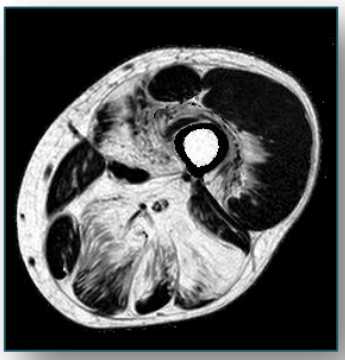
* p = 0.05 compared to male ST

** p = 0.01 compared to male SAR

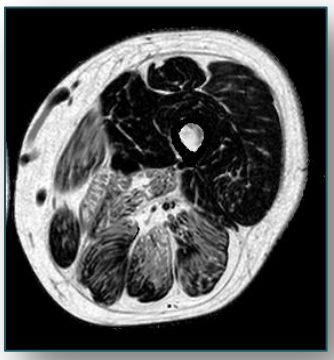
§ p < 0.005 compared to male VL

† p = 0.05 compared to male MG

Figure 5.6: These MRI scans illustrate the medial vastus changes seen in the male (i) and the preservation seen in the female (ii).



(i)



(ii)

Interfamilial differences

There were changes also seen with two sisters who were of similar ages (58 years, 55 years), who were both similar with regards to their strength and functional ability however on MRI their lower leg images were very different with one sister obtaining results within normal limits (**figure 5.7**), whilst the other had a significant increase in fat fraction of both her gastrocnemius muscles (**figure 5.8**).

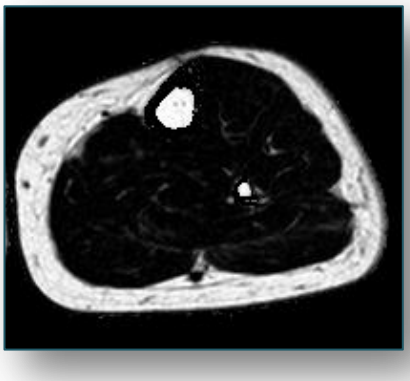


Figure 5.7: Sister 1 (58 years); within normal limits.

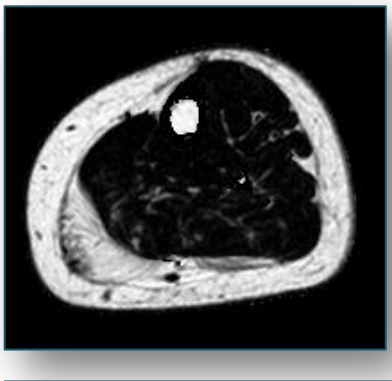


Figure 5.8: Sister 2 (aged 55 years); increased fat fraction in the medial and lateral gastrocnemius muscles.

5.2.2 – Correlation with the patients age and duration of symptoms.

Cross sectional analysis was made on the 38 patients at baseline, correlating the degree of fat fraction with age, duration of symptoms and the standardised physical testing (as previously reported in chapter 3).

There was a positive correlation seen between average fat percentage and age (r =.48, p<0.01) (**figure 5.9**), but there was no correlation between average fat percentage and the duration of symptoms (**figure 5.10**).

Figure 5.9: Age correlated with average fat%. This shows a correlation r =-.48 (p<0.01).

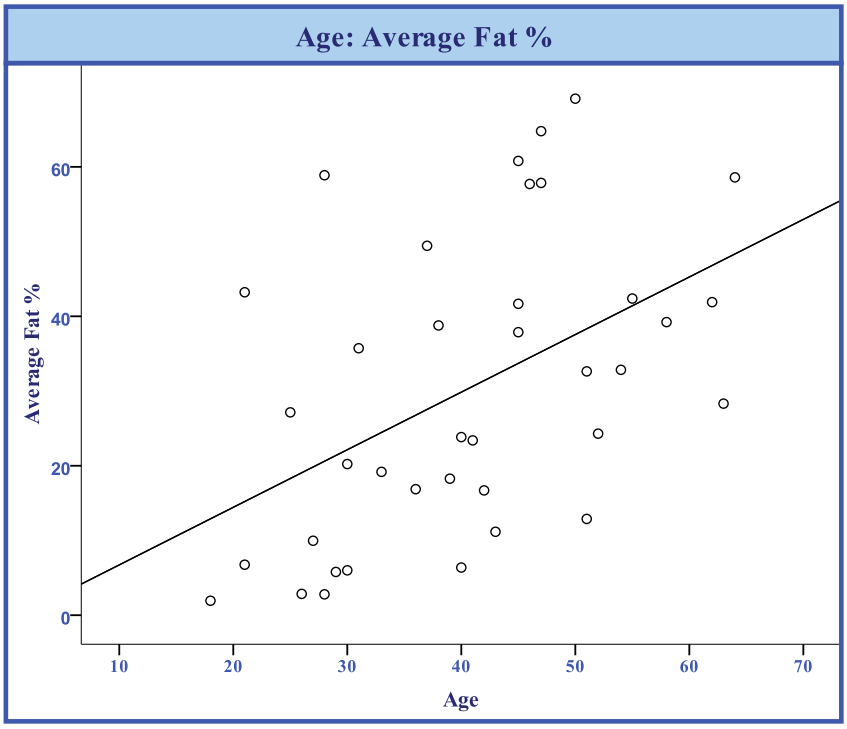
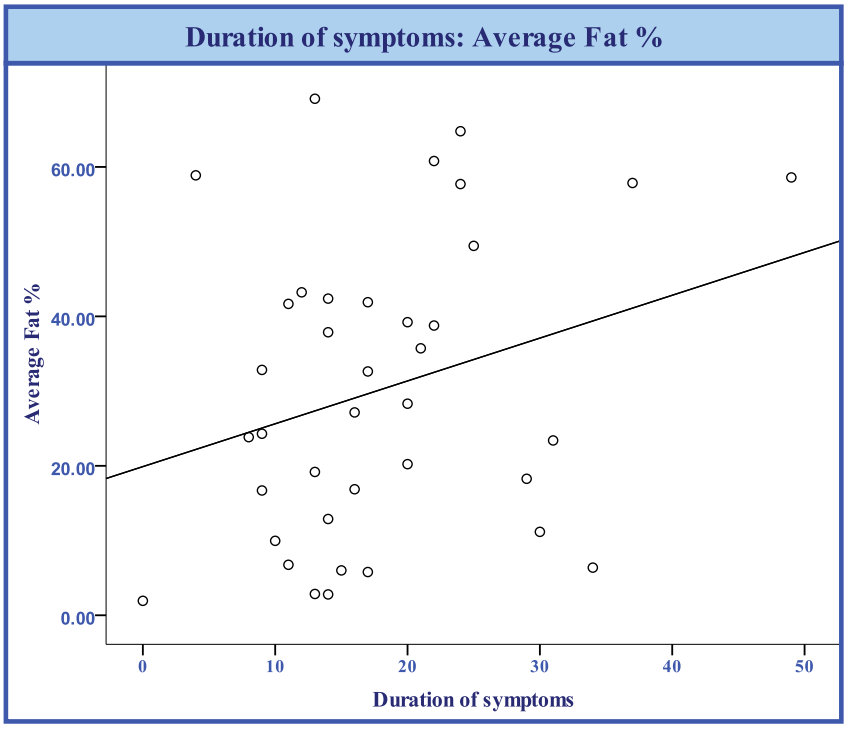


Figure 5.10: Duration of disease symptoms correlated with average fat%. This shows no correlation.



5.3 - Discussion

This is the largest reported cross sectional MRI study (n=38) of patients with LGMD2I due to the common mutation in the *FKRP* gene. Both traditional qualitative grading and quantitative fat imaging have been employed to measure the differing degrees and pattern of muscle involvement, quantitative fat percentage and the relationship with functional tests. Disease progression and severity of individual muscle involvement in patients with LGMD2I can often be difficult to assess clinically and therefore there is need for reliable objective measures.

Within the literature T_1w MRI analysis of patients with LGMD2I demonstrated pathology in the posterior thigh muscles (Bushby and Beckmann, 1995, Wicklund and Hilton-Jones, 2003, Wattjes et al. 2010, Fischer et al. 2005, Mercuri et al. 2005b). More

specifically the changes seem to occur in the biceps femoris and internal adductor muscles first and with further disease progression the rest of the hamstring muscles become involved and to a lesser degree the vastus intermedius and lateralis muscles. Fischer et al. (2005) reported that involvement of the vastus medialis and rectus femoris muscles was only observed in those patients with advanced disease. The results from this study confirmed the qualitative findings of these previous studies; however we have also demonstrated that in the male group there was a more severe and earlier involvement of the vastus medialis muscle compared to the vastus lateralis muscle unlike in the female group. This has been detected as a difference in the functional assessments, as reported in chapter 4, that the female patients descend the box step better than the male patients, regardless of their severity. Descending steps depend upon functioning quadriceps and if there is a difference in the level and pattern of fraction within the quadriceps muscles, this will alter the way in which this task is performed.

In the gastrocnemius muscles, diffuse and equal involvement (Wattjes et al. 2010, Fischer et al. 2005) has previously been reported. However in our study, the male group demonstrated a predominantly medial involvement.

This cross sectional part of the study has demonstrated that there are a few potential target muscles that could be used for the assessment of pathological changes for clinical trial purposes. This will be explored further in chapter 6 when reporting on the longitudinal data. **Figure 5.4** highlights the suitability of certain muscles for these assessments based upon the fat fraction. The muscles that appear to be the most suitable demonstrate a wide range of fat fraction within the cohort. The biceps femoris long head, semimemebranosus and semitendinosus muscles may be considered less suitable to monitor as they have more than a 50% fat fraction in at least 50% of the LGMD2I population. Conversely the tibialis anterior muscle would also be considered less suitable due to limited pathology even in the severely affected patients.

There have been a number of studies addressing the more severe type of muscular dystrophy, DMD, highlighting both the natural history and its effect on muscles assessed by MRI (Mercuri et al. 2005c, Fischer et al. 2005). DMD is now subject to

clinical trials and at present therapeutic success is assessed by muscle biopsy and by functional outcomes, such as the 6MWD. These tests however are either invasive or are highly dependent on patient cooperation. MRI is both non-invasive and objective and is therefore a powerful quantitative tool for trials when considered along with the strength and functional measures. Wren et al. (2008) demonstrated that the quantitative Dixon technique correlated well with functional outcome measures in DMD as well as age. The severity of muscle involvement in our cohort was not related to the onset of symptoms (disease duration) but was associated with age of the patient. There were strong correlations seen between fat percentage and functional timed tests which may reflect disease severity, such as the 6MWD (Chapter 4; **figure 4.12**) and the TUG test ($r = .633$, $p<0.01$). Whilst some of these tests are now being employed in therapeutic drug trials in patients with DMD (McDonald et al. 2010), with the 6MWD as a primary outcome, the correlations need to be taken in conjunction with both the clinical examination and findings.

Myometry was found to significantly correlate with some muscle MRI fat percentages as previously described in chapter 4. Our cohort of patients was well motivated and the functional tests were performed to their best. This demonstrated that these tests were well matched and correlated if the patients were performing to their full potential. In the usual clinic setting this may not be possible in all patients.

There remains variability between the two methods of assessment which is not accounted for on an individual patient basis: a result of 250m on the 6MWD can be associated with a fat fraction between 10 and 70%. This is a wide range and whilst MRI is an objective measure of muscle health, it cannot be interpreted in isolation.

MRI therefore may be useful as a monitoring tool on an individual patient basis to assess change/deterioration or in the advent of a therapy to monitor improvement.

Longitudinal analysis of this cohort will therefore assess whether change has occurred over time in this cohort, and this is reported in Chapter 6.

Limitations; This MRI study, although carried out in the largest LGMD2I cohort to date, was still small and would ideally need to be extended to include more asymptomatic and paediatric patients. The assessment was also limited to analysis of the quantitative Dixon scans at one level mid point in the lower leg and thigh. Ideally one would assess the ROIs at all levels and analyse the variability that is seen within each muscle. This may give further information as to how the process of muscle damage occurs in this condition and potential muscles that can be used in clinical trials as biomarkers.

The cross sectional part of this study has shown that quantitative fat imaging provides an objective measurement of the fat fraction that is more sensitive than the previous qualitative technique. We have found a similar pattern of muscle involvement as previously described in the literature but have also demonstrated gender differences not previously reported. These quantitative techniques could be used on an individual patient basis to monitor both disease progression and in the future, improvement in the advent of a therapeutic agent.

Chapter 6 – The longitudinal MRI results.

The aims and objectives of this study were to assess the progression of muscle pathology in patients with LGMD2I by muscle MRI, both qualitatively and quantitatively. I also compared quantitative MRI to qualitative MRI and analysed whether MRI would be a sensitive enough biomarker to detect change over a 12 month period of time, and hence a possible outcome measure to monitor disease progression. The results of this work are outlined in this chapter and include the longitudinal qualitative results, the longitudinal quantitative results and discussion of these findings.

6.1 - Analysis of T_1w images

The follow up analysis was limited to 32 participants of the original 38 due to the following reasons. Two patients could not take part due to early pregnancy since the first visit and this was one of the contraindications to undergoing an MRI in a research setting and was outlined in the ethical approval for the study. MRI during pregnancy has not be proven to cause deleterious effects on human embryos or foetuses and represent an excellent imaging mode if the clinical need dictates and ultrasound scanning is not sufficient (Levine et al. 1999, Amin et al. 1999). Due to limited data on MRI safety within the first trimester, it was agreed that for study purposes, patients in the early stages of pregnancy be excluded (Levine et al. 1999). One further participant was unable to complete the follow up MRI as he was undergoing treatment for a recently diagnosed malignancy and one participant did not want to remain in the study.

34 participants therefore underwent the follow up MRI scans; however two of the follow-up scans were not adequately repositioned for analysis and therefore were excluded from the final statistical testing.

The statistical analysis has therefore been performed on the 32 paired participants (17 male and 15 female; aged between 19 years and 65 years, mean ages of 40.9 years in the male group and 42.3 years in the female group) and their qualitative and quantitative results.

Pelvis, thigh and lower leg T_1w images were scored for the patient group (n=32), as in the cross sectional study. The whole muscle was analysed and scored according to the Mercuri et al. (2002a) grading scheme (**table 6.1**). 48 muscles (5%) were assessed to be normal (grade 0) and 94 (9.8%) had early changes (grade 1), compared to 414 (43.1%) scoring grade 3 and 4. Although quantitative 3 point Dixon data for the gluteus maximus muscle was not acquired, the scoring of the T_1w images revealed that 81.3% of the patients scored grade 3 or 4.

Table 6.1: Percentage of LGMD2I patients in each category of each semi-quantitative grade for individual muscle groups using the Mercuri et al. (2002a) scale, with the median grade for each muscle. Follow up data 12 months from baseline

Semi quantitative grade	0	1	2a	2b	3	4	Median grade
Muscle							
Gluteus Maximus (GM)	0	3.1	12.5	3.1	25.0	56.3	4
Biceps Femoris long head (BFLH)	3.1	3.1	9.4	9.4	9.4	65.6	4
Semitendinosus (ST)	3.1	6.3	9.42	9.4	18.8	53.1	4
Semimembranosus (SM)	3.1	6.3	12.5	15.6	18.8	43.8	3
Biceps Femoris short head (BFSH)	3.1	9.4	21.9	25.0	21.9	18.8	2b
Sartorius (SAR)	3.1	6.3	37.5	18.8	21.9	12.5	2b
Vastus Medialis (VM)	9.4	12.5	18.8	6.3	28.1	25.0	3
Gracilis (GRAC)	3.1	12.5	34.4	12.5	25.0	12.5	2b
Vastus Lateralis (VL)	6.3	12.5	9.4	25.0	28.1	18.8	2b
Rectus Femoris (RF)	9.4	15.6	21.9	21.9	15.6	15.6	2b
Medial Gastrocnemius (MG)	6.3	6.3	12.5	28.1	18.8	28.1	2b
Lateral Gastrocnemius (LG)	6.3	12.5	15.6	18.8	37.5	9.4	2b
Peroneus Longus (PL)	6.3	6.3	40.6	25.0	15.6	6.3	2a
Soleus (SOL)	3.1	12.5	28.1	28.1	21.9	6.3	2b
Tibialis Anterior (TA)	9.4	25.0	56.3	9.4	0	0	2a

In the semitendinosus muscle, the median score changed from grade 3 to grade 4. In reviewing **table 6.1**, it can be appreciated that there was a shift in percentages of scans scored a grade 4 at follow up (53.1%) compared to at baseline (39.5%). The majority of this increase has come from previously graded scans being scored a grade 3, 31.6% at baseline and 18.8% at follow up. The percentages of muscles scored in grade 0-2b are fairly constant for this muscle from baseline to follow up.

In the vastus medialis muscle, the median score changed from a grade 2b to a grade 3. This is due to an increase in scans scored a grade 4 at follow up (25%) compared to at baseline (18.4%). The majority of this increase appears to come from previously graded scans scoring a grade 2b, 13.2% at baseline and 6.3% at follow up. This represents a 7% increase of scans scoring a grade 3 at follow up from a grade 2b at baseline, and likewise a subsequent 7% increase in scans scoring grade 4 from grade 3 at baseline, therefore maintaining a constant percentage scoring grade 3 at baseline and follow up.

Peroneus longus, unlike the other muscle groups, the median score changed from 2b to a follow up score of grade 2a. Whilst the percentage scoring a grade 0 and 1 appears to be greater than the baseline scan, the numbers of patients actually scoring a grade 0 and 1 is a total of 4, compared to the baseline where a total of 7 patients scored grade 1. There also appears to be an increase in scoring a grade 2a in the follow up scan, 40.6%, compared to the baseline of 26.3%, however this refers to 10 and 13 patients respectively and in total the number of patients scoring grade 0, 1 and 2a equates to 17 patients in both the baseline and follow up scans.

Further analysis of the changes from baseline to the follow up scan in the qualitative T_1w scans show no significant differences with little change either increasing or decreasing in the scoring and grades (**table 6.2**).

Whilst overall the grades remain the same with no significant changes, there are some grades that increase and others that decrease. This is seen particularly in the peroneus longus muscle, as discussed earlier. There is an apparent increase in the numbers of patients scored a grade 0, 1 and 2a where in fact patient numbers scoring these grades in

Table 6.2; Number of patients with a decreased, increased or 'same grade' qualitative grade at from baseline to follow up. (Non-parametric paired Wilcoxon signed rank test)

Muscle	Number decreased grade	Number increased grade	Number same grade
Gluteus Maximus (GM)	3	4	25
Biceps Femoris long head (BFLH)	2	1	29
Semitendinosus (ST)	5	4	23
Semimembranosus (SM)	7	5	20
Biceps Femoris short head (BFSH)*	5	4	20
Sartorius (SAR)	5	8	19
Vastus Medialis (VM)	8	8	16
Gracilis (GRAC)	5	4	23
Vastus Lateralis (VL)	5	10	17
Rectus Femoris (RF)	4	6	22
Medial Gastrocnemius (MG)	4	7	21
Lateral Gastrocnemius (LG)	4	10	18
Peroneus Longus (PL)	10	7	15
Soleus (SOL)	4	5	23
Tibialis Anterior (TA)	3	3	26

* BFSH was graded in 29 participants. 3 were scans were impossible to grade due to poor image resolution severe infiltration or positioning.

both baseline and follow up scans are the same. It is the distribution of these grades that is altered. This reflects the difficultly and the poor specificity of the grading system when performing longitudinal studies. A scan that appears to have a fat fraction of approximately 30% may be given a grade of a 2a or a 2b as the cut off limit is 30%, as with grade 1 and 2a, a mild degree of pathology and less than a 30% fat fraction respectively. It can be difficult to appreciate when a scan changes from mild to less than 30% and hence a score of 1 or 2a.

6.2 - Quantitative analysis

As in the cross sectional study (Chapter 5.2) nine muscles mid thigh and five muscles mid lower leg, a total of 14 muscles per patient, were analysed in all 32 subjects. In each scan the midpoint of the thigh and lower leg was identified and the ROI highlighted from the baseline (S0) scan was matched to the follow up (S1) scan. The 'goodness of fit' depended on the positioning of the patient at follow up and landmarks used as set out in the protocol. For some patients the original ROI shapes required adjustment for the altered shape of the patient leg.

The degree of muscle pathology, as at baseline, varied significantly from severe, in the biceps femoris long head muscle (median fat fraction 75.2%), to very mild involvement in the tibialis anterior muscle (median fat fraction 5.2%).

As can be seen in **table 6.3**, the median fat fraction increased from baseline (S0) to follow up (S1) and was significant in 9 out of the 14 muscles and in 2 there was a non-significant increase. The 3 muscles that did not increase were the peroneus longus muscle, the tibialis anterior muscle and biceps femoris short head muscle. Whilst the median fat fraction appeared to decrease in these muscles, none of them reached significance levels.

The biceps femoris short head muscle decreased by 0.6% however the minimum and maximum level of pathology increased from baseline (2.7% - 78.1%) to follow up (4.1% - 82.3%), likewise in the peroneus longus muscle.

In the tibialis anterior muscle, there was only at 0.3% decrease in the median fat fraction, which is within the limits of error of the technique (0.5%) (Lim et al. 2011).The minimum and maximum values at baseline and follow up were closely matched.

Table 6.3: Median values of the fat fraction at baseline (S0) and follow up (S1). (Non-parametric paired Wilcoxon signed rank test)

Muscle	S0 min	S0 max	S0 median	S1 min	S1 max	S1 median	S1-S0 difference (sig)
TA	1.4	24.6	5.5	1.3	23.5	5.2	0.627
MG§	1.1	90.3	21.7	1.3	90.3	22.6	0.009
LG§*	0.8	88.4	19.3	0.8	87.8	23.9	0.009
PL	2.8	55	15.1	3.2	61.8	14	0.896
SOL	1.5	84.9	9.1	2.1	86	10.9	0.246
SAR§*	0.85	88.9	24.2	3.4	87.5	25.3	0.010
GRAC§*	2.3	81.7	25.3	3.9	84.2	26.6	0.018
SM§	2.6	94.1	49	2.9	95.6	54.2	0.015
ST§	2.3	100	55.7	2.1	96.1	59.7	0.021
VL§*	0.6	82.1	15.6	1.2	82.1	20.9	0.025
VM	1.1	89.1	25.6	0.8	83.5	30.9	0.065
RF§*	0.4	81.3	10.9	0.8	82.1	12.3	0.028
BFSH	2.7	78.1	25.5	4.1	82.3	24.9	0.065
BFLH§	1.5	97.3	71.6	2.2	94.4	75.2	0.004
Av Fat PC§	1.9	69.1	28.5	2.4	67.1	31.3	0.003

§ - significant differences between S1 and S0

BFLH=Biceps Femoris long head, ST= Semitendinosus, SM= Semimembranosus, BFSH=Biceps Femoris short head, SAR= Sartorius, VM=Vastus Medialis, GRAC=Gracilis, VL=Vastus Lateralis, RF=Rectus Femoris, MG=Medial Gastrocnemius, LG=Lateral Gastrocnemius, PL=Peroneus Longus, SOL=Soleus, TA=Tibialis Anterior.

* - possible muscles for future longitudinal analysis; In lower leg; LG, In thigh; SAR, GRAC, VL and RF.

The nine muscles identified above were those that reached significant differences between S0 and S1.

The semitendinosus, gracilis and rectus femoris muscles which had increased significantly quantitatively did show a shift in qualitative grades from a 3 to a 4, 2a to 2b and 2a to 2b respectively, although these were not significant. Although the soleus and vastus medialis muscle also showed a shift in qualitative grading from a 2a to a 2b and 2b to 3 respectively, they did not show a significance change when analysed quantitatively. Six of the muscles that had increased significantly on quantitative analysis did not show any change in qualitative grades. These included the lateral and medial gastrocnemius muscles, vastus lateralis muscle, sartorius muscle, semimembranosus muscle and biceps femoris long head muscle.

In considering future longitudinal studies and even larger therapeutic trials, the data presented in **table 6.3** has identified potential muscle groups that might be suitable as outcome measures in further longitudinal studies in LGMD2I. The muscles that have been identified from **table 6.3** have been further defined based on the amount of fat fraction on the quantitative MRI scan and the range of spread of that fat fraction. In the lower leg the soleus, the Peroneus longus and tibialis anterior muscles did not change significantly, whilst both medial gastrocnemius and lateral gastrocnemius muscle did. Both were similarly affected however the medial gastrocnemius muscle would be preferential as the ROI mid calf was easier to delineate.

In the thigh there was more significant change seen in the sartorius, gracilis, semimembranosus, semitendinosus, vastus lateralis, rectus femoris and the biceps femoris long head muscles. The semimembranosus, semitendinosus and the biceps femoris long head muscles generally had a high fat fraction with median levels of 54.2%, 59.7% and 75.2% respectively and hence would be more difficult to monitor on a longitudinal basis.

The gracilis and sartorius muscles showed overall very similar levels of change over the 12 month period, however the gracilis muscle is the easier of the two muscles to

identify midthigh as the sartorius muscle often had ill defined fascial borders and baseline ROIs appeared to have a better fit on follow up scans with the gracilis muscle.

The rectus femoris and vastus lateralis muscles are also easy muscles to delineate in the thigh and could be useful in monitoring.

As in **chapter 6.1**, the quantitative scores were analysed to assess the number of participants with either increased or decreased fat fraction percentage values. Unlike the qualitative scoring there were no participants with figures identical to the baseline, however those within the limits of error of the technique, (0.5%) were regarded as unchanged. As demonstrated in **table 6.4**, most of the participants have had an increase in their fat fraction; however some have had a decrease. The average decrease in fat fraction was 4.88%, with some muscles such as semitendinosus, biceps femoris short head and peroneus longus having the highest fat fraction decrease and this may represent poor repositioning on the follow up scan as these muscles were some of the more difficult ones to realign, and hence could result in the fat fraction being analysed and recorded at a different level. This therefore highlights that the precision in realignment is vital for accurate analysis.

Amongst the participants that had a decrease in fat fraction there were 8 who appeared to be more commonly associated with a decrease in fat fraction and this may reflect the repositioning of those participants. Out of the 8 participants that had the majority of the recorded decreases in fat fraction, 2 were mildly affected and in these participants, 1/3 of the muscles remained unchanged and 1/3 decreased, 2 were moderately affected participants and again approximately 1/3 remained unchanged and 1/3 had a decrease in fat fraction, the other 4 patients were more severely affected. The most changes were seen in semimembranosus, with a fat fraction decrease of 8.6% in these patients and this muscle was the more difficult to delineate especially in those patients who were more severely affected. Therefore the majority of decreases in fat fraction or unchanged fat fractions were seen in a 8 of the participants, this therefore could reflect overall poor repositioning and particularly in those more severely affected, difficultly in ROI delineation and hence accurate measurements.

Table 6.4: Number of patients with a decreased or increased fat fraction at 12 months.

Muscle	Number % decreased	Number % increased	Unchanged
Biceps Femoris long head (BFLH)	4	23	4
Semitendinosus (ST)	6	19	7
Semimembranosus (SM)	6	20	5
Biceps Femoris short head (BFSH) *	8	15	6
Sartorius (SAR)	5	19	8
Vastus Medialis (VM)	6	16	10
Gracilis (GRAC)	5	21	6
Vastus Lateralis (VL)	6	17	9
Rectus Femoris (RF)	5	21	6
Medial Gastrocnemius (MG)	5	19	8
Lateral Gastrocnemius (LG)	7	18	7
Peroneus Longus (PL)	9	14	9
Soleus (SOL)	8	11	13
Tibialis Anterior (TA)	9	9	14

* BFSH was graded in 29 participants. 3 were scans were impossible to grade due to poor quality, severe infiltration or positioning.

6.3 - Gender differences

As reported in chapter 5 (section 5.2.1) striking gender differences were described. These differences of muscle pathology were seen in the sartorius and gracilis muscles, the medial lateral gastrocnemius muscles, the hamstring muscles and most significantly between the vastus lateralis and vastus medialis muscles ($p<0.005$). The gender differences (**table 6.5**) seen in the follow up scans were not as significant as seen at baseline. At baseline there were 38 patients analysed, however as the follow up data was

only on 32 patients, there is a less significant difference seen. This maybe due to the loss of key patients or possibly the need for greater numbers to illustrate this.

The female subjects continued to demonstrate diffuse involvement of both the gastrocnemii muscles (22% and 23.5%) with no significant difference. The medial gastrocnemius muscle in the male group continues to have a fat fraction of 38.7% which is higher compared to the lateral gastrocnemius muscle (24.2%) which has a similar fat fraction to the female subjects, however the result is not significant (p=0.08).

In the female subjects there was a trend towards semimembranosus being spared relative to semitendinosus at baseline (median 28.2% compared with 47.3%), however this value was not seen when only the 32 at follow up were analysed at baseline, indicating that possibly the loss of 4 female patients has influenced these values. The differences has now equalised and there is similar fat fraction in the hamstrings both in the male (60.3% and 59.2%) and the females (51.7% and 51%), with no significant differences.

In the male subjects the gracilis muscle continued to be better preserved (15.8%) compared to the sartorius muscle (24.2%) but does not reach significance (p = 0.06). In the female group the gracilis and sartorius muscle are equally affected and similar to the male fat fraction in the sartorius muscle (28.8% and 26.3% respectively).

Table 6.5: Median values of fat fraction for the study cohort (17 males and 15 females) at baseline (S0) and at follow up (S1). It illustrates significant differences between the anterior thigh muscles in the male group and preferential sparing of the gracilis muscle compared to the sartorius muscle. These features are not found in the female group.

Muscle	Male S0	Female S0	Male S1	Female S1
Semitendinosus (ST)	60.4	50.9	60.30	51.70
Semimembranosus (SM)	51.8*	43.8	59.2*	51.0
Sartorius (SAR)	24.0	24.2	24.2	26.3
Gracilis (GRAC)	15.5**	26.1	15.8**	28.8
Vastus Lateralis (VL)	14.3	18.2	20.5	24.6
Vastus Medialis (VM)	45.6§	23.3	50.0§	26.2
Medial Gastrocnemius (MG)	28.7	17.8	38.7	22.0
Lateral Gastrocnemius (LG)	19.6†	19.3	24.2†	23.5

* p = 0.03 compared to male ST	* p = NS compared to male ST
** p = 0.02 compared to male SAR	** p = 0.06 compared to male SAR
§ p = 0.001 compared to male VL	§ p = 0.02 compared to male VL
† p = 0.08 compared to male MG	† p = 0.08 compared to male MG

BFLH=Biceps Femoris long head, ST= Semitendinosus, SM= Semimembranosus, BFSH=Biceps Femoris short head, SAR= Sartorius, VM=Vastus Medialis, GRAC=Gracilis, VL=Vastus Lateralis, RF=Rectus Femoris, MG=Medial Gastrocnemius, LG=Lateral Gastrocnemius, PL=Peroneus Longus, SOL=Soleus, TA=Tibialis Anterior.

The anterior thigh muscles continued to demonstrate gender differences; in the female subjects the vastus lateralis and vastus medialis muscles are affected similarly (24.6% and 26.2%) whilst in the male subjects there continues to be a greater degree of fat fraction, 50.0%, in vastus medialis muscle compared to 20.5% in vastus lateralis muscle ($p=0.02$).

6.4 - Case study

Case A was a 33 year old male that was initially diagnosed as having a 'liver problem' following a biochemical profile which suggested liver inflammation. He had raised liver enzymes identified after routine bloods before donating blood. Following this he underwent lengthy gastroenterology investigations and cut out any alcohol he was drinking. His CK continued to be raised at 12,000units/l and eventually a muscle problem was suspected. He had no family history and no symptoms of note. In his early 20's he noticed some slowing of his running ability, however he continued to be very active running 9 miles out of a 13 mile run in 2009. On formal testing, he did have weakness in his hip flexors, hip adductors and abductors, but was otherwise strong. He ran the 10m walk and achieved normal values for the 6MWD and other timed tests. He has obvious proximal weakness when getting up from lying, however is not limited in his chair rise or TUG test.

He was assessed on four occasions for the standardised physical examination, the second examination, not recorded here, was within four weeks of the first examination for inter-rater reliability and test-retest validity. As it can be seen from **figure 6.1**, he showed no changes over the 12 months in his myometry strength recordings. He also showed no change in his 6 MWD and NSAA as shown in **figures 6.2** and **6.3** respectively.

Figure 6.1: Baseline, 6 months and 12 months myometry results in patient A. This shows no deterioration in results. (flex=flexion, add=adduction).

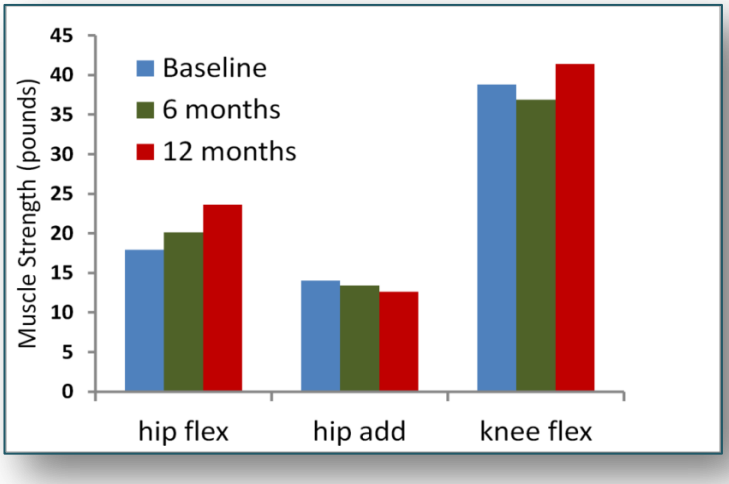


Fig 6.2: Six minute walk test (6MWD) at baseline, 6 and 12 months, showing stability over 12 months

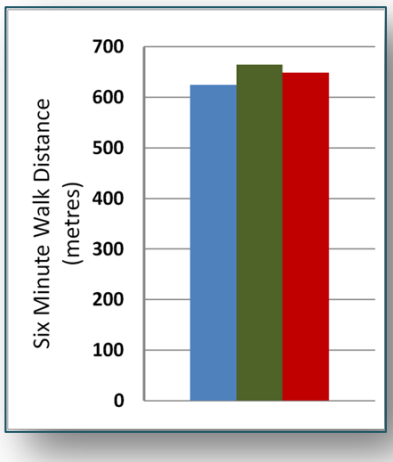
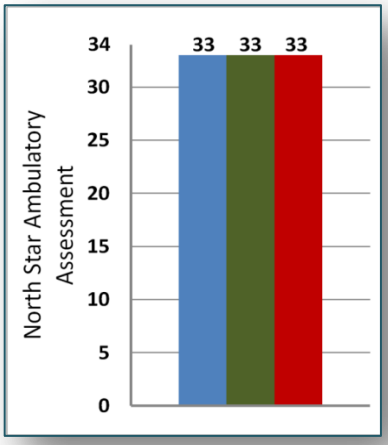


Fig 6.3: adapted NSAA at baseline, 6 and 12 months, which is stable scoring 33/34 for all assessments.



Whilst Case A's strength and functional testing remained stable, his quantitative MRI results showed significant changes (**figure 6.4**) particularly in his medial gastrocnemius muscle (28.7% fat fraction at baseline and 49.7% at follow up) and biceps femoris long head muscle (77.1% fat fraction at baseline and 86.5% at follow up). These changes can

be appreciated in the 3 point Dixon **figures 6.5** and **6.6.** There is also a significant increase in the fat fraction in the medial and lateral vastus muscles as well as peroneus longus muscle. The changes in the medial gastrocnemius muscle were evident on the T_1w image, but the other changes were not visible. Neither were they reflected in the qualitative scores, where the biceps femoris long head, vastus lateralis and Peroneus longus muscles remained the same (grade 4, 2b and 2b respectively). The medical gastrocnemius muscle scoring changed from a grade 2b to a 3, however this was because the change occurred across a boundary point of 30%, which was reflected in the score.

Figure 6.4: Baseline and 12 months quantitative MRI results, showing an increase in the fat fraction of MG (Medial Gastrocnemius), BFLH (Biceps Femoris long head), PL (Peroneus Longus), VM (Vastus Medialis) and VL (Vastus Lateralis) muscles. There was no change in the TA (Tibialis Anterior)

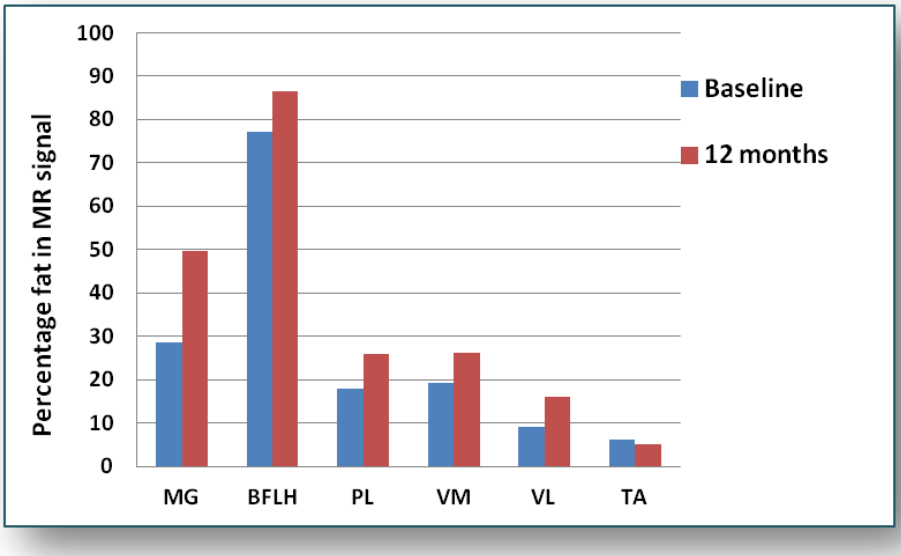
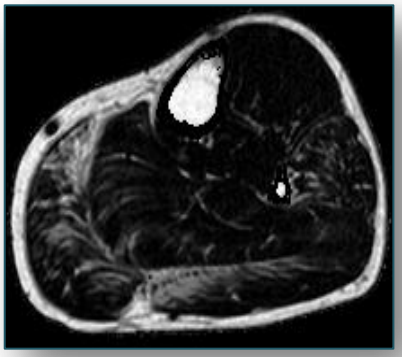
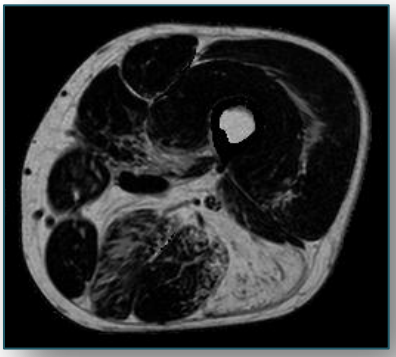


Figure 6.5 - Baseline MRI using 3 point Dixon mid lower leg (i) and mid thigh (ii).

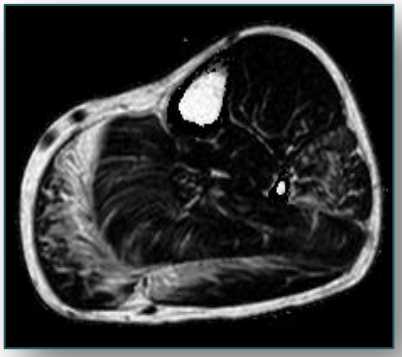


(i)

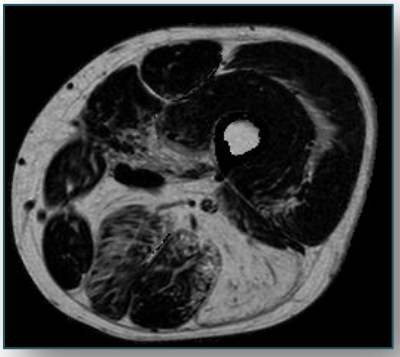


(ii)

Figure 6.6: Follow up MRI 3 point Dixon mid lower leg (i) and mid thigh (ii) illustrating in 6.6(i) the obvious fat increase in the medial gastrocnemius muscle



(i)



(ii)

This case illustrates that quantitative MRI imaging longitudinally by 3 point Dixon does pick up changes prior to clinical change. These changes were significant and seen in a patient who was one of the less affected participants and hence the ideal candidate if a therapeutic trial was imminent. Current clinical trials have functional measures, such as the 6MWD, and strength measurements as primary and secondary outcome measures (Chapter 4), but these would not have detected any change in case A over a 1 year period. MRI could become a useful adjunct in defining the outcome of a study.

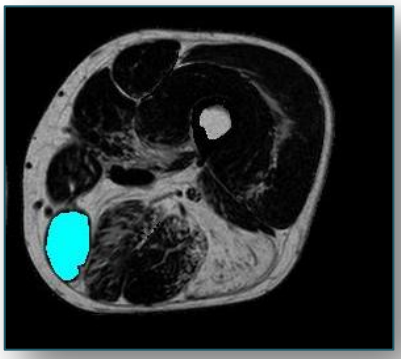
6.5 - Discussion

The longitudinal quantitative study demonstrated changes to a significant level in the majority of muscles. This was in contrast to the longitudinal qualitative study which did not demonstrate any significant change in any muscle group. The lack of significant change in the qualitative study is mainly due to the broadness of the grading system which does not enable small changes to be detected unless they occur across one of the boundary points of the scale, such as 30% or 60% muscle fat. Some of the grades are less well defined such as grade 1 and 2a, this therefore reduces the power of the qualitative scoring scheme for longitudinal studies or accurate monitoring of early disease progression, which is potentially the crucial time for effective treatment and prevention of progression.

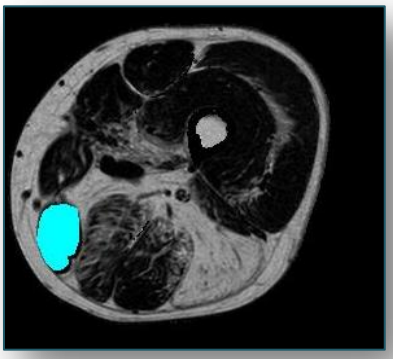
Even though LGMD2I is a slowly progressive condition, the results revealed that significant changes in fat infiltration did occur over the twelve month period. The changes occurred in the majority of the muscle groups but whilst some muscles were severely affected at baseline, others demonstrated a more modest level of infiltration and showed a variable spread.

Limitations; As highlighted earlier two participants from the Copenhagen cohort were excluded after being scanned, as the positioning of the patient at follow up was different to baseline and this made it difficult to accurately compare the two scans. Other repositioning issues were of a different order of magnitude making placement of the ROI drawn at baseline difficult to apply to the follow up scan. Using the baseline ROI ensured that the slice level that was being assessed on the follow up scan was at the same level as the baseline. ROI placements, in well positioned patients and with a good quality scan, fitted well with little need for adjustments in some cases (**figure 6.7**), some however were less well aligned and needed some adjustments to get a good fit (**figure 6.8**).

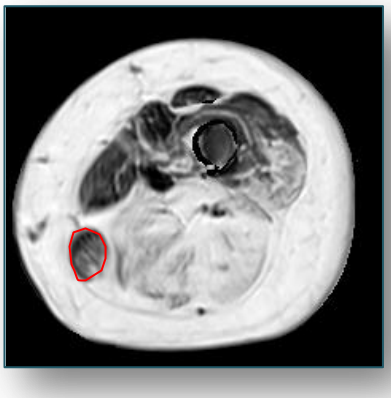
Figure 6.7: Baseline (i and iii) and follow up (ii and iv) MRI scans using the 3 point Dixon technique – example of a good ROI fitting due to precise leg repositioning.



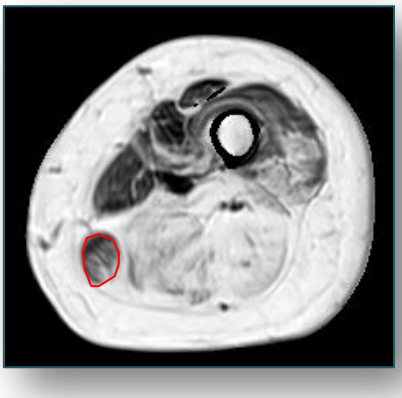
(i)



(ii)

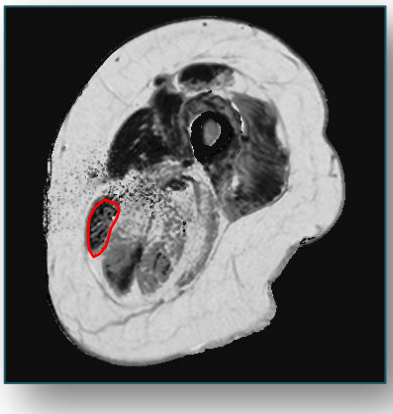


(iii)

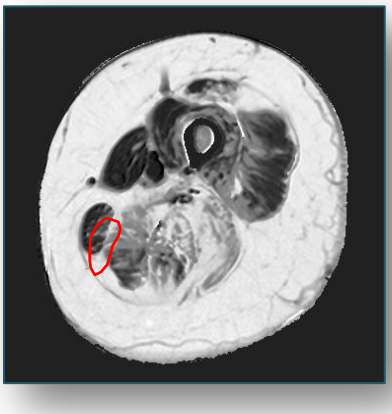


(iv)

Figure 6.8: Baseline (i) and follow up (ii) MRI scans using the 3 point Dixon technique – example of a poor ROI fitting due to imprecise leg repositioning.



(i)



(ii)

Registration is vitally important and recording measurements from baseline scans is paramount. Whilst the legs can be kept still and in position with straps, foam pads or vacuum bags, care needs to be taken not to induce any outward pressure on the legs as seen in some scans (**figure 6.8(i)).**

As seen in **table 6.3** significant changes were seen after only 12 months in the LGMD2I cohort and these were seen in a majority of muscles. In a large multicentre longitudinal study or therapeutic trial, measurements of all these muscles would not necessarily be feasible and on this basis we would aim to highlight three or four possible candidate muscles that would illustrate the changes. Reviewing the table this would therefore mean that the medial gastrocnemius muscle in the lower leg and the gracilis muscle, vastus lateralis muscle and rectus femoris muscle in the thigh would be potential candidate muscles for future analysis. These muscles have been identified due to their significant change over time, level of fat fraction, ease of identification and consistent ROI placements on a longitudinal basis

Chapter 7 - Skeletal MRS Results

Skeletal muscle MRS was performed in two of the centres taking part as it was not possible to perform MRS in the other centres. As a result 20 of the patients from NCL and Paris underwent MRS as part of the MRI examination. As discussed in chapter 3, this was conducted in different but comparable ways in NCL and Paris. MRS was primarily performed to assess whether any metabolic abnormalities could be detected in this cohort of patients and in particular whether any of the changes observed predated the changes seen on the qualitative and quantitative MRI scans or clinical changes. If this were the case this may indicate that MRS could be used as a preclinical/early biomarker.

In this chapter I will therefore report on the results that were found in the two cohorts, both as a whole and separately.

7.1 - The patient cohort

The NCL cohort was older at a mean age of 43.3 years and percentage fat in the MR signal was increased compared to the Paris cohort (mean age 38.3 years). The MRS method, as discussed in chapter 3, obtained spectra from the gastrocnemius and soleus muscles under rest, exercise and recovery conditions. The mean fat fraction for the soleus muscle was 20.8% in the NCL cohort versus 7.8% in the Paris cohort, the gastrocnemius muscle was 39% in the NCL cohort versus 16% in the Paris cohort and for their 'average fat percentage' was 32.8% in the NCL cohort versus 12.5% in the Paris cohort.

7.2 - Resting metabolite concentrations

PDE resting concentrations for the LGMD2I patients were on average 44% higher than those of the control group (**table 7.1**, $p = 0.002$). The concentration of inorganic phosphate (Pi) was also raised (by 36%, $p = 0.0002$) at rest, whilst the concentration of phosphocreatine (PCr) showed no significant change at rest. The ratio of the initial Pi/PCr reflected these contributions, rising by 35% ($p = 0.0002$).

7.3 - Oxidative function during exercise

There was no significant difference in the half-time for PCr recovery (**table 7.1**), either uncorrected or corrected for end-exercise pH, indicating that there is no impairment in maximal oxidative function in the LGMD2I patients.

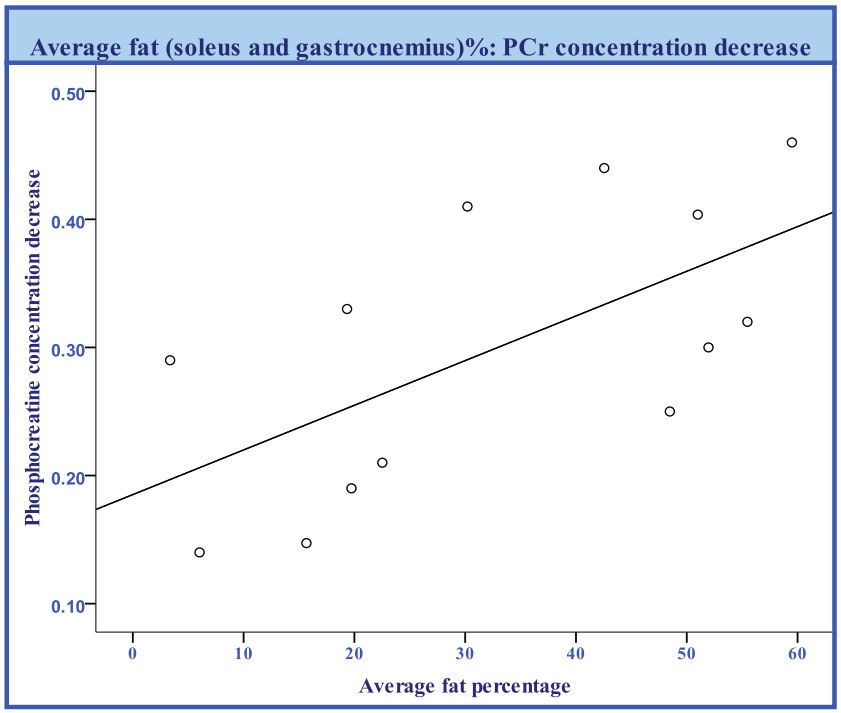
7.4 - pH handling

The baseline pH prior to exercise was found to be raised for the LGMD2I patients as a whole group (7.056 for patients with LGMD2I vs 7.039 for controls, $p = 0.05$) and continued to remain high at the cessation of exercise (7.077 for patients with LGMD2I vs 6.9763 for controls, $p = 0.025$) and at the minimum pH obtained during the recovery (6.990 for patients with LGMD2I vs 6.835 for controls, $p = 0.03$).

7.5 - Correlation with degree of fat fraction

Neither the percentage fat in the MR signal of the gastrocnemius, soleus or their average was found to correlate with the concentration of PDE, Pi or the ratio of Pi/PCr when applied to the group as a whole. When analysed separately, the NCL group did have some correlations with the fat fraction in their lower legs. The decrease in PCr positively correlated with the fat fraction in the gastrocnemius muscle ($r = .599$, $p<0.05$), and also with the average fat fraction of the soleus and gastrocnemius muscles together ($r = .615$, $p<0.05$) (**figure 7.1**). These correlations were not seen in the Paris cohort.

Figure 7.1: This illustrates the correlation seen between the average fat percentage in the soleus and gastrocnemius muscles and the drop in PCr concentration. (r = .615, p<0.05)



There were also negative associations between the inorganic phosphate and the pH (r =-.835, p<0.01) and the PDE was also negatively associated with the Pi/PCr (r = -.659, p<0.05). Neither of these associations were seen with the Paris cohort of patients. **Table 7.2** therefore shows the two groups split into the Paris cohort and the NCL cohort

7.6 - Discussion

The skeletal muscle MRS part of this study has examined both the phosphorus metabolite metabolism at rest and under exercise in just over half (n=20) of the total LGMD2I cohort.

Resting metabolite concentrations

The alterations in resting metabolite concentrations are consistent with measurements made in other muscular dystrophies. The 36% increase of inorganic phosphate concentration is smaller than that reported for DMD (68%, Younkin et al. 1987, 109% in Kemp et al. 1993, and 60%, Banerjee et al. 2010), and to some extent in BMD (16% Lodi et al. 1999, 47% Kemp et al. 1993). This is in contrast to a previous study on LGMD due to sarcoglycan deficiency, where a fall in inorganic phosphate concentration was reported (Lodi et al. 1997).

There was a 44% increased concentration of PDE seen in the patients with LGMD2I and again this is less marked compared to the changes found in DMD (2300% increase in Younkin (1987), and 162% increase in Banerjee (2010). The PDE measurements are not reported by Lodi and colleagues (1999) in BMD patients, however Kemp et al. (1993) does not show a significant difference compared to controls. The study on LGMD due to sarcoglycan deficiency did not measure the PDE (Lodi et al. 1997).

Although the Pi/PCr ratio was increased, the resting phosphocreatine (PCr) concentration was not significantly different to the control group. The raised Pi/PCr ratio was solely due to the raised Pi concentration. In the previous studies in patients with DMD and BMD (Kemp et al. 1993, Younkin et al. 1987, Griffiths et al. 1985, Newman et al. 1982) it has demonstrated that there is both an increase in the Pi concentration as well as a decrease in PCr concentration, suggesting a decrease in total creatine concentration. The changes seen in LGMD2I suggest that the total creatine concentration is less affected than in DMD and BMD.

The raised Pi concentration and PDE suggest a loss of membrane integrity and hence membrane breakdown (Younkin et al. 1987). This appears less marked in the LGMD2I cohort compared to the more severely affected patients with BMD and DMD where the increases are more significant.

Oxidative function during exercise

Comparable depletions of phosphocreatine were achieved in the LGMD2I patients and controls, suggesting mitochondrial oxidative function post-exercise is not impaired in LGMD2I. This has been described in previous studies, with normal mitochondrial function reported in BMD (Lodi et al. 1999), DMD (Kemp et al. 1993) and sacoglycanopathy-deficient limb girdle muscular dystrophy patients (Lodi et al. 1997).

pH handling

Resting pH is higher in LGMD2I patients compared to the controls ($p=0.05$). pH at the cessation of exercise was also higher for LGMD2I than controls and remained higher at minimum exercise.

Analysis method

The acquisition hardware and analysis were not identical at the two sites, which reflects the established practice at the two centres. In this analysis, 'peak picking' of the Pi resonance has been used to calculate pH. The Pi peak is usually symmetrical conventionally as shown in **figure 7.2**. In some LGMD2I patients with advanced disease, however, the Pi resonance is not symmetrical at baseline and there is a small component which has a chemical shift, appearing as a shoulder, suggestive of a more alkaline component.

This component has been disregarded in the present analysis: in principle it would be possible to account for this component by integrating the Pi resonance and finding the centre of mass of the resonance. This process has been attempted on the NCL and Paris data, but owing to its sensitivity to the baseline of the phosphorus spectra, no robust and unified approach could be agreed. Resolving this issue will form the basis of future studies in this cohort.

In conclusion therefore, this cohort of LGMD2I patients does have similar, albeit less marked, changes to those seen in DMD and BMD. There is an increased concentration of resting inorganic phosphate and raised PDE levels at rest, which suggest a loss of membrane integrity and hence membrane breakdown. There was no evidence of mitochondrial dysfunction. Whilst there was evidence of some correlations with the fat

fractions seen on the quantitative MRI, this was only present in the NCL cohort, who were more severely affected than the Paris cohort. Further longitudinal work will need to be conducted to assess whether skeletal muscle MRS is a valuable early biomarker of the disease process.

Table 7.1: ^{31}P MRS results for the two centres (NCL and Paris) as one group with controls (* control fat fraction data only available for NCL controls n=7).

	LGMD2I patients (n = 20)	**Controls (n = 14)**	**p-value (not corrected for multiple comparisons)**
Age	41 ± 12	38 ± 12	ns
PDE concentration prior to exercise (mM)	2.91 ± 0.90	1.96 ± 0.69	0.002
Pi concentration prior to exercise (mM)	4.24 ± 1.04	3.09 ± 0.45	0.0002
PCr concentration prior to exercise (mM)	32.7 ± 3.6	33.2 ± 1.9	ns
Ratio of Pi/PCr (-)	0.1298 ± 0.0294	0.0944 ± 0.013	0.0002
Baseline pH prior to exercise (-)	7.056 ± 0.033	7.039 ± 0.015	0.05
pH at cessation of exercise (-)	7.077 ± 0.060	6.976 ± 0.144	0.025
Minimum pH during exercise (-)	6.990 ± 0.045	6.835 ± 0.231	0.03
Depletion of PCr during exercise (%)	34 ± 11	42 ± 15	ns
Half-time for PCr recovery, uncorrected (s)	36.6 ± 11.4	38.7 ± 9.3	ns
Half-time for PCr recovery, corrected for end-exercise pH (s)	36.6 ± 11.4	35.2 ± 7.1	ns
Percentage fat in gastrocnemius (%)	31.8 ± 22.7	*2.5 ± 0.9**	-
Percentage fat in soleus (%)	16.4 ± 14.5	*2.9 ± 1.0**	-
Average fat in gastrocnemius and soleus (%)	26.1 ± 19.0	2.6 ± 0.9*	-

Table 7.2: ^{31}P MRS results for the two centres (NCL and Paris) considered separately, together with their controls.

	LGMD2I Paris (n = 7)	**LGMD2I Newcastle (n = 13)**	**Controls Paris (n = 7)**	**Controls Newcastle (n = 7)**
Age	38 ± 9	43 ± 14	35 ± 10	40 ± 15
PDE concentration prior to exercise (mM)	2.64 ± 0.86	3.04 ± 0.92	1.72 ± 0.83	2.20 ± 0.46
Pi concentration prior to exercise (mM)	4.87 ± 0.95	3.95 ± 0.98	3.26 ± 0.43	2.92 ± 0.42
PCr concentration prior to exercise (mM)	34.0 ± 0.7	32.1 ± 4.2	33.4 ± 2.2	33.0 ± 1.6
Ratio of Pi/PCr (-)	0.143 ± 0.029	0.123 ± 0.028	0.100 ± 0.013	0.089 ± 0.012
Baseline pH prior to exercise (-)	7.080 ± 0.035	7.046 ± 0.027	7.035 ± 0.016	7.044 ± 0.035
pH at cessation of exercise (-)	7.086 ± 0.111	7.073 ± 0.030	6.907 ± 0.179	7.045 ± 0.038
Minimum pH (-)	6.975± 0.043	6.996 ± 0.046	6.681 ± 0.242	6.989 ± 0.042
Depletion of PCr during exercise (%)	43 ± 7	30 ± 11	50 ± 16	34 ± 8
Half-time for PCr recovery, uncorrected (s)	38.1 ± 10.7	36.1 ± 12.0	41.8 ± 11.3	35.6 ± 6.0
Half-time for PCr recovery, corrected for end-exercise pH (s)	41.2 ± 11.9	34.9 ± 10.3	36.3 ± 9.0	34.0 ± 4.9
Percentage fat in gastrocnemius (%)	17.3 ± 16.1	38.7 ± 23.4	*n/a*	2.5 ± 0.9
Percentage fat in soleus (%)	7.8 ± 7.9	20.8 ± 15.7	*n/a*	2.9 ± 1.0
Average fat in gastrocnemius and soleus (%)	12.5 ± 10.3	32.8 ± 19.6	*n/a*	2.6 ± 0.9

Figure 7.2: Phosphorus spectrum from the gastrocnemius and soleus of a healthy control at 3.0T showing relative concentrations of inorganic phosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr) and adenosine triphosphate (ATP)

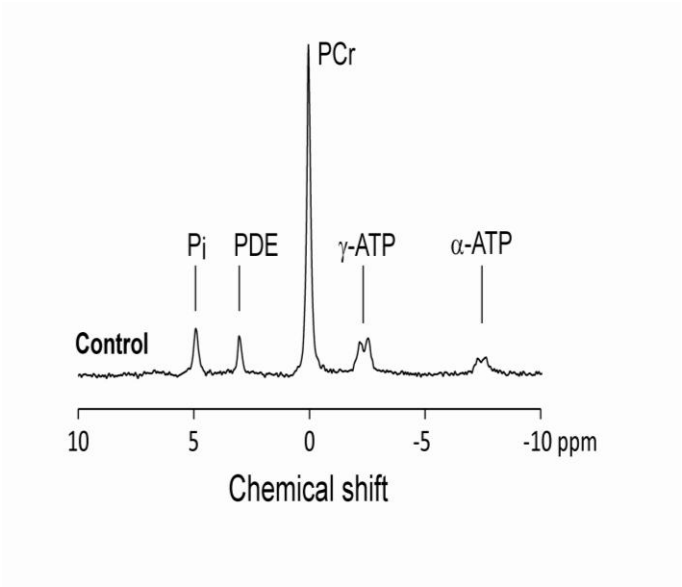
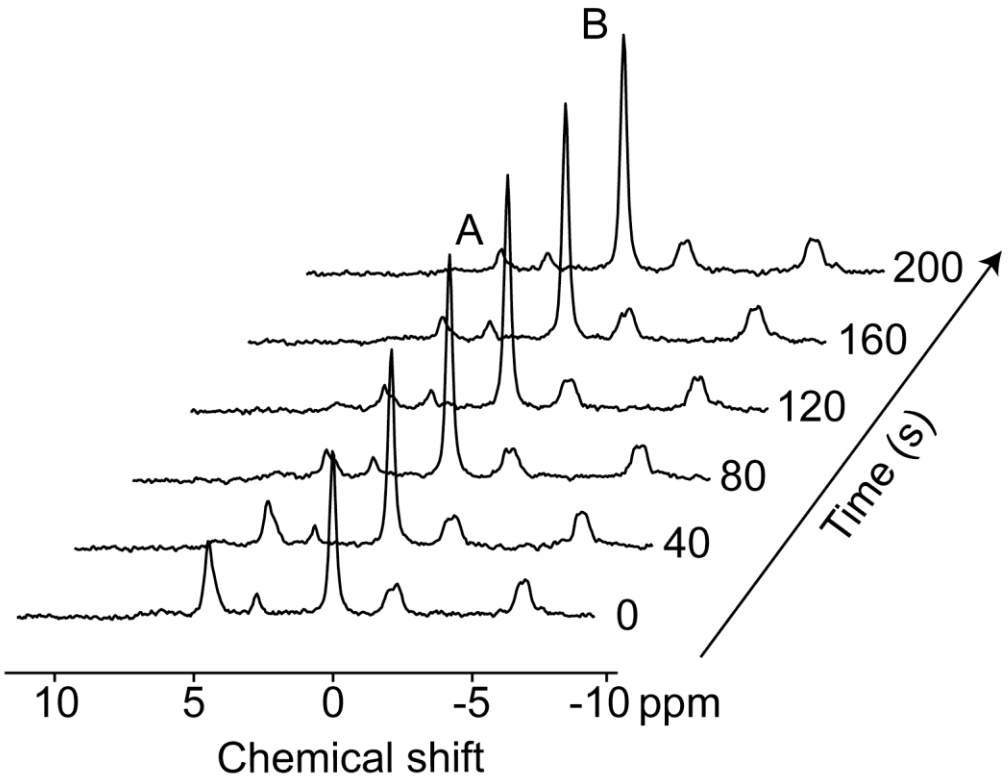


Figure 7.3 : A selection of phosphorus spectra acquired from the gastrocnemius and soleus of an LGMD2I patient at 3.0T during recovery from a plantar flexion exercise. A decreasing concentration of inorganic phosphate (A) and the replenishment of phosphocreatine (B) can be measured. The rate of recovery of the phosphocreatine acts as a surrogate measure for maximal mitochondrial function.



Chapter 8 - Cardiac muscle imaging and spectroscopy results

Cardiac MRI was performed on the NCL cohort following the 12 month follow up skeletal MRI. The main objective of this part of the study was to assess whether cardiac MRI is more sensitive in detecting cardiac change in these patients as reported by Gaul et al. (2006). If this is the case then early intervention with cardioprotective drugs could be commenced, preventing further damage.

8.1 - Cardiac Morphology and function by standard cine-MRI

Cine-MRI was successfully interpreted in all ten LGMD2I subjects. One male patient with LGMD2I was excluded from the study due to previous use of recreational drugs which is known to have an effect on cardiac function and can cause a cardiomyopathy (Ghuran and Nolan, 2000). The patient cohort therefore comprised of 7 male patients and 3 female patients, with a mean age of 47 years, ranging from 22 - 65 years (**table 8.2**). Five of the patients were known to have a cardiomyopathy and were on appropriate medication as listed in **table 8.1.** Five did not have a known cardiomyopathy and reportedly had normal echocardiograms.

Table 8.1: List of patient medication

Subject	Medications
1	perindopril 4mg, fluvostatin, torteridine, fenesterate
2	perindopril 8mg, nebivolol 2.5mg
3	perindopril 6mg, bisoprolol 5mg, calcium with Vitamin D
4	lisinopril 10mg, bisoprolol 5mg, losec 20mg, frusemide 40mg
5	lisinopril 10mg (for BP), arimidex (breast Ca)
6	perindopril 8mg, bisoprolol 5mg
7	nil
8	painkillers (solpadol)
9	nil
10	nil

10 gender, age-, weight- and BMI- matched subjects were recruited by advertisement as controls (**table 8.2**). All the control subjects were screened with a 12-lead ECG to exclude cardiac abnormalities and blood pressures were recorded to exclude those with hypertension (systolic blood pressure greater than 150 mmHg and/or diastolic blood pressure greater than 90 mmHg).

The most significant functional impairments found by cine-MRI in the LGMD2I patients were reduced ejection fractions (mean 47% vs 58% in controls, $p = 0.017$) and reduced stroke volume (61ml vs 81ml, $p = 0.038$, **table 8.2**).

The decrease in stroke volume arose mostly from the trends of increased end-systolic volume and reduced end-diastolic volumes in LGMD2I patients, though neither of these reached statistical significance in themselves. The resting heart-rates were no different between the patients and controls. Neither left ventricular mass nor left ventricular index were raised significantly for the group as a whole and there was no significant difference in cardiac wall thickness at either diastole or systole between the LGMD2I patients and the control group

In diastole, the peak early filling rate was reduced by 26% in LGMD2I (283 ml/s vs 380 ml/s). This was not significant under multiple correction and there was no significant difference in any other measure of diastolic function (late filling rate, E/A ratio or early filling percentage).

Table 8.2: Cardiac parameters for LGMD2I and controls subjects (mean ± s.d.)

p values result from Student t-tests with Bonferroni correction for multiple comparisons

Parameter	LGMD2I	Controls	p
n	10	10	
Age (years)	47 ± 14	48 ± 12	ns
Weight (kg)	80 ± 2	81 ± 1	ns
Body mass index (kg/m^2)	26.8 ± 4.0	27.5 ± 3.6	ns
Body surface area (m^2)	1.92 ± 0.24	1.88 ± 0.21	ns
Systolic blood pressure (mmHg)	137 ± 10	132 ± 15	ns
Diatolic blood pressure (mmHg)	86 ± 11	76 ± 9	ns
Ejection fraction (%)	47.3 ± 7.4	58.1 ± 4.4	0.017
Stroke volume (ml)	61.1 ± 1.1	80.9 ± 1.3	0.038
Resting heart rate (bpm)	62 ± 9	58 ± 9	ns
Cardiac output (l/min)	3.8 ± 0.7	4.6 ± 0.5	ns
End diastolic volume (ml)	134 ± 38	140 ± 27	ns
End systolic volume (ml)	72 ± 28	59 ± 16	ns
Left ventricular mass (g)	116 ± 31	109 ± 21	ns
Left ventricular index (g/m^2)	60 ± 15	58 ± 7	ns
Left ventricular mass/end diastolic volume (g/ml)	0.88 ± 0.08	0.78 ± 0.10	ns
Torsion to endocardial strain ratio (rad)	0.31 ± 0.05	0.51 ± 0.14	0.028
Peak torsion (deg)	3.9 ± 1.3	6.4 ± 1.5	0.038
Longitudinal shortening (%)	17.0 ± 3.0	18.4 ± 3.5	ns
Peak whole wall circumferential strain (%)	16.4 ± 3.2	18.3 ± 3.5	ns
Peak endocardial circumferential strain (%)	21.4 ± 4.2	22.2 ± 2.7	ns
Peak circumferential strain rate in systole (%/s)	0.082 ± 0.016	0.082 ± 0.010	ns
Diastolic wall thickness (mm)	7.08 ± 0.85	6.84 ± 1.01	ns
Systolic wall thickness (mm)	10.86 ± 1.17	10.69 ± 1.92	ns
Radial thickening (%)	54 ± 14	56 ± 13	ns
PCr/ATP ratio	1.50 ± 0.24	1.94 ± 0.12	0.0001
Peak ejection rate (ml/s)	271 ± 69	366 ± 109	ns
Early filling rate (ml/s)	283 ± 87	380 ± 114	ns
Late filling rate (ml/s)	192 ± 63	226 ± 69	ns
E/A ratio	1.67 ± 0.79	1.89 ± 0.92	ns
Early filling percentage	67 ± 10	71 ± 10	ns

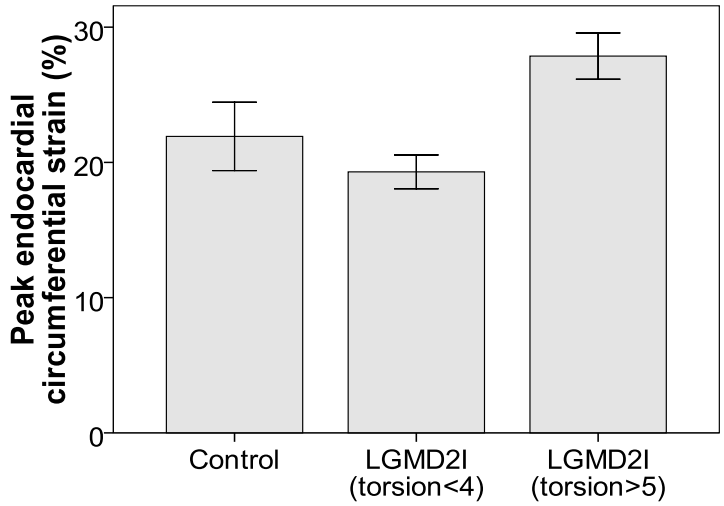
8.2 - Cardiac tagging measurements: Selective reduction in torsion related to changes in global left ventricular volumes and function.

Cardiac tagging was successfully analysed in eight subjects, two of the datasets had respiratory artefact. The peak cardiac torsion was significantly reduced in the LGMD2I patients compared to the controls (mean 3.9^{o} vs 6.4^{o}, $p = 0.038$, **table 8.2**), while there was no significant change in peak circumferential strain (whole wall) achieved (16.4% vs 18.3%, ns).

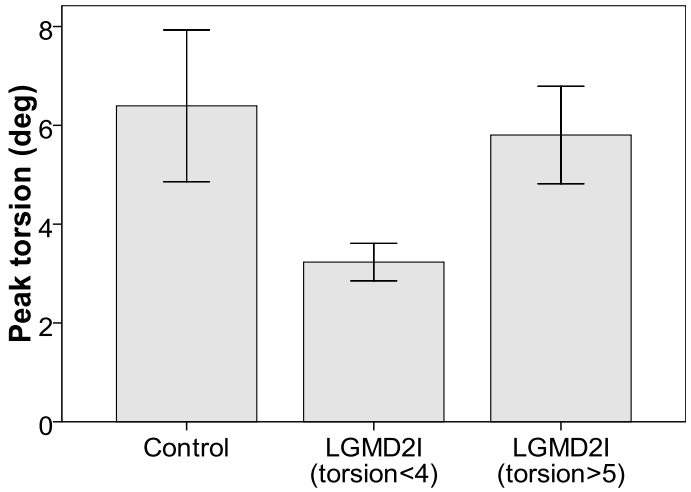
The torsion to endocardial strain ratio (TSR) was also found to be significantly reduced in LGMD2I patients (mean 0.31 ± 0.05 vs 0.51 ± 0.14, $p = 0.028$). This was due to reductions in torsion alone, as there were no changes in the endocardial circumferential strain (normal controls $22.2 \pm 2.7\%$, and LGMD2I $21.4 \pm 4.2\%$). Longitudinal shortening, which is generally reduced in association with age-related cardiac changes, was not significantly altered in LGMD2I patients compared to controls (17.0 ± 3.0 vs 18.4 ± 3.5 %). Therefore the LGMD2I patients appear to have a selective loss of torsion with other strains preserved. In the two LGMD2I patients with normal values of torsion, endocardial circumferential strain rates were actually higher than those of normal controls ($p = 0.008$, **figure 8.1**). This suggests a loss of subepicardial function in these patients, though the interactions between layers is preserved, or even increased.

Figure 8.1: (i) Peak endocardial circumferential strain compared between the controls, LGMD2I patients with markedly reduced peak torsion (< 4 degrees) and those with normal peak torsion (> 5 degrees). (ii) Plot showing the basis of division of the LGMD2I group, where 2 subjects have normal peak torsion compared to the rest.

(i)



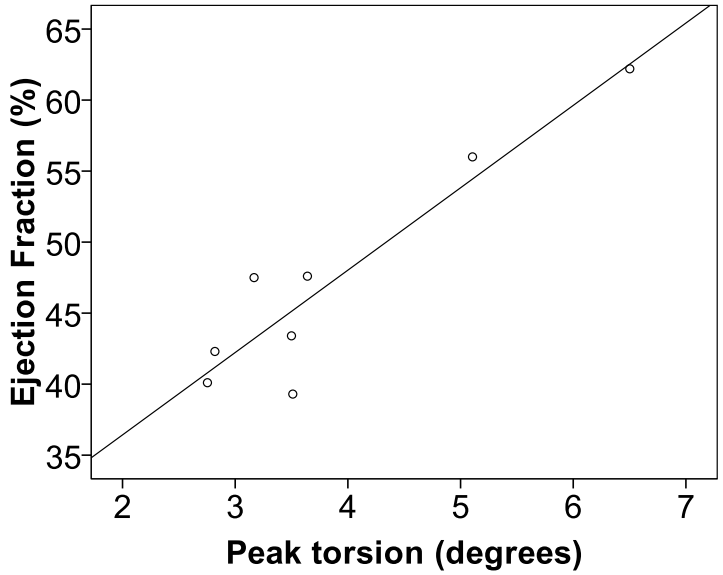
(ii)



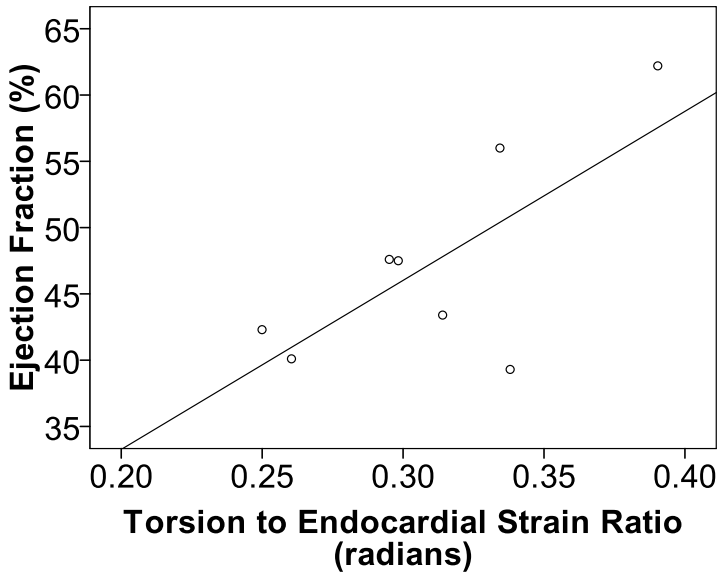
Impairments in peak cardiac torsion and the torsion to endocardial strain ratio correlated strongly with impairment in ejection fraction ($r = 0.93$, $p < 0.001$ and $r = 0.88$, $p < 0.004$ respectively, **figure 8.2**), though the change in peak torsion and TSR was much larger than that of LVEF (39% for torsion, 39% for TSR and 19% for LVEF). Peak torsion and circumferential strain were strongly correlated ($r = 0.94$, $p = 0.001$).

Figure 8.2: For the LGMD2I patients, correlation between LV ejection fraction and (i) peak torsion, (ii) torsion to endocardial strain ratio.

(i)



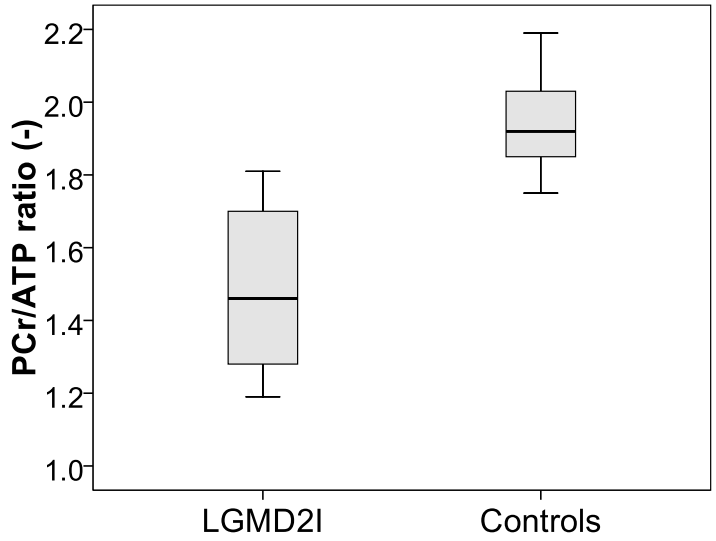
(ii)



8.3 - Cardiac spectroscopy

The cardiac spectrum for one LGMD2I patient had insufficient (signal to noise ratio) SNR for analysis. The ratio of PCr/ATP was significantly reduced in the LGMD2I patients compared with the control group as a whole (mean 1.50 vs 1.94, $p < 0.0005$, and **figure 8.3**). Cardiac energetics were not correlated with peak cardiac torsion, though there was a borderline correlation between reduced PCr/ATP ratio and reduced cardiac output ($r = 0.66$, $p = 0.05$)

Figure 8.3: PCr/ATP ratio for the LGMD2I patients and controls

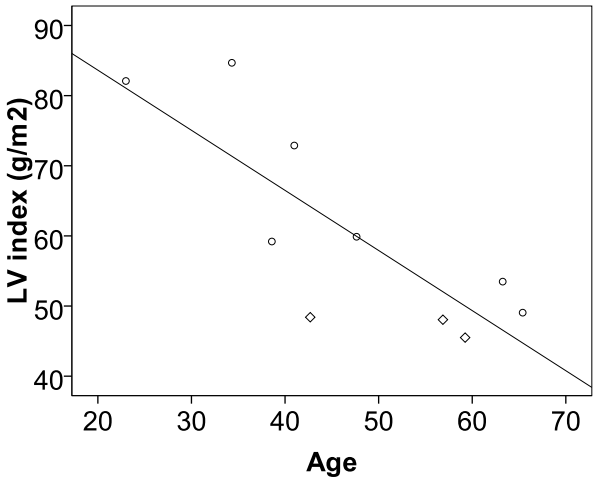


8.4 - Correlation of cardiac parameters with age

Within the LGMD2I group, reduced stroke volume correlated strongly with increasing age (r = -0.78, p = 0.007) and a reduction in LV mass and LV index correlated with increasing age (r= -0.77, p = 0.009, **figure 8.4i),** which was not found in the control group (**figure 8.4ii**). However, there was no direct correlation between ejection fraction or peak cardiac torsion with age. By dividing the LGMD2I patients into two groups about their median age, 45 years, and considering the LV index and PCr/ATP ratio (**table 8.3**), there was a trend of worsening cardiac energetics with increasing age, which may suggest an initial rise in LV index in younger patients before a decrease with ageing (**table 8.2**).

Figure 8.4: Correlation between LV index and age for (i) LGMD2I patients and (ii) control subjects.

(i)



(ii)

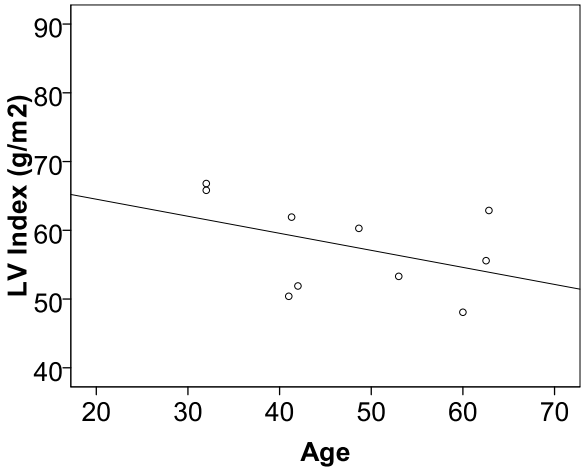


Table 8.3: The two LGMD2I groups; younger (<45 years) and older (> 45 years).

	Control	Younger LGMD2I	Older LGMD2I
PCr/ATP ratio (-)	1.94 ± 0.12	1.65 ± 0.19	1.37 ± 0.21 *
Left ventricular index (g/m^2)	58 ± 7	69 ± 15	51 ± 6 ‡
Stroke volume (ml)	81 ± 13	67 ± 6	55 ± 12
Cardiac output (l/min)	4.6 ± 0.5	4.1 ± 0.4	3.4 ± 0.8
Peak torsion (^{o})	6.4 ± 1.5	4.0 ± 1.7	3.8 ± 1.0
Ejection fraction (%)	58 ± 4	46 ± 9	48 ± 6
‡ p = 0.07 vs young LGMD2I by ANOVA, * p = 0.02 vs young LGMD2I by ANOVA. PCr = phosphocreatine, ATP = adenosine triphosphate.			

8.5 - Ultrasound findings

Routine clinical cardiac ultrasonography, performed for screening purposes, was available for 7 of the 11 subjects recruited, including the one patient subsequently excluded from the study. The main abnormalities noted were dilation of the left and right atria (4/7) and low ejection fraction (5/7): these features were also identified on cine MRI. The right ventricle was reported to have normal size and function for all patients. The four valves were reported to have normal structure and function in 6 reports with trace/mild mitral regurgitation in 5 subjects. The diastolic function as measured by E/A and E/Ea ratios correlated strongly with MRI measures but was only available for 5 of the LGMD2I patients.

8.6 - Discussion

This is the first time cardiac energetics and myocardial motion by MR tagging in patients with LGMD2I has been examined. It has demonstrated impaired cardiac energetics, and significantly reduced torsion and torsion to endocardial strain ratio in the absence of a significant reduction in peak circumferential strain or longitudinal shortening. Cine imaging revealed that left ventricular ejection fractions and stroke volumes were significantly reduced, however there was no significant increase in myocardial mass, whether or not it was normalized for body surface area.

The combination of reduced torsion and preserved circumferential strains found in LGMD2I is unique compared to changes found in other cardiomyopathies: in idiopathic cardiomyopathy there is a significant reduction in peak circumferential strain without significant change in peak torsion (MacGowan et al. 1997). A similar finding was reported for one study of DMD patient (Ashford et al. 2005), though other DMD studies have not reported torsion results, but only reduced circumferential strains (Hor et al. 2009, Hagenbuch et al. 2010).

The study of the development of cardiac disease in the *mdx* mouse model of DMD (Li et al. 2009) showed a biphasic change in cardiac torsion and strain, where *mdx* mice at 2 months showed increased peak torsion and circumferential strain, at 7 months demonstrated comparable torsion and strain to control mice and at 10 months torsion and strain were decreased compared to controls. This was accompanied by reduction in ejection fraction and stroke volume, and with continued increase in cardiac mass with age. There was no evidence in this study for any of the cohort having increased torsion compared to the controls, though the endocardial circumferential strain was significantly raised in two of the LGMD2I patients with normal peak torsion. In a δ-sarcoglycan null mouse model, circumferential strains were mostly impaired at 8 months with reduction in ejection fraction, but torsion was not assessed (Wansapura et al. 2011).

The timely release of torsion and strain during diastole is crucial for good early diastolic filling, and the low peak torsions achieved by the LGMD2I patients may underlie the reduced early filling rate observed (26% lower than control subjects, in comparison with

a 18% reduction for the mdx mice vs wild-type) though the peak ejection rate was also 26% lower than controls.

In previous work (Hollingsworth et al. 2010) on healthy young (mean age 31 ± 6y), middle-aged (mean age 50 ± 9) and older groups (mean age 62 ± 2y), it was found that the TSR remained constant between the young and middle-aged groups (mean 0.45 and 0.46 respectively), while it was raised by 41% in the oldest group (mean 0.62). The increase in torsion and TSR in older control adults indicated that there had been an alteration in the transmural distribution of strain, and, in particular, that subendocardial fibres were not making a proportionate contribution to systolic ejection compared to the subepicardial fibres, with a consequent increase in torsion. It has been observed that the endocardium is particularly sensitive to insult, as has been shown in histopathology of ischemic tissue (Ishijima et al. 1990), by studies of transmural wall motion in stunned myocardium that has recovered from an ischemic insult (Bolli et al. 1989, Mazhari et al. 2001) and in patients with aortic valve stenosis (Van der Toorn et al. 2002). The significantly reduced torsion and torsion to endocardial strain ratio in LGMD2I patients are in contrast to the above studies and indicate that the pathogenesis and localization of disease is rather different to the ageing phenotype. This indicates that, rather than a preferential dysfunction in the endocardium as per ageing, myocardial impairment in LGMD2I is concentrated at the epicardium and that there is abnormal transmission of mechanical forces from endo to epicardium.

Impairments in cardiac energetics have been measured previously in DMD and BMD patients (Crilley et al. 2000). In that study the PCr/ATP ratio was 36% lower for both patients and carriers compared to matched controls, in contrast to 23% lower obtained in this study. Crilley et al (2000) demonstrated that there was no correlation between energetics, ejection fraction, or left ventricular mass. The origins of changes in cardiac energetics were investigated by a study in 8 months old *mdx* mice (Zhang et al. 2008). The PCr/ATP ratio was reduced by 29%: this was concurrent with a 22% fall in total creatine and a 38% fall in PCr. This was not accompanied by any change in left ventricular dimensions or systolic function. In this study cardiac total creatine was not analysed, due to the techniques used. The wall stresses arising from unequal fibre shortening between endo and epicardial walls in the LGMD2I patients may alter oxygen

consumption within the tissue, leading to changes in cardiac energetics as found elsewhere (Strauer et al. 1977, Arts et al. 1982).

8.7 - Conclusion

The limitations of this study include the small number of patients studied (n=10), and, the fact that this data was acquired at the same time as a comprehensive skeletal muscle MRI and a full physical examination. It was therefore not possible to collect delayed late gadolinium enhancement (LGE) images which look for the presence of myocardial fibrosis. There are few studies demonstrating this in LGMD2I (Yilmaz et al. 2009), but it is also clear from cardiac tagging studies of DMD that abnormalities in cardiac tagging parameters can be detected before myocardial fibrosis is visible on LGE MRI (Ashford et al. 2005). Due to the limited quantitative information reported from routine ultrasound imaging, it was not possible to objectively compare the two techniques.

This data gives an insight into the pathogenesis of cardiac dysfunction, with early reduction in myocardial energetics which worsens with age, and early reductions in cardiac torsion and ejection fraction. Despite the small numbers, there is the suggestion that cardiac mass increases early in the disease course before decreasing. These results therefore demonstrate, for the first time, the strength of cardiac spectroscopy and cardiac tagging to distinguish changes in cardiac function in LGMD2I patients. Further longitudinal studies in large multi-centre cohorts will be required to assess the cardiac progression in LGMD2I.

Chapter 9 – The International FKRP patient registry

In this chapter I will discuss the importance of patient registries for rare diseases, such as LGMD2I and the setting up of the International FKRP patient registry as part of my project. I will discuss how the International FKRP patient registry works and how such a registry may contribute to "trial readiness" for this group of patients.

9.1 Preparing the patients for future trials/registries

Those diseases that are termed 'rare', whilst clinically heterogonous and individually rare make up a substantial proportion of the population as a whole, 6-8% at some stage in life (Schieppati et al. 2008). Within the neuromuscular world, inherited neuromuscular diseases are thought to affect between 152,000 and 228,000 people, with prevalence between 75-2,800 people with LGMD2I, based on an EU population of 501million. There is no curative treatment, however with clinical trials now ongoing in Duchenne muscular dystrophy, Spinal Muscular Atrophy and Pompe disease, collaboration is essential.

Cooperation with partners both in Europe and International is key to projects working in such rare diseases. Such collaborations enable consensus documents to be created with addressing protocols for molecular diagnoses, patient assessments and outcome measures and most important, standards of care that can be applied to all patients around the world (Butcher et al. 2007).

The level of care provided around the world varies greatly and whilst some of this is due to financial difficulties, some is purely due to knowledge gaps in these extremely rare conditions. Patient registries are a useful way of filling in these knowledge gaps and translating these to the health care providers. Patient registries and databases constitute a vital role in establishing clinical research in these rare diseases. They provide longitudinal natural history data and benchmarks by which to design clinical trials. They provide demographic data when assessing the feasibility of trials and with orphan drugs serve as safety monitoring in place of large phase 3 studies. They serve to disseminate knowledge both to patient and clinician and improve the quality and

outcomes of treatments in these rare conditions by standardising practice and reducing practice variation.

The International FKRP registry is a global patient registry that has been set up under the auspice of TREAT NMD. The TREAT NMD (Translational Research in Europe-Assessment and Treatment of Neuromuscular Diseases) initiative is a pan-European organisation made up of 250 researchers from 11 countries and was established in 2007 with funding from the European Union's sixth framework programme (FP6). TREAT NMD now has 22 partner organisations including academic institutions, industry and patient groups (Rubinstein et al. 2010).

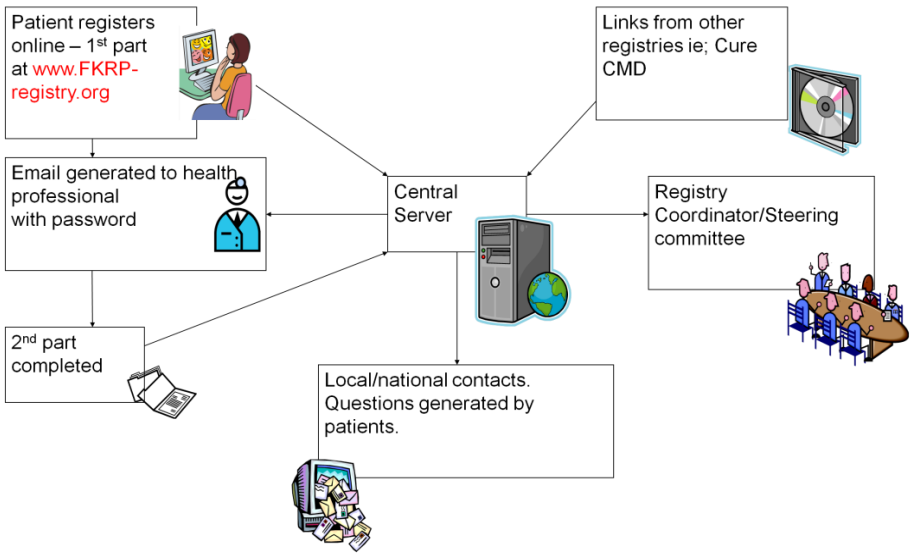
9.2 The FKRP registry

Previous neuromuscular registries have been run on a national basis feeding into a larger International database, the FKRP registry however has been designed to be purely Global, with the server being in Munich (**figure 9.1**). The registry is also unique in that it combines both a patient and a professional part with the patient initiating the registration. Many registries have difficulties with curation and time taken either entering medical data that have been sent in by patients or by editing data entered by patients themselves. I designed the registry to combine both the patient and professional involvement as this will curtail the amount of time required curating the data as professionals with an interest in neuromuscular conditions will be entering the data. The patient, on the other hand, will act as a catalyst and continue to provide prompts to the clinician if the data is not entered.

Within the patient part of the FKRP registry we have included optional questionnaires on quality of life and pain. The quality of life is a validated neuromuscular questionnaire developed by Michael Rose and his group and copyright for use of this was obtained. The short McGill pain questionnaire is also a well validated questionnaire and in this setting would only take a few minutes to complete. The mandatory questions refer to the patients' demographics, diagnosis and symptoms. These symptoms refer to their presenting ones as well as their current symptoms and also their current motor ability, past best motor ability and respiratory and cardiac status.

After consent for their respective doctor to enter their medical data onto the registry an email is generated and sent to the professional chosen by the patient from a drop down menu of doctors who have previously consented to this. The professional, if already holding an account, logs on with their password and completes their patient's details on the doctor's form. The doctor is able to view all his patients and their data but no other patients looked after by other doctors. Whilst the doctors are able to view their patient's entries on the patient form, they are discouraged from altering the data.

Figure 9.1: This figure illustrates how the FKRP registry works; patient initiation and completion of part one of the registry, health professional notification and completion of part two of the registry. All these are directed through the central server in Munich, with links to other relevant registries such as the CMDIR (congenital muscular dystrophy international registry). There is also an oversight committee and steering committee for the registry to deal with any enquiries and changes to the registry.



If the doctor has not already created an account and is not on the drop down menu, they will be contacted by the registry and requested to do so in order to enter their patient's medical data. The doctor's form includes item such as their latest respiratory function tests, including FVC both sitting and lying, cardiac ECHO results, molecular result, and motor function including timed tests if possible. The registry is also automated so that if a doctor fails to fill in the relevant data sets, an email is sent to prompt them and

likewise after one year a reminder email will be sent to the patient and professional to re-enter their data for the longitudinal natural history study.

Rare disease registries are essential for industry as well. More recently there has been interest generated around developing drug treatments for these rare conditions and registries, especially global, are of vital importance for assessing the feasibility of a clinical trial, to facilitate the planning of the trials and most importantly to assist in the enrolment of suitable patients. Orphan drugs can also be monitored in this fashion by using the register to recognise any long-term complications and side effects.

In 1983, the Orphan Drug Act was established, this provided incentives for the pharmaceutical companies to develop orphan drugs for rare diseases, due to its tax incentives and 7 year exclusive right to treat a rare or orphan disease. The pharmaceutical companies are also aware that these drugs whilst they are labour intensive and expensive to develop are almost indefinitely used life-long in these patients and therefore proving profitable long-term (FDA, Haffner et al. 2006). As well as patient registries, which can be used for feasibility studies pre-clinical trials, Bio-banks and tissue repositories linked to patient registries means that more accelerated research into potential drug therapies can be achieved.

9.3 Strengths and Benefits

It has become increasing evident that patient registries are an important resource for patients, professionals and industry. They are an efficient, powerful tool for evaluating patient change over time, response to treatment and variations in the disease course as well as demographics and disease pockets (Watson et al. 2008). Less than a fifth of rare diseases have registries, and most of these are operated by patient organisations or researchers (Wrobel et al. 2009).

In rare diseases, such as LGMD2I, collaboration and coordination between major centres means that studies and trials performed are large enough for statistical power to be achieved. These registries can also be interrogated when a researcher is contemplating a study in order to target where the main study /trial sites would be

suitable. These would be centred on clusters of patients with the disease, enabling easier enrolment.

Registry data and the software employed, means that alteration or addition of data sets is achievable, especially if a new treatment becomes available and through the registry we would like to monitor the response.

As with any registry, patient and clinician awareness is vital as is retention once registered, however with many rare conditions patients are usually motivated to influence the course of their disease. There is also an added incentive to help future generations as in the majority, these conditions are genetic. With online registries this retention is easier to manage, as emails can prompt re-entry of the data, when following up longitudinally and can easily be built into the programme.

In the international FKRP patient registry the patient initiates the enrolment process and consents online. As it is the patient that initiates the registration and fills in a small number of key items that would need updating to monitor disease progress, this would hopefully keep the patient motivated. Potentially this means that on a yearly basis following an automated email, they would update their data. Other enticements include the potential to be enrolled in a clinical trial should one become available and in time the ability to print off personalised graphs that highlight the change with time that the patient may want to show their clinician. The patient would also act as a prompt to the professional to enter the data as they will be keen for the dataset to be as complete as possible to be meaningful. With the clinician filling in the second part this means that the medical data should be accurate, negating the intensive curation step required in patient only registries.

Incentives for the clinician may include the ability to produce a report on their patient. Once the clinician has entered the data, the registry can be instructed to produce a clinic letter/formal report for their patient. This could either be inserted into the local clinician's standard letter or act as a standalone report. This would therefore save the clinician having to produce a separate one or dictate a clinic letter after their patient's appointment.

9.4 Limitations

Although the technology for creating massive databases is feasible, the agreement between professionals on the standardised dataset required for rare diseases can be more problematic. The registry ideally needs to be standardised in a way that is accessible to all users with and without translations and leaves no room for error in the recording of the data. Registries are only as good as the data recorded and if this is poor quality data then this will prove useless in the future. In a recent rare diseases workshop in the US, this was the concern that if a mega database made up of 7000 rare diseases with 9 million registrants, based on 30% enrolling, was created then it would take "a seismic shift" to get individuals to commit to the effort and agree on the standardised terminology (Rubinstein et al. 2010).

In the FKRP registry there have certainly been delays in launching due to technical difficulties and standardising the questions after feedback from various specialists, and whilst it has been launched in all the English speaking countries and Germany the other European languages remain to be launched, although the registry questions are translated.

9.5 The Future and discussion

Patient registries for rare diseases are the way forward if research is to progress and be meaningful. It has been said that patient registries can be identified as "an organised system that uses observational study methods to collect uniform data (clinical and other) to evaluate specified outcomes for a population defined by a particular disease, condition or exposure, and that serves one or more predetermined scientific, clinical, or policy purposes" (Gliklich et al. 2010).

Ideally these registries would be linked centrally and be identical in format, with a common subset of questions for all patients, and more disease specific questions depending on disease/gene registry. With formatting being the same, this would make the ease of addition and deletion of questions easier as well as curation.

Most importantly if these registries could be linked to the government health systems, such as the NHS in the UK, this would mean that patient data could be transferred and

stored on systems that are compatible with each other, which result in a decrease in the manpower time required as well as eliminate the element of human error. This would also mean that data entry would be possible whilst the patient is in the clinical setting and if the two systems were compatible and able to 'talk to each other' then this would negate double entry of data, both on the registry and in the doctor's notes/hospital system.

Worldwide, this would require harmonisation to enable communication between the different health care systems and exchange data in a sensitive and secure manner. This then would enable international collaboration which is key to understanding the natural history of these complex and rare diseases, developing gold standards of care, defining patients and trials with clinically relevant outcome measures and ultimately discovering a cure for these rare diseases (Bushby et al. 2009b).

Chapter 10 – Conclusion

This study represents the largest cohort to date of patients with LGMD2I studied longitudinally with functional, physical and imaging assessments. These patients represent a sub-group of the wider population of patients with LGMD2I. The results from this study could prove useful when considering future developments in the treatment of patients with LGMD2I and the construction of well designed trials and outcome measures. Definition of powerful and specific trial end-points requires a detailed knowledge of the natural history of the disease and ideally the muscles that are being imaged and analysed should have a wide dynamic range of pathology, be easy to delineate and sensitive enough to pick up change over the time course of a therapeutic trial.

The group as a whole had similar characteristics as described in the literature regarding the age of presentation with almost half recognising symptoms in childhood, the presenting symptoms with difficulty climbing stairs, running and myalgia being the commonest. The percentage demonstrating respiratory and cardiac involvement were also similar to the figures quoted in the literature (Poppe et al. 2004, Sveen et al. 2006, Walter et al. 2003). 42% of our cohort had cardiac involvement with approximately three quarters of them male. A third had respiratory involvement with a FVC </=75%, with almost half demonstrating a drop in their FVC on lying, indicating diaphragmatic involvement.

The standardised physical testing and functional scales did not show any significant difference longitudinally, however they did correlate strongly with muscle pathology seen on the MRI. They also provided insight into the particular difficulties these patients have as well as identified compensatory mechanisms that they all appear to adopt independently, such as supporting the weakened pelvic girdle with their hands in their trouser pockets or using their relatively strong neck muscles to walk themselves up a wall to achieve an upright position.

Whilst it is useful to document strength and functional abilities, they only give a snap shot of that particular patient's daily level of functioning. Many patients will perform well in that hour of assessment as they have rested pre assessment and will be fatigued

for days afterwards. The physical and functional tests are subjective and a number of factors contribute to the success or failure at assessment. These include fatigue, pain, illness, contractures and respiratory impairments and can all contribute to a patient's level of activity (Johnson et al 1992, Tiffreau et al 2006, McDonald et al. 1995, Feasson et al. 2006). Personal factors such as, emotion, motivation, cognition as well as environmental factors also play a role in a patient's level of functioning. In our patients this was most evident on the first round of testing when the floor surface appeared to be shiny and hence potentially slippery, they were therefore wary about walking too fast in the 10 metre test.

Clinical trials require robust rating scales that measure the health constructs that they claim to (i.e. the scales are valid) and they require that the health constructs are clinically meaningful and can be interpreted. (Hobart et al. 2007). Rasch methods have the advantage over classical rating scales. This Rasch analysis was applied to the NCL cohort who completed a total of 48 adapted NSAA over the 12 months. Rasch can measure stability (Andrich 1988), testing convergent and discriminant construct validity, which is deemed the strongest statistical evidence of scale validity (Campbell and Fiske, 1959).

The cross sectional quantitative analysis of the fat fraction using the 3 point Dixon technique has shown that quantitative fat imaging provides an objective measurement of the fat fraction that is more sensitive than the previous qualitative technique. We have found a similar pattern of muscle involvement as previously described in the literature but have also demonstrated gender differences not previously reported. These quantitative techniques could be used on an individual patient basis to monitor both disease progression and in the future, improvement in the advent of a therapeutic agent.

The longitudinal quantitative analysis of the fat fraction changed significantly over the 12 month period with significant increases in fat fraction seen in 9 out of the 14 muscles analysed. The qualitative grading did not change significantly longitudinally and this possibly represents the broadness of the grades. As a result of this analysis I was able to identify possible muscles that would be candidates for longitudinal studies in this cohort. These included the medial gastrocnemius muscle in the calf, the gracilis muscle, the rectus femoris muscle and the vastus lateralis muscle in the thigh. These muscles

were identified as they showed a significantly increase in pathology over time, were easy to delineate on the 3 point Dixon image and generally had good longitudinal ROI placement and did not have extensive pathology within the muscle.

The skeletal MRS results demonstrated metabolic abnormalities at rest, while demonstrating no impairment in maximal mitochondrial function. This cohort of LGMD2I patients did have similar, albeit more modest, changes to those seen in DMD and BMD. There was also an increased concentration of resting inorganic phosphate and raised PDE levels at rest, which suggest a loss of membrane integrity and hence membrane breakdown, similar to those seen in BMD and DMD.

The cardiac MR results from the small cohort of LGMD2I patients gives an insight into the pathogenesis of cardiac dysfunction, with early reduction in myocardial energetics which worsens with age, and early reductions in cardiac torsion and ejection fraction. Despite the small numbers, there is the suggestion that the cardiac mass increases in the early disease course before decreasing. These results demonstrate, for the first time, the power of cardiac spectroscopy and cardiac tagging to distinguish changes in cardiac performance in LGMD2I patients.

In coordinating the setup of the international FKRP patient registry and the development of the mandatory and highly desirable questions, I was aware that longitudinal capture of data and preparation for clinical trials was important. Since the launch earlier this year the registry has had 87 patients register from 14 countries. It is now increasingly evident that patient registries are an important resource for patients, professionals and industry. They are an efficient, powerful tool for evaluating patient change over time, response to treatment and variations in the disease course as well as demographics and disease pockets (Watson et al. 2008).

Limitations

This study, whilst being the largest cohort of patients with LGMD2I involved in longitudinal imaging, is still small and the time period between the scans was only 12 months. As LGMD2I is a slowly progressive disorder, unlike DMD, pathological changes may therefore become more obvious over a longer time period. It would therefore be interesting to follow these patients up over a five year period and re-image them as well as perform the standardised physical assessment and functional assessments, including the adapted NSAA.

The quantitative MRI was only analysed at one level, mid thigh and mid calf. As I have identified possible candidate muscles, future work should be directed at analysing these particular muscles at all levels to establish the proximal and distal distribution of muscle pathology.

The cardiac results, whilst providing a possible pathogenesis of cardiac dysfunction, was only carried out on the NCL cohort (n=10) and therefore should ideally be expanded to include the total cohort of 38 patients.

The FKRP international registry was delayed in launching due to technical difficulties, and whilst it has been launched in all the English speaking countries and Germany the other European languages remain to be launched, although the registry questions are translated.

Future work

This study has provided longitudinal quantitative MRI data on a large cohort of patients with LGMD2I. Further work will be required to continue to define the natural history of this condition, which can be achieved both by interrogating the global FKRP patient registry as well as by the follow up of this cohort. In the future one would ideally like to re-image these patients at year 3 and year 5 with the standardised physical testing and functional assessments as well as perform the adapted NSAA across the whole cohort. It would be interesting to relook at this gender difference detected and monitor whether this does correlate with the gender differences seen in the MRI's, particularly in the anterior thigh muscles. Expanding the cohort to include paediatric patients as well as

those who are asymptomatic would also provide further insight into the pathomechanisms of the muscle pathology in LGMD2I.

It is the patients that are mildly affected and asymptomatic that will probably benefit most from therapeutic interventions, rather than the severely affected patients who have little to no muscle left to preserve. Therefore it is vitally important to detect sensitive biomarkers that will highlight these changes and progression. A biomarker is an objectively measureable parameter that indicates the pathogenic process and potentially serves as a surrogate endpoint in a treatment trial. Candidates for biomarkers include motor functional scales, serological parameters, electrophysiological data, histopathological findings and imaging parameters. Takeuchi et al. (2008) states that it is feasible to analyse combinations of biomarkers to monitor disease progression, such as the functional scales and the quantitative MRI.

Functional assessments alone, may not demonstrate a change until a threshold of fat fraction is reached and only then is a decrease in function observed. We have demonstrated in this study that pre clinical changes are seen on MRI. We ideally want to be intervening before this point to preserve as much of the muscle tissue as we can whether this is by upregulating other proteins, such as LARGE (Barresi et al. 2004, Kanagawa et al. 2009), stem cell therapy (Benchaouir et al. 2007, Quenneville et al 2007, Denti et al. 2008, Goyenvalle et al. 2009) or mutation specific therapies, such as the antisense-oligonucleotide-induced exon skipping in DMD (van Deutekom et al. 2007, Kinali et al. 2009).

In conclusion, this study has therefore established that quantitative MRI is a sensitive biomarker longitudinally, even in a slowly progressive disorder such as LGMD2I. It has been possible to identify potential target muscles to analyse longitudinally in LGMD2I patients. The MRI findings are clinically relevant and do reflect the ability of the patients and the MRI fat fraction correlates well with functional and strength assessments. MRI is also able to pick up significant changes in patients with only mild disease whereas the physical testing and functional assessments did not. MRS demonstrated that whilst there was no mitochondrial dysfunction, resting abnormalities were seen suggesting loss of membrane integrity and hence membrane breakdown. The cardiac MRI results indicate that an there is an early reduction in myocardial energetics

and also reductions in cardiac torsion and ejection fraction seen early in the disease course in patients with LGMD2I that have not been reported before.

Further longitudinal work is required to continue to define the natural history of this condition both from following this cohort longitudinally as well as interrogating the international FKRP patient registry. This is key in designing a powerful clinical trial with sensitive outcome measures and biomarkers that are clinically relevant and to prepare these patients for “trial readiness”.

Appendix A

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

REC reference number:
Committee; Newcastle and North Tyneside REC 1
Protocol version 4, 30/04/08
Patient identification number for this trial;

Institute of Human Genetics
International Centre for Life
Central Parkway
Newcastle upon Tyne NE1 3BZ

Newcastle University

A study using Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) to assess muscle damage in patients with Limb girdle muscular dystrophy type 2I

Patient Information Sheet

Why have I been chosen?
As a known patient with Limb Girdle Muscular Dystrophy 2I, we are inviting you to take part in this research study. Before you accept or decline the invitation, it is important for you to understand why the research is being done and what it will involve. Please read the following information and discuss it with relatives, friends and your General Practitioner, if you wish. If there is anything that is not clear, or you have any further questions, please ask us (our contact details are at the end of this information sheet). Take time to decide whether you would like to take part, or not. This study will have no direct benefit to you, but will provide important information for possible future therapeutic trials.

Who is taking part in the study?
We are asking all patients from our local database who have a diagnosis of LGMD2I and who are ambulant to take part in the study. This is part of a larger study involving patients from London, Paris, Denmark and Munich. All the data will be stored and analysed locally. The information will be anonymised with no risk of patient identification.

What is the purpose of the study?
The aim of the study is to assess the damage that occurs in the muscle of patients with LGMD2I, this is going to be achieved in two parts;

Firstly, we will perform standard MRI scans to assess overall muscle damage and, in particular, which muscles are involved and to what extent they are damaged. This scan will look at the muscles in your mid thigh and mid calf area. These scans are a

standard MRI which looks at what muscles are involved and a technique called 3 point Dixon which looks at the percentage of fat within your muscles.

Secondly, we will look at the energy levels within your muscles and how they respond to exercise. This is called Magnetic Resonance Spectroscopy (MRS). MRS allows doctors to obtain biochemical information about the tissues of the human body in a non-invasive way (without the need for a biopsy), whereas MRI only gives them information about the structure of the body (the distribution of water and fat).
This will involve lying in the MRI scanner and flexing and extending your ankle whilst we take measurements with a monitor strapped to your legs. It is not painful and the movement required is like depressing a clutch pedal on the car. You will be required to do this for 3 minutes.

What is a Magnetic Resonance Imaging (MRI) scan?
MRI is a special technique that uses powerful magnets, radio waves and computers to produce detailed images (or scans) of the inside of your body. MRI does not use X-rays and doesn't cause pain or discomfort.

The scanning session takes approximately two hours and you will be need to lie on your back on a moveable table, which slides inside the cylinder shaped scanner. The scanner is open ended and as we are only scanning your leg, you will not be completely enclosed at any time.

A radiographer will operate the scanner from behind a window but will be able to hear you and see you during the scan. You will be given a call button to hold during the scan, which you can press to get the radiographer's attention, should you need to.

It can take several minutes for each image to be taken, and it is important to lie still and breath gently during the process. The machine is noisy and will make a loud knocking or buzzing sound throughout the scan. However, we will provide you with earplugs or headphones which will help block a lot of the sound out.

When the scans are complete, the table will be removed from the scanner, whilst you are on the table and then you will be able to get off once safely out. There will be someone to help you off the table if required.

What happens now?
If you are happy to participate in the study, we will ask you to sign a consent form indicating your willingness to take part. We will also arrange a time for you to come for your scan. There will be a total of 2 scans with a twelve month interval, assessing the extent of the muscle damage and also assessing the individual muscles which are affected. The scanning session will take approximately two hours and will also include a clinical examination of your muscle strength prior to the scan, as well as a timed walk for six minutes and a breathing test.

You will be able to eat and drink as usual and will not need to take any special precautions.
Are there any side effects to taking part in the study?
MRI scans are commonly performed and generally safe. However you need to be aware of the possible side-effects.

Some people are slightly claustrophobic in the scanner, however with the scan of your leg you will not be completely enclosed. The radiowaves used in the MRI can cause metal and tissues to heat up, you will therefore need to inform us if you have any of the following;

Surgical clips,
A previous history of metal fragments in your eyes.
Any pacemakers or heart defibrillators,
Inner ear implant (a hearing aid),
Medicine infusion pump (insulin pump),
Neurostimulator,
Aneurysm clip (a metal clip on an artery),
Shunts (tubes) in the brain,
Joint replacements/large metal implants,
Stents (tubes) in the heart or arteries,
Eye, penis or breast implants,
An intrauterine contraceptive device or coil,
Any allergies.
You will also need to inform us if you have any body piercing, tattoos or transdermal patches, metal fragments anywhere in your body or gun/shrapnel wounds.

If you are pregnant or possibly pregnant please let us know, as you will not be able to be included in the study.

You may find that teeth fillings may tingle during the scan but are safe.

Please note that there will someone to help you onto and off the table if needed.

Confidentiality

All data collected will be anonymised – you will not be able to be identified from the information we collect. The results of the study will be published, but again, you will not be able to be identified from this information.

Do I have to agree to take part in the study?

You do not have to agree to take part in this study. If you do not wish to take part, your future care will not be affected in any way. You may also wish to change your mind and withdraw from the study at any time. Again this will not affect your care in any way. If you choose to withdraw from the study, we will not use any of the data already collected on you.

If you have any further questions or concerns, please do not hesitate to telephone Professor Volker Straub on 0191 2418762, or you can write to him:

Professor Volker Straub,
Institute of Human Genetics
International Centre for Life
Newcastle upon Tyne
NE1 3BZ.

Thank you for reading this information sheet.

Appendix B

REC reference number:
Committee; Newcastle and North Tyneside REC 2
Protocol version 3, 04/06/09
Patient identification number for this trial;

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Institute of Human Genetics
International Centre for Life
Central Parkway
Newcastle upon Tyne NE1 3BZ

Newcastle University

Consent form - patients

Title of Study: Assessment of muscle damage in patients with Limb girdle muscular dystrophy 2I using MRI and MRS

Name of researchers: Professor Volker Straub,
Institute of Human Genetics
International Centre for Life,
Newcastle upon Tyne NE1 3BZ.

Please initial box if in agreement.

1. I confirm that I have read the information sheet dated ……..(version 5)for the above study and have had the opportunity to ask questions.

2. I understand that participation in this study is voluntary and that I am able to withdraw at any time, without the need to give reasons for withdrawal. This will not affect my future clinical care.

3. I understand that my research medical notes may be reviewed by responsible individuals from the research team or from regulatory authorities where it is relevant to taking part in this research. I give permission for these individuals to have access to my records.

4. I understand that the coded MRI images and MRS data obtained are stored indefinitely as DVDs or CD-ROMs by Professor Straub.

5. I agree for the MRI images and MRS data obtained to be stored indefinitely.

6. I agree for any photographs to be stored indefinitely.

7. I understand that there is a small possibility that the MRI research may produce unexpected results that are clinically important for me. In this event, I will be contacted via the research team in Newcastle and appropriate follow-up arranged.

8. (Optional – follow up physiotherapy assessment within one month of 1st/2nd scan date)
9. I agree to take part in this study.

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Name of person taking consent.	Date	Signature

Appendix C

Limb Girdle Muscular Dystrophy 2I Study

Draft Manual of Operations
Clinical Evaluation

Dr Michelle Eagle
Consultant Physiotherapist
Dr Anna Mayhew
Research Physiotherapist

Newcastle Muscle Centre
UK

INTRODUCTION

Clinical evaluation will include myometry, functional assessment (which includes timed up and go, timed 10 metre walk and rise from the floor and stairs), 6 minute walk test and activity monitoring.

General Testing Guidelines

- Tests should be performed at approximately the same time of day and after similar pre-test activities and routines.
- Tests should be performed in the order provided in this manual.
- If any test must be repeated, a 30-second rest period is allowed.
- To avoid bias, the clinical evaluator (CE) should not review any previous testing results.
- The testing environment should be standardized. It is recommended that family members or friends not be present in the room during testing, unless test compliance is affected by the absence of the parent/caregiver. A second CE or staff member may be in the room during testing.
- The subject should wear loose clothing. It is preferable for the subject to wear a short-sleeved shirt and shorts.

Equipment Required

Myometry	*Hand held myometer: CITEC* A weight of ~5 lbs or 2.5 kg for calibration testing
Range of Movement	Examination table with a firm testing surface that is accessible to the CE from both sides
Functional and timed tests	Digital stopwatch A quiet hallway with at least 12 meters of straight, uninterrupted walking space, with a starting line and finish line exactly 10 meters (32 feet and 9 and 10/16th inches) apart A sturdy chair for use in the Gowers maneuver, if required. The same chair must be used for all evaluations. Floor mat. It is recommended that the floor be used, but if a floor mat is required to obtain the cooperation of the subject, then the same floor mat should be used throughout the study. A box step of approximately15 cms height
Six Minute Walk Test	Small orange cones (12 inch or 20 cm): 2 Two 25 metre tape measures Stop watch: 2 Soft cloth surgical tape: Marking pens: Wide 3M vinyl tape: Clip board: A chair or wheelchair that can be easily moved along the walking course 6MWT worksheet with checklist.
Step Activity Monitoring	To be provided by Orthocare or company providing activity monitors

Recommended Sequence of study procedures

Study procedures should be performed in the same order and at approximately the same time of day for each subject. All procedures should be conducted by a trained physiotherapist.

Order of evaluations

1. Myometry
2. Ankle Range of Movement

3. Functional and Timed Tests
4. 6 minute walk test
5. Step activity monitoring calibration and placement (when required, according to protocol).

1; Myometry

1.1; Testing Guidelines

- Tests should be performed in the order listed on the myometry worksheet.
- The myometer is a very sensitive measuring tool therefore care must be taken to be consistent with test method.
- Allow at least 5 seconds of rest between trials.
- Repeat the test if the subject moves out of the testing position during a trial.
- The subject should be vigorously coached to push or pull in the desired direction while the myometer is held stable by the CE ("make" test).
- If the CE encounters problems during a trial, the trial must be repeated. Three "valid" trials will be performed, and the value from the myometer will be recorded after each trial. The unit of measurement should be the pound, and values should be recorded with 1 decimal place (eg, 1.0 lbs).
- Calibration dependent on myometer The test is explained to the patient in a way that they understand. The intent is to build a maximum isometric hold, so the command is effectively **'HOLD'** or **'Keep still/don't let me move you'**. The words push or pull should not be used.
- The patient will be encouraged verbally to build to and maintain a maximum hold over a period of approximately 5 seconds, to allow for full physiological recruitment of muscle.
- Following one 'trial' test to allow for learning, the **best** of 3 tests shall be noted. Test results should be fairly closely grouped – a 10% variation is not unusual, e.g. 30N +/- 3
- Any discomfort will limit the patient's ability to offer maximum resistance. As much as is possible, ensure comfort when applying the myometer. The applicator can be padded to allow for comfort.
- If the evaluator does not feel that they have been able to gain compliance from the patient for any reason (e.g. understanding or poor concentration), this should be noted.

1.2; Order of Tests

A ‘make’ test will be used unilaterally (dominant side) for the following muscle groups:

1. Hip flexors
2. Hip abductors
3. Hip adductors
4. Knee extensors
5. Knee flexors
6. Ankle dorsiflexion

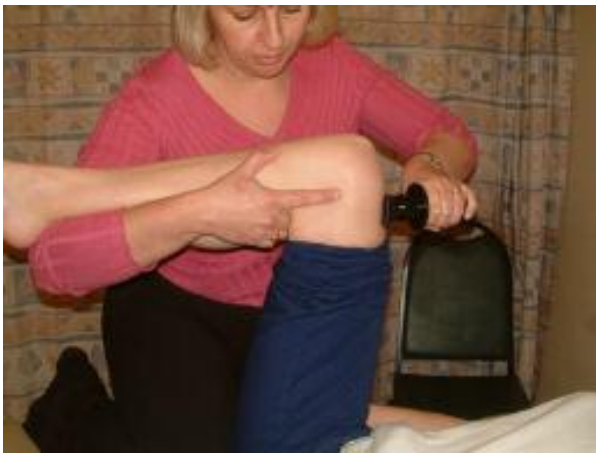
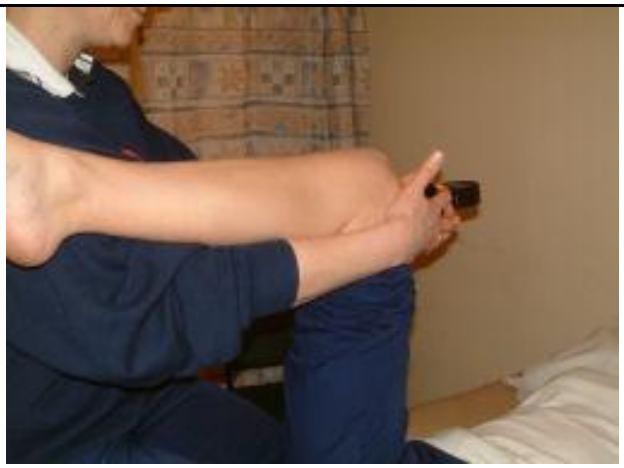
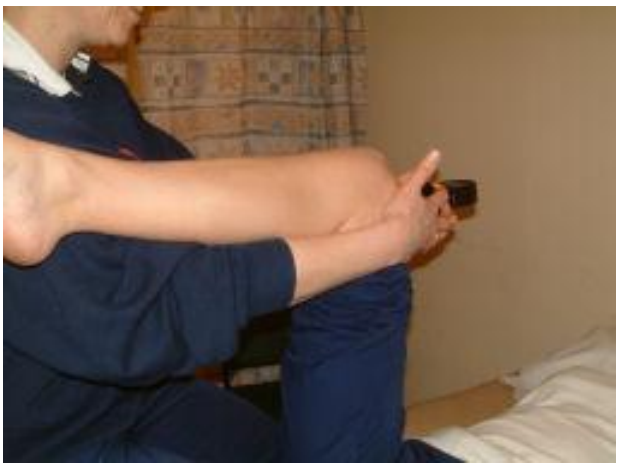
Hip Flexors	
Patient position	Supine with hip and knee at 90º. Femur in neutral rotation. Patient is asked to concentrate on keeping knee steady, not to move foot, as this discourages hamstring involvement
Stabilisation	*Under knee and calf to support weight of leg and prevent unwanted hip movement*
Myometer Position	*Anterior aspect of lower thigh, just proximal to condyles*
Therapist Position	At side of, or kneeling on, plinth. Facing the patient
Figure 2a: Hip Flexors - Option 1	

Figure 2b: Hip Flexors - Option 2	

Hip Abductors	
Patient position	Supine, arms by side, leg straight, knee caps pointing towards the ceiling. Patient is asked to concentrate on keeping knee steady, not to move foot.
Stabilisation	Around heel if necessary
Myometer Position	10 cm above lateral maleolus of ankle
Therapist Position	Either at end of plinth or standing at edge of plinth on side which is to be tested
Figure	Not available

Hip Adductors	
Patient position	Supine, arms by side, leg straight, knee caps pointing towards the ceiling. Patient is asked to concentrate on keeping knee steady, not to move foot.
Stabilisation	Around heel if necessary
Myometer Position	10 cm above medial maleolus of ankle
Therapist Position	Either at end of plinth or standing at edge of plinth on side which is to be tested
Figure	Not available

Knee Extensors	
Patient position	Patient sitting with thigh supported and hip and knee at 90º, on plinth, chair or in wheelchair. Feet must be clear of the floor. Femur in neutral rotation. Patient can hold onto front of plinth or chair to stabilise himself
Stabilisation	If needed, given by therapist over lower third of thigh, just above knee
Myometer Position	Anterior surface of tibia, at junction between middle and lower third of tibia
Therapist Position	Sitting in front of patient, on a chair or on floor
Figure 1: Knee extensors	

Knee Flexors	
Patient position	Patient sitting with thigh supported and hip and knee at 90º, on plinth, chair or in wheelchair. Feet must be clear of the floor. Femur in neutral rotation. Patient can hold onto front of plinth or chair to stabilise himself
Stabilisation	If needed, given by therapist over lower third of thigh, just above knee Watch for: hip external rotation, trunk flexion
Myometer Position	
Therapist Position	Sitting in front of patient, on a chair or on floor
Figure	Not available

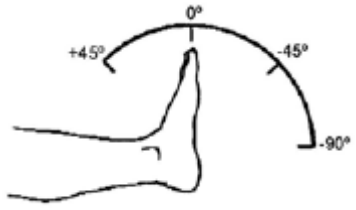
Ankle Dorsiflexors	
Patient position	Patient sitting with thigh supported and hip and knee at 90º, on plinth, chair or in wheelchair. Feet must be clear of the floor. Femur in neutral rotation. Patient can hold onto front of plinth or chair to stabilise himself Supine?
Stabilisation	If needed, given by therapist over lower third of thigh, just above knee Watch for: hip external rotation, trunk flexion, ankle eversion and inversion
Myometer Position	Anterior surface of foot, just in front of the ankle joint
Therapist Position	Sitting in front of the patient, on a chair or on floor
Figure	Not available

Ankle Dorsiflexion Range of Movement

1.3; Testing Procedure

Patient position: Supine. As we are interested in the effects of gastrocnemius shortening on ankle dorsiflexion, this test is undertaken with the knee in full extension. The calcaneum is held in neutral alignment whilst pressure is applied over the mid-section of the foot to dorsiflex the ankle as much as possible, preventing inversion.

Goniometer position: Axis of the goniometer over the lateral malleolus. Stationary arm aligned with the fibular head, along the lateral aspect of the lower leg. "Moving arm" held parallel to the lateral aspect of the 5th metatarsal, aligned with the posterior third of the foot (this is to ensure that gastrocnemius range is being monitored and not that of the planter structures of the foot). Note range of dorsiflexion past plantergrade as **+xº's,** range lacking from plantergrade as **-xº's**



Normal range: 20º dorsiflexion to 50º planterflexion.

End feel: firm due to joint capsule, Achilles tendon and ligaments

1; Functional and Timed Tests

1.1; Order of Tests

- Timed up and go
- 10 metre walk / run
- Stair Ascend
- Stair Descend
- Timed rise from the chair

Testing procedure

Timed up and go 'TUG' test

Preparation	Place a piece of tape or other marker on the floor 3 meters away from the chair so that it is easily seen by the subject. The chair should be stable and positioned such that it will not move when the subject moves from sitting to standing. The subject wears their regular footwear, may use any gait aid that they normally use during ambulation, but may not be assisted by another person. There is no time limit. They may stop and rest (but not sit down) if they need to. Normal healthy elderly usually complete the task in ten seconds or less. Very frail or weak elderly with poor mobility may take 2 minutes or more. The subject should be given a practice trial that is not timed before testing
Starting position	Subject sitting in a chair with arms. The subject's back should resting on the back of the chair.
Instructions	On the word "*GO"* you will stand up, walk to the line on the floor, turn around and walk back to the chair and sit down. Walk at your regular pace
Timing	Start timing on the word "*GO*" and stop timing when the subject is seated again correctly in the chair with their back resting on the back of the chair.

10 metre walk/run

Preparation	Orthoses should not be worn for this assessment. However if the person regularly uses them the test could be repeated with orthoses for comparison Mark out 10m distance in quiet area if possible
Starting position	Standing at start of marked distance
Instructions	On the word "*GO" go as fast as you can to "x". Define point x.*
Timing	Start timing on the word go and stop when the first foot passes the 10 metre mark.
Grading	1 Unable to walk even with aids 2 Able to walk with a walking aid (sticks, Frames) 3 Walk but cannot pick up speed 4 Walking but able to pick up speed 5 Picking up speed, nearly running but still using double stance phase 6 Running - no double stance phase, no excessive trunk and upper limb movement

Stair Climb

Preparation	Use standard steps four steps with handrail where possible
Starting position	Standing upright at bottom of steps. Arms by side
Instructions	When I say "GO" climb the stairs as quickly as you can, safely and stand up straight with your arms by your side when you get to the top
Timing	Start the watch on the word go and stop it when they are standing straight with their arms by their side.
Grading	1. Unable to climb 4 standard stairs 2. Climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step), uses both arms on one or both handrails 3. Climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step), using one arm on one handrail 4. climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step), not

needing handrail
5. Climbs 4 standard stairs alternating feet, needs handrail for support
6. Climbs 4 standard stairs alternating feet, not needing handrail support

Stair Descend

Preparation As stair ascend

Starting position Standing at the top of the stairs with arms by side

Instructions When I say "GO" go as fast as you can down the stairs safely and at the bottom stand up straight with your arms by your side

Timing Start the watch on the word go and stop it when they are standing straight with their arms by their side.

Grading

1. 1.Unable to descend 4 standard stairs
2. Descends 4 standard stairs "marking time" (descends one foot at a time, with both feet on a step before moving to next step), requires both arms on one or both handrails.
3. Descends 4 standard stairs "marking time" (descends one foot at a time, with both feet on a step before moving to next step), requires one arm on a handrail
4. Descends 4 standard stairs "marking time" (descends one foot at a time, with both feet on a step before moving to next step), not needing handrail
5. Descends 4 standard stairs alternating feet in both directions, needs handrail for support
6. Descends 4 standard stairs alternating feet, not needing handrail support.

Timed rise from a chair

Preparation Chair

Starting position Hips and knees at 90 degrees with arms folded

Instructions When I say "GO" stand up as fast as you can, stand up straight with your arms by your side

Timing Start the watch on the word "GO" and stop it when they are standing up straight with their arms by their side

Grading

1. Unable to stand up even with the use of furniture
2. Can stand up using additional furniture or turms to face chair
3. Uses two arms to stand up on leg or on chair
4. Uses one arm to stand up on leg or on chair
5. Adapt starting position to a wide base and use more flexion to rise from chair
6. Gets up normally without using arms

6-MINUTE WALK TEST

This evaluation is a modified version of the 6MWT adapted from the protocol specifically designed for DMD in the PTC 124 2b trial [ATS, 2002, Ref Henriksen, Mc Donald).

Testing Guidelines

- Subjects should wear comfortable clothing and appropriate shoes for walking (ie, trainers, tennis shoes, etc). Since subjects will be tested at multiple time points, they should make an effort to wear the same type of shoes each time.
- A light meal or snack is permissible at least 1 hour before testing.

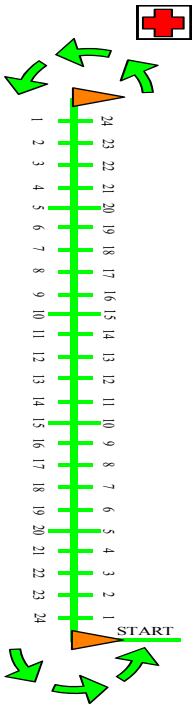
Set up of course

- The test should be performed indoors, along a flat, straight, enclosed, and seldom traveled corridor at least 6 feet wide with a hard surface. The test area will be marked with a 25-meter tape line. The tape line should be placed in the middle of the corridor. Arrows indicating the counterclockwise direction and path of movement should be placed in a half-circle at the ends of the course. Note that due to the possibility of subject falls, the course should be within easy access of appropriate medical assistance.

Testing Directions

- Set the stopwatch to zero.
- Ask the subject to stand quietly with his toes at the starting line, immediately adjacent to axis of the "home" cone.

  "**Remember, you will be walking back and forth around these cones without crossing the line in the middle. You will walk around the cone in a half circle without slowing down. Then you will go back the other way.**



Remember that the object of this test is to walk as fast and as far as you can for six minutes without running."

- The clinical evaluator should follow the subject whilst he is walking around the course.
- When the subject is ready, say "**Ready, set, go**!", and start the stopwatch.
- Every time the subject reaches the each end of the course, mark the worksheet to record the time at each 25 metres completed.
- Give positive verbal encouragement at approximately 15-second intervals. Encouragement should be similar to any of the following phrases:
    - "**You're doing great (subject name)! Keep it up!**"
    - "**Remember, walk as fast as you can!**"
    - "**Fantastic job (subject name)! Keep Going!**"
    - "**Keep up the good work!**"
- let the subject know how long he has been walking. For example **"three minutes done, only three to go, you are half way there, one minute left"**
- If the subject stops to rest, say:
- **"You can lean on the wall to rest. Just start walking again as soon as you feel like you can.**"
- If the subject falls:
- **Record the time of fall**
    - The evaluator should ensure that the subject is OK, and then should assist him back to a standing position as soon as it is safe to do so.
    - If the subject is injured or cannot rise from the floor, the test is over. Total time and distance should be recorded, and any necessary medical attention should be given to the subject.
    - If the subject is uninjured, he should resume walking as soon as he is able. Say: **"If you are alright, you should start walking again as soon as you feel like you can."**
- At the final seconds of the test count down, the timer will announce:
- **"Five fifty seven, five fifty-eight, five fifty-nine, six minutes! Great job, you can stop now.**"

- Bring a chair or wheelchair for him to sit and rest. Offer the subject a drink of water.
- Measure the distance from the marker to the point at which the subject stopped at 6 minutes. Multiply by 25 the number of completed 25 metre laps and add to the distance reached on the final lap. This is recorded as the total distance walked in 6 minutes.
- Document the number of falls during the 6 minute walk test

Safety Considerations and Additional Information

- Subjects should have medical clearance from the study investigator prior to testing.
- Testing staff should know the location of the nearest resuscitation cart and institutional emergency care procedures. Emergency contact numbers should be immediately accessible.

Adapted North Star Scale for LGMD 2I (NSAA for LGMD 2I)

09/06/09

Name **DOB**
Date of assessment **Assessor**
Time test took to complete

Test	***Instruction***	**2**	**1**	**0**	**Comments**
1 Stand unsupported	*Can you stand without holding onto anything for the count of 3?*	Stands upright and symmetrically, without compensation (with heels flat and legs in neutral) for minimum count of 3	Stands but with some degree of compensation	Cannot stand independently, needs support	
2 Walk 10 m	*Can you walk as far as …..(give 10 m marker)*	Able to complete the distance with no aids	Able to complete the distance with aids Specify aids	Unable to walk 10 m	Aids = ……
3 Stand up from chair	*Stand up from the chair keeping your arms folded if you can*	Able to stand up not using arms	With help from thighs or push on chair or prone turn	Unable	
4 Stand on one leg - Right	*Can you stand on your right leg for as long as you can?*	Able to stand in a relaxed manner (no fixation) for count of 3	Stands but either momentarily or needs a lot of fixation e.g. by knees tightly adducted or other trick	Unable	
5 Stand on one leg - Left	*Can you stand on your left leg for as long as you can?*	Able to stand in a relaxed manner (no fixation) for count of 3	Stands but either momentarily or needs a lot of fixation e.g. by knees tightly adducted or other trick	Unable	
6 Climb box steps – Right	*Can you step onto the top of the box using your right leg first?*	Faces forwards, climbs up– no support needed	Goes up sideways or needs support	Unable	
7 Climb box steps – Left	*Can you step onto the top of the box using your left leg first?*	Faces forwards, climbs up– no support needed	Goes up sideways or needs support	Unable	
8 Descends box step - Right	*Can you step down from the box using your right leg first?*	Faces forward, climbs down controlling weight bearing leg. No support needed	Goes down sideways or skips down or needs support	Unable	
9 Descends box step - Left	*Can you step down from the box using your left leg first?*	Faces forward, climbs down controlling weight bearing leg. No support needed	Goes down sideways or skips down or needs support	Unable	
10 Lying to sitting	*Can you get from lying to sitting?*	Able - may use one hand to push	Self assistance e.g. pulls on legs or turns into prone / towards floor	Unable	
12 Lifts head from supine	*Lift your head to look at your toes keeping your arms folded*	In supine, head must be lifted in mid-line. Chin reaches chest	Head must be lifted in mid-line. Chin moves towards chest	Head is lifted but through side flexion or with no neck flexion	

13 Standing on heels	*Can you stand on your heels?*	Both feet at the same time, clearly standing on heels only for count of 3	Flexes hip and only raises forefoot	Unable	
14 Jumps	*How high can you jump?*	Both feet at the same time, clear the ground simultaneously	One foot after the other (skip)	Unable	
15 Hops – Right leg	*Can you hop on your right leg?*	Clears forefoot and heel off floor.	Able to bend knee and raise heel, no floor clearance.	Unable	
16 Hops – Left leg	*Can you hop on your left leg?*	Clears forefoot and heel off the floor.	Able to bend knee and raise heel, no floor clearance	Unable	
17 Runs 10m	*Go as fast as you can to…….(give point)*	Can run – both feet off the ground – no double stance phase during running	Speeds up walk but maintains double stance phase	Walks with no extra speed.	Time ……secs
S1 Squat **Score**	*Can you squat? Pretend you are going to sit in a very low seat.*	Squats with arms free (at least 90 degrees of hip and knee flexion) **No. of 1's =**	Initiates squat (more than 10 degrees), uses arm support. **No. of 2's**	Unable/ Unable to initiate	**Total = /34**
18 Step ups TT	*How many step ups can you do in 30 seconds from when I say "go" to when I say "stop"*	Number = …….		One step up = floor to both feet on step to both feet back down	

Comments

Appendix D

Institute of Human Genetics
International Centre for Life
Central Parkway
Newcastle upon Tyne
NE1 3BZ

Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) in patients with Limb girdle muscular dystrophy 2I: Assessment of muscle damage

Respiratory Function

Name	Date ddmmyy and time of test 24 hour clock _ _ / _ _ / _ _ Time _ _ . _ _
Date of Birth ddmmyy	Examiner
Study Number	Signature of examiner
Contractures	Yes ☐ No ☐
Details of contractures	

Height (cm) ……	**Weight (kg) ……..**
FVC Sitting	
% predicted FVC in sitting	
FVC Lying	
% predicted FVC in lying	

Institute of Human Genetics
International Centre for Life
Newcastle upon Tyne
NE1 3BZ

Myometry and Range of Movement Worksheet

Name	Date ddmmyy and time of test 24 hour clock _ _ / _ _ / _ _ Time _ _ . _ _
Date of Birth ddmmyy	Examiner
Study Number	Signature of examiner
Test dominant side. Dominant side Right ☐ Left ☐	If unable to test a muscle/muscle group, record "ND". To convert pounds to kilograms, use the following: 1 pound = 0.4536 kilograms

Tested in supine	Muscle / Muscle Group	Result (Maximum Force)
Myometry ☐ Kilograms ☐ Newtons ☐ Pounds	Hip Flexors __ __ . __ __ __ . __ __ __ . __	Highest __ __ . __
	Hip Abductors __ __ . __ __ __ . __ __ __ . __	Highest __ __ . __
	Hip Adductors __ __ . __ __ __ . __ __ __ . __	Highest __ __ . __
	Ankle dorsiflexion __ __ . __ __ __ . __ __ __ . __	Highest __ __ . __

<table>
<tr><td>Ankle Dorsiflexion ROM
(Range 20° + to 50° -)</td><td colspan="2"><table><tr><th>Right °</th><th>Left °</th></tr><tr><td></td><td></td></tr></table></td></tr>
<tr><th>Tested in sitting</th><th>Muscle / Muscle Group</th><th>Result (Maximum Force)</th></tr>
<tr><td rowspan="2">Myometry</td><td>Knee extensors __ __
. __
__ __
. __
__ __
. __</td><td>Highest
__ __ . __</td></tr>
<tr><td>Knee flexors __ __
. __
__ __
. __
__ __
. __</td><td>Highest
__ __ . __</td></tr>
<tr><td colspan="3">Comments:</td></tr>
</table>

Functional and Timed Tests

Name	Date ddmmyy and time of test 24 hour clock _ _ / _ _ / _ _ Time _ _ . _ _
Date of Birth ddmmyy	Examiner
Study Number	Signature of examiner

Timed up and go	Comments	Time (seconds)	_ _ . _ _

Grade	**1**	**2**	**3**	**4**	**5**	**6**	**Time (seconds)**
10 metre walk / run Please circle	Unable	Walk with aids	Walk No extra speed	Walk extra speed	Nearly running Double stance	Running	_ _ . _ _
Comments							
Stair Climb Record Height of Hand rail from floor to top of rail ……..cm							
Stair Climb Please circle	Unable	Marking time Using two hands	Marking time Using one hand	Marking time No handrail	Alternating feet Needs handrail	Alternating feet No handrail	_ _ . _ _
Comments							
Stair Descend Please circle	Unable	Marking time Using two hands	Marking time Using one hand	Marking time No handrail	Alternating feet Needs handrail	Alternating feet No handrail	_ _ . _ _
Comments							
Rise form Chair Record height of chair from floor to middle of cushion or pad ……..cm							
Timed rise from chair Please circle Standard chair with no arms	Unable	Stand up using additional furniture or person	Uses two hands to stand up	Uses one hand to stand up	Adapts standing position to stand	Gets up normally	_ _ . _ _

Institute of Human Genetics
International Centre for Life
Central Parkway
Newcastle upon Tyne

Six minute Walk Test

Name	Date ddmmyy and time of test 24 hour clock _ _ / _ _ / _ _ Time _ _ . _ _
Date of Birth ddmmyy	Examiner
Study Number	Signature of examiner
BP and HR before test _ _ _ /_ _ _mmHg. _ _ _ bpm	**BP and HR after test** _ _ _ /_ _ _mmHg. _ _ _ bpm

See manual for details

Distance	**Time** minutes . seconds	**Distance**	**Time**	**Falls**	
25	_ _._ _	325	_ _._ _		Time
50	_ _._ _	350	_ _._ _	1	_ _._ _
75	_ _._ _	375	_ _._ _	2	_ _._ _
100	_ _._ _	400	_ _._ _	3	_ _._ _
125	_ _._ _	425	_ _._ _	Comments	
150	_ _._ _	450	_ _._ _		
175	_ _._ _	475	_ _._ _		
200	_ _._ _	500	_ _._ _		
225	_ _._ _	525	_ _._ _		
250	_ _._ _	550	_ _._ _		
275	_ _._ _	575	_ _._ _		
300	_ _._ _	600	_ _._ _		

Distance at six minutes				
Measure the distance from the marker to the point at which the subject stopped at 6 minutes. Multiply by 25 the number of completed 25 metre laps and add to the distance reached on the final lap. This is recorded as the total distance walked in 6 minutes				
Distance from marker (a)	_ _ metres	Number of completed laps (n)	_ _	(25 x n) + a = Total distance 25 x _ _ + _ _ =

Institute of Human Genetics
International Centre for Life
Central Parkway

Activity Monitor given (number)	
Information sheet given	Yes ☐
Functional sheet (NSAA for LGMD) **IPAQ**	Yes ☐ Yes ☐
Activity Monitor returned and data downloaded	Yes ☐

Appendix E

Institute of Human Genetics
International Centre for Life
Central Parkway
Newcastle upon Tyne
NE1 3BZ

Medical Information Sheet

A study using Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) in patients with Limb girdle muscular dystrophy 2I; an assessment of muscle damage.

Name	Date ddmmyy and time of test 24 hour clock _ _ / _ _ / _ _ Time _ _ . _ _
Date of Birth ddmmyy	Examiner
Study Number	Signature of examiner
	First Appointment Yes
Address Telephone: Mobile number:	GP Telephone:

Age at first symptoms	……years
First symptoms were	
Age at diagnosis	……years
Best motor function Including participation in sports, ability on stairs walking distance etc.	
Family history	Yes
Family History details	
Genetic Mutation	
Highest recorded CPK	
Date of highest CPK	

test	
Most recent CPK Level	
Date of recent CPK test	

Current motor function/symptoms	
Walk Distance	……..metres
Able to run	Yes ☐ No ☐
Able to climb stairs	Yes ☐ No ☐
Arm involvement	Yes ☐ No ☐
Participates in sports	Yes ☐ No ☐
Details of sports	
Myalgia	Yes ☐ No ☐

Respiratory involvement	
Respiratory Involvement	Yes ☐ No ☐
Details of respiratory involvement	
Ventilated at night	Yes BiPAP ☐ Yes CPAP ☐ No ☐
Number of hours ventilated each night	……hours
Last FVC in sitting	
% predicted in sitting	
Last FVC in lying	
% predicted in lying	
Diagnosed with sleep	Yes ☐ No ☐

apnoea	
Latest oximetry if known Amount of minutes below 90% and lowest SaO2.	

Other Medical	
Echo results. Ejection fraction (%)	
Details of any cardiac involvement	
Any other medical conditions Renal, vision, diabetes, peripheral vascular disease etc.	
Medications	
Additional Comments	

Appendix F

FKRP website July 2011 – http://www.fkrp-registry.org

Information for patients

General information

Background and purpose

TREAT-NMD aims to improve treatment and find cures for patients with neuromuscular disorders. For the treatment of LGMD2I, MDC1C and other conditions caused by FKRP, promising new therapeutic strategies are currently being developed which need to be tested in clinical trials/research studies. This international registry will make the recruitment of FKRP patients for trials or studies easier by helping to identify suitable patients for particular trials or studies and by enabling them to be contacted and informed quickly when there is a trial or study they might be interested in. More information

Target group

The registry is for patients affected by LGMD2I (Limb girdle muscular dystrophy 2I), MDC1C (congenital muscular dystrophy 1C) and other conditions caused by a mutation in the FKRP gene. There have been two registries launched which are collecting data, preparing for trial readiness. These are in patients with Duchenne Muscular Dystrophy and Spinal Muscular Atrophy. Because this registry, as with the other two, is primarily designed to register patients who might be suitable for participation in future clinical trials of new therapies, and to help the researchers find the best way of caring for patients with LGMD2I/MDC1C and other FKRP-related conditions, this registry is intended for patients currently living with the condition and not a record for those who have already died.

Advantages for patients

If you are a patient affected by LGMD2I, MDC1C or an other FKRP-related condition and register here, we can direct you to clinical trials and research results (such as new treatments) that might be of specific interest to you. In addition, by registering you are helping researchers obtain precise data about the prevalence of LGMD2I, MDC1C and other FKRP-related conditions, which could be of benefit to all affected patients. You are also helping achieve equal care for all patients. More information

Required data

The required data will be asked for in a two-part questionnaire.

Part 1
This part is to be completed by the patient or carer / guardian / parent of the patient.

You can view all the questions in advance:

Questionnaire FKRP Registry

Part 2
This part is to be completed by the professional involved either geneticist or neurologist/neuromuscular specialist. The information required will be related to test results such as respiratory function, cardiac status, mutation analysis and outcome scores such as the 6 minute walk distance which would not be readily available to the patient.

Registration process

Patients can register on their own by clicking "Registration", which leads them to the web application for the international FKRP patient registry, which is an online (internet-based) self-report registry. A self-report registry is one where patients enter their details themselves, which is often quicker and easier for everybody concerned, but you can of course talk to your own doctor or to the registry staff at any time if you have any questions.

Roughly speaking, the registration process contains the following steps. After reading the patient information, you agree to the online consent form and enter your personal and contact details.Then you fill in Part 1 of the questionnaire.

If necessary, you can save and interrupt your registration any time, for instance in order to consult a doctor to discuss questions. More information

Data access

Staff in charge of the registry might need to gain access to your medical records to obtain information necessary to the project (for example we will need to ask your geneticist/physician for a copy of your genetic report and also information on your respiratory and cardiac function). More information about data protection

Data updates

To make sure that the data in the registry are correct and up to date, it is essential that we update them regularly. To do this, we will send you emails once a year asking you to tell us about any changes in your medical condition. If there are any major changes in your details that might occur in the period between updates, for example change of address or loss of ambulation, you can easily update them yourself by logging into the registry and changing your data.

Data protection

Your data is stored on a specially secured computer in the University of Munich, which can be accessed only by selected people. Information that you enter online via the Internet is encrypted while being transferred, so that it cannot be intercepted. More information about data protection

Help

Here you will find answers to some questions that you might have when using the

registry.

I had to temporarily interrupt my registration. When I wanted to continue, I had to type in my password. Why?

For security reasons you are logged out automatically if you have been idle for two hours, i.e. not clicked on a link or button or typed in any text. After you type in your password as requested, in most cases you should be back on the page you were on before you were logged out. If not, you can click through to the desired page via the navigation bar on the left. If at the time you were automatically logged out you had made changes in a form without clicking the "Save" button, you will have to make these changes again.

I have forgotten my password. What should I do?

If you forget your password, please follow the instructions on the "Forgot password" page.

I have forgotten my username. What should I do?

You can find your username on the list of backup passwords you should have received by mail after registration. Please also see the page "Forgot password".

If you have any more general questions about TREAT-NMD patient registries, you may like to view the "Patient Registries - Questions and Answers" section of the main TREAT-NMD website.

Background and purpose

Background and purpose

What is the patient registry and why do we want to create one?

Scientific advances over recent years have led to substantial changes in the treatment of many disorders. New therapeutic strategies are being developed and, for some of these treatments, plans for large studies involving patients from more than one country are already in place.

Several new therapeutic strategies for neuromuscular disorders like LGMD2I target specific gene defects. When a clinical trial is being planned, it is very important that patients suitable for that trial can be found and contacted quickly. The best way to insure that this happens, is to make sure that patients' details are all collected in a single database or "registry" that contains all the information that researchers will need, including each patient's particular genetic defect and other key information about that disorder.

The Treat-NMD network is creating this kind of global registry, which means that all patients who register will be contacted if their profile fits the requirements for the clinical trial. In addition, the registry will help researchers answer questions such as how common disorders like LGMD2I are internationally, and will support other activities to improve patient care, and establish a standard of care worldwide.

Advantages for patients

Advantages for patients

How will I benefit from registering?

The registry is intended as a public service for the benefit of patients living with LGMD2I or MDC1C. You will not receive any payment or any other financial benefit as a result of submitting your data to the registry. The results of research facilitated by the registry may be patentable or may have commercial potential. However, you will not receive patent rights and will not receive financial benefits from future commercial development.

Nevertheless, there may be other benefits from participating, including the following: We will inform you if (on the basis of the information that you and your doctor provide) you might be a suitable candidate for a certain clinical trial. We will also inform you if we receive any new information on your disorder which might be of interest to you - for example if we find better ways for caring for patients with LGMD2I or MDC1C. The data collected might also provide benefits for other patients with your disorder, for example by revealing statistics on how many people worldwide have the same condition, or providing information for researchers interested in the best standards of care for your disorder.

I want to take part in a clinical trial. If I register, is this guaranteed?

Although one of the main aims of this registry is to make it easier for patients to be recruited for trials, there is no guarantee that registering your details will ensure that you will be involved in a clinical trial. If you are interested in receiving details of trials that you may be eligible for, please tick the box at the end of the online consent form.

However, it is important that you understand that even if the coordinators of a clinical trial believe that you might be eligible for that trial, based on your data about you stored in the registry, it is possible that at a later date it will turn out that you do not meet the inclusion criteria for the trial after all.

I do not want to take part in a clinical trial. Should I still register?

We hope that you will be interested in registering even if you do not want to take part in a clinical trial. Your information will still be useful to researchers who are trying to find out more information about patients living with LGMD2I and MDC1C, and we will still provide you with other information that might be relevant to your disorder. If you do not want to receive any information about clinical trials that you might be eligible for, please tick “no” at the end of the online consent form.

Questionnaire

Questionnaire

Here you can find a preview of the questions you will be asked if you sign up as patient with LGMD2I, MDC1C or an other condition caused by a mutation in the FKRP gene. When filling in the real questionnaire online, you can click on the answers and enter your details in text boxes.

Part 1 (to be completed by the patient or carer / guardian / parent of the patient)

Please answer all the questions, as this information is necessary for including you in the international registry. But if there is a question that you can not answer right away, just leave the option 'not specified' checked and continue with the other questions. You can enter the missing data at a later date.

1. What is your diagnosis according to your doctor?

LGMD2I (Limb Girdle Muscular Dystrophy 2I)

MDC1C (Congenital Muscular Dystrophy 1C)

Other FKRP-related condition (please specify below)

Other diagnosis (please specifiy below)

Not specified

Other diagnosis : _____

2. What is your current best motor function?
Motor function describes a person's ability to move his or her body. Sitting independently means that you can stay in the sitting position for several minutes, without being supported by either another position or stabilizing device (chair back, corset or brace).

I am able to run

I am currently able to climb stairs without assistance

I am currently able to climb stairs with the use of hand rails

I am currently able to walk without support

I am currently able to walk with support

I am currently not able to walk, but I am able to sit independently (without support)

I am currently neither able to walk nor to sit independently

Not specified

3. What was the best motor function you achieved?
This question is not about the current situation, but about the time when your ability to move was the best. What was your best motor function ever? Please also state the age at which you were able to perform this motor function.

I was able to run

I was able to climb stairs without assistance

I was able to climb stairs with the use of hand rails

I was able to walk without support

I was able to walk with support

I was not able to walk, but I was able to sit independently (without support)

I was neither able to walk nor to sit independently

Not specified

This was possible from the age of ___ years and ___ months up to the age of ___ years and ___ months (leave the last two fields blank if this is your current motor ability).

4. Do you currently use a wheelchair?

I always use a wheelchair

I sometimes use a wheelchair, but I am able to walk

I never use a wheelchair

Not specified

5. Do you have muscle aches or pains (myalgia)?

I don't have muscle aches or pains

I have muscle aches or pains at rest

I have muscle aches or pains during exercise

I have muscle aches or pains after exercise

Not specified

6. Do you regularly use a non-invasive ventilation device?
Some patients with LGMD2I or MDC1C have trouble with their breathing. To support their breathing, they get a ventilation device that they either use full-time, i.e. 24 hours a day, or only for several hours a day or at night. "Non-invasive" means that they use this

device without having had an operation. Usually this means they wear a mask that can be removed at any time.

I do not regularly use a non-invasive ventilation device

I regularly use a non-invasive ventilation device for several hours a day or at night

I regularly use a non-invasive ventilation device full-time, i.e. 24 hours a day

Not specified

This has been the case since the age of ___ years and ___ months.

7. Do you regularly use invasive ventilation?
"Invasive ventilation" means that the patient had to have an operation (an incision in the wind-pipe, also known as tracheotomy) to use the ventilation device. Again, this ventilatory support system can be used either all day or a few hours per day.

I do not regularly use invasive ventilation

I regularly use invasive ventilation for several hours day or at night

I regularly use invasive ventilation full-time, i.e. 24 hours a day

Not specified

This has been the case since the age of ___ years and ___ months.

8. Do you know of any family members who have similar symptoms, or a diagnosis of LGMD2I, MDC1C or a different condition caused by a mutation in the FKRP gene?
FRKP is a a gene that when mutated (faulty) can cause LGMD2I or a more severe congenital muscular dystrophy (MDC1C). It is important to know if any other family members have similar conditions, or raised creatine kinase (muscle enzyme) levels, or the same diagnosis.

Yes

No

Not specified

9. Would you like to be contacted if you might be suitable for a clinical trial/research study?
If you do not want us to inform you if there is a clinical trial/research study that you might be eligible for, please turn off the following option. But note that by leaving this option on, you are not placing yourself at any risk or under any obligation to take part in a trial or a study. This option only means you will be informed about trials/studies. In order to take part in any trial/study, you would be given precise details about that trial/study and you would have to sign a separate consent form. Also note that we will never give away your contact data to anybody, whether you are interested in clinical

trials/research studies or not. You can switch this option off or on at any time.

Yes

No

Part 2 is to be completed by the professional involved either geneticist or neurologist/neuromuscular specialist and is available for viewing by the patient

1. When were the first presenting symptoms?
At the age of: ___ years ___ months

What were the presenting symptoms?

Weakness in upper limb

Weakness in lower limb

Proximal weakness

Distal weakness

Myalgia

Respiratory problems

Myoglobinuria

HyperCKaemia

Cramps

Stiffness

Other: _____

2. What is the result of the last pulmonary function test?
Forced vital capacity (FVC)
_____ litres
_____ %
Type

In sittting position

In lying position

Not specified

Date of the test: year-month-day

3. What was the result of the last cardiac check (ultrasound examination)?

Normal

Impaired function, no treatment

Impaired function and started treatment

Deterioration and medication changed

Not specified

Results:
Fractional shortening (FS) _____ %
Ejection fraction (EF) _____ %
Further results: _____

4. What medication are you currently on?

ACE inhibitors

Beta blockers

Steroids

Other medication: _____

5. Has a brain MRI been performed?

Yes, it showed normal results

Yes, it showed structural brain abnormalities (please specify below)

No brain MRI has been performed

Not specified

Structural brain abnormalities: _____

6. Is your cognitive function normal?

Yes

No

Not specified

7. Has a muscle MRI been performed?

Yes, it showed normal results

Yes, it showed abnormal results (please specify below)

No, a muscle MRI has not been performed

Not specified

The MRI showed abnormal results in the following muscle groups:

Biceps femoris and/or internal adductors

Rest of the hamstring muscles involved

Vastus medialis and/or lateralis muscles

Other: _____

8. Do you have contractures?

Yes (please specify below)

No

Not specified

Elbow

Wrist

Fingers

Knee

Ankle

Hip

Other joints : _____

9. Are there any other medical problems?

Yes (please specify below)

No

Not specified

Vision

Hearing

CNS

Vascular

Endocrine

Renal

Others: _____

10. What is your current 6 minute walk distance?
_____ metres
This is a validated test that is easy to perform and can be accurately and reproducibly assessed. Standard procedures for conduct of the test and for analysis of the data in adults and children have been developed (ATS 2002, Geiger 2007). Space and equipment are minimal (stop watch, hallway and traffic cones) and physiotherapists will be easily able to perform this.

11. What are your current MRC scores?

Hip flexor ___ /5

Hip extensor ___ /5

Hip adductors ___ /5

Hip abductors ___ /5

Shoulder flexion ___ /5

Shoulder abduction ___ /5

Ankle dorsiflexion ___ /5

Ankle plantar flexion ___ /5

Registration process

Registration process

Creating a user account

In the first step of the registration process, you create your user account. This means that you enter your name, date of birth and gender and choose a user name and password. You only need to create your user account once; using the user name and password you create now, you can log in again later at any time in order to continue an incomplete registration, update your clinical details and/or contact information or simply view your data at any time, whether your registration is complete or not.

Note that a parent/guardian can have one or more patients added to his/her account (e.g., siblings with FKRP can be added to the same parent's account). When creating a user account, the following situations are possible:

If you are a patient aged 18 or over, then please create a user account in your own name.

If you are a patient aged under 18, please ask your parent or guardian to create a user account under their name and add you as a patient to their account.

If you are a parent or guardian of a patient aged 18 or over, then please ask the patient to create a user account under their own name.

If you are a parent or guardian of a patient aged under 18, please create a user account under your own name and add the patient to your account.

Whenever we have any new information for a patient, we will get in touch with the owner of the respective user account.
In a later registration step we will ask not only the patient (if old enough), but also the account owner (if different from the patient) to sign the informed consent form, which is a form that asks you to confirm that you are happy to have your data stored in the registry and whether or not you want to be contacted if we have information e.g. about a clinical trial that might be relevant to you.

Entering contact details

In the next step, you as the user account owner will enter your contact details. We need your postal address, a telephone number and an e-mail address. You are encouraged to enter more than one postal/e-mail address and/or phone number; this makes it easier for us to contact you if you move house but forget to update your data.

Adding patient(s)

Now you can add patients to your user account. You can only add either yourself or a child of whom you are a parent or guardian. (If you know someone else in your family

who might benefit from being added to the registry, you might want to suggest that they register themselves, as you cannot register on their behalf.) If you are entering a patient who is not yourself, you will need to provide some further personal and contact details.

Informed Consent

Next, an informed consent form is automatically created for each patient. The consent forms are personalised, containing the name of the respective patient as well as of the user account owner (if different from the patient). You will be able to agree online. The informed consent is a PDF document, which you can download and print for your own documentation.

Filling in the questionnaire

Finally, the questionnaire appears. You can complete it directly on-screen. Where possible, automatic checks are performed to make sure your data entries make sense and do not contradict each other. For example, if you by mistake enter an impossible date of birth, you are alerted to this immediately. The questionnaire asks questions about each patient's genetic and clinical state. You can view the questionnaire in advance.

Updating your details

You can return to each registration step at any time, for instance to complete, update or simply view your/the patient's data. In this way you can also check whether we have received your informed consent form and/or genetic report. Once a year we send out a letter asking for a clinical data update but you are encouraged to inform us about major changes whenever they occur (including any change in your contact details) by logging into the registry and changing your data. We also send out a letter when a patient turns 18, asking them whether they would like to move to their own user account.

It is possible to withdraw your data from the FKRP registry at any time - please contact us if you wish this to be done.

Data protection

Data protection

Where will my data be stored?

In the questionnaire we ask you for some personal data and some information about your condition. The information that you enter will be entered into an international registry which is supervised by TREAT-NMD. Your data will be stored securely and no unauthorized people will be able to gain any information about you. When planning clinical trials, researchers can search the registry for participants eligible for the trial, based on the patients' clinical and genetic data. Only researchers who have been approved by their local ethics committee and by the TREAT-NMD governing board and ethics are allowed access to the registry.

In the registry, your data will only be identified by an anonymous code, not by your name. This means that when researchers search the registry they will not be able to find out your personal information (name, address, etc.), but only the information they need to know about your condition that will help them decide whether you might be suitable for the trial. If they think that you meet the criteria and might benefit from the trial, they will contact the person in charge of the registry. Staff working for the registry will "de-code" the data to find out the personal details and will contact you to give you information about the trial or about any other issues relevant to your condition. They will not give your name or personal information to the researchers.
If you are interested in the information that you receive about a particular clinical trial, you will be given information about how you can contact the researchers running the trial. If you decide to take part in the trial, you will need to review and sign a separate consent form. You are completely free to make your own decision about any trial we inform you about. If you decide not to take part in a particular trial, your data will still be kept in the registry and we will continue to inform you about other trials unless you tell us not to. Please note that if we tell you about the existence of a trial, this does not imply that we endorse it.

Who will have access to my medical records?

Staff in charge of the registry might need to gain access to your medical records to obtain information necessary to the project (for example we will need to ask your geneticist/physician for a copy of your genetic report and also information on your respiratory and cardiac function).

How will I be identified in the registry?

Your personal details (name, address etc.) have to be stored in the registry so that we can contact you if we need to inform you about possible clinical trials or anything else that might be relevant to your disorder.

This data will be stored in a secure manner and your records will be assigned a unique code. Your records will only be identified by this unique code. Researchers searching in the registry therefore cannot identify you personally from the information you have access to. Only the person in charge of the registry (Priv.- Doz. Dr. M. Walter M.A.) and persons explicitly appointed by her will be able to "de-code" the data to get access to your personal details.

Will my data be kept confidential?

Your data will be kept for an indefinite period at Munich, under the responsibility of Priv.- Doz. Dr. M. Walter M.A..

Creating a registry requires the existence of a file containing a patient's personal and medical data. This file will be subject to the regulations on data protection (national laws related to EU directive 95/46). All information we receive from you will be treated confidentially. The information will be encrypted and stored on a secure server.

Third parties wishing to have access to the data in the registry (such as researchers or companies planning clinical trials or conducting research on new therapies) will only have access to anonymous information identifiable by a code. Before they are granted access even to this anonymous information, they have to have permission from the ethics committee. Your data will **not be made available** to employers, government organisations, insurance companies or educational institutions, nor to your spouse, other members of your family or your doctor!

Can I withdraw and have my data erased if I change my mind?

Your participation in this project is completely voluntary. The data protection act grants you the right to access your own data and to rectify them or withdraw them completely at any time. Should you wish to withdraw your data from the registry you will be free to do so without having to provide any explanation. If you wish to withdraw, you should get in touch with the staff in charge of the registry.

Information for Doctors

Information for doctors

The global registry for patients affected with FKRP mutations collects genetic and clinical data about patients either affected with a (Limb Girdle Muscular Dystrophy) LGMD, (Congenital Muscular Dystrophy) MDC1C or any other condition caused by a mutation in the FKRP gene. For research into treatments for LGMD2I, MDC1C and other FKRP-related conditions, it is important that the researchers have precise information about the genetic mutation that causes the condition. This information will be validated by experts. In anonymous form, the valuable medical data will be available to selective researchers around the world, thereby accelerating the research into LGMD2I and MDC1C and other conditions caused by mutations involving the FKRP gene.

Additionally with the advent of clinical trials and research studies for some of the neuromuscular conditions, patient registries mean that patients who are eligible for certain clinical trials and research studies are readily identifiable. The registries contain accurate and updated information about the patients' genetic mutations and clinical conditions. Without a patient registry for these conditions it means that finding enough patients for a meaningful trial can take years to recruit and therefore delay potential therapies.

In the global FKRP patient registry this information is both provided by the patient and the professionals involved in the patient's care after full consent by the patient.

Genetics

Useful information on reporting the mutation for the registry

Most mutations in the FKRP gene are substitutions, the common mutation being c.826C>A, p.Leu276Ile, denoting a change from cytosine to adenine at position 826. There is however many other mutations detected in the FKRP gene however substitutions remain the more commonly reported abnormality.

In recording the mutation, there are a few guidelines so that all reporting is universal across the registry.

Firstly,
Identify the method used for testing:

Sequence analysis

quantitative methods [MLPA (Multiplex ligation-dependent probe amplification), Real time PCR]

RFLP (Restriction fragment length polymorphisms)

Other methods

Secondly,
Describe the mutation (see www.hgvs.org)

Indicate the level of description of mutation:
c. coding sequence
g. genomic DNA
r. RNA
p. protein: use the three-letter amino acid code

To discriminate between different levels, DNA, RNA or protein, descriptions are unique:
At **DNA** level, in capitals starting with a number referring to the first nucleotide affected (eg c.76A>T)
At **RNA** level, in lower case starting with a number referring to the first nucleotide affected (eg r.76a>c)
At **protein** level, in capitals, starting with a letter referring to the first amino acid (one letter code) affected (eg p.thr26pro)

Two sequence variations in the same allele are listed between brackets, separated by a ";" , whereas in recessive conditions when there are sequence changes in different alleles, the characters are separated by a "+".

Use the correct symbols:
">" for substitutions
Eg: c.826C>A (p.Leu276Ile) denotes a change cytosine to adenine at nucleotide

position 826.

"_" for small deletions, duplications or insertions.
Eg; c.918_929del, c.242_244del.
Eg; c.1234dupC , denotes a duplication of nucleotide C at 1234
Or c.154_158dup, p.(Val53_Arg54ins).

Insertions/deletions(indels) are described as a deletion followed by an insertion after the nucleotides affected; eg. 112_117delinsTG (alternatively 112_117delAGGT-CAinsTG or 112_117>TG denotes the replacement of nucleotides 112 to 117 (AGGTCA) by TG

"+" and "-" for intronic mutations
The current recommendations for the description of intronic mutations suggest identifying them relative to the coding DNA reference sequence (c.889+6T>G or c.1603-…) rather than relative to the intron number (IVS3+……or IVS14-.)The coding DNA reference sequence position used is either the first or last nucleotide of a given exon, as follows;

"+" beginning of the intron: the number of the last nucleotide of the preceding exon, a plus sign and the position in the intron.
"-" end of the intron. : the number of the first nucleotide of the following exon, a minus sign and the position in the intron.

At the **RNA** level, changes are basically the same as those described at the DNA level with the following modifications;
An "r" is used to indicate that this is at the RNA level
Nucleotides are designated by the bases (in the lower case); a (adenine), c (cytosine), g (guanine) and u (uracil).
When one change affects RNA processing, yielding two or more transcripts, these are described between square brackets, separated by a "," character.

In summary

Identify the technique use.

Identify the exact exons tested:

Was the entire gene directly sequenced, or screened by another technique?

Was the entire gene sequenced, or just one exon?

Collect all available data:

Mutation class (substitutions, insertion/deletion, missense)

Exon or Intron number

Nucleotide position (DNA level)

Amino acid position (protein level)

Confirm the correct mutation nomenclature using International Mutation Nomenclature.

Determine if the mutation has already been described in the literature.

Determine if the testing was conclusive.

Contact us

Contact us

If you have a question that we have not answered to your satisfaction on our information pages or if you want to give us any feedback, you can contact us directly by sending us an e-mail to the following address:

uk@fkrp-registry.org

If the question you are contacting us about concerns a particular patient, please give us the full name and date of birth of the patient.

It is also possible to contact the following e-mail-addresses according to your country or language:

australia@fkrp-registry.org

belgium@fkrp-registry.org

canada@fkrp-registry.org

catalan@fkrp-registry.org

croatia@fkrp-registry.org

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The team

The Team

Steering Committee

The committee is responsible for reviewing all requests for data from the global database. It is composed of four neuromuscular specialists plus a representative from CureCMD. Read more about the steering committee.

Operator

The global FKRP registry is operated by the Friedrich-Baur-Institut of Munich University. Read more about the operator.

The steering committee

The FKRP Registry Steering Committee

About the Global FKRP Database Steering Committee

The FKRP Global Database Steering Committee is composed of four neuromuscular specialists plus a representative from Cure CMD. The committee is chaired by Volker Straub, from TREAT-NMD.

The committee is responsible for reviewing all requests for data from the global database. This is intended to be a streamlined and rapid procedure in order not to delay approval. Requests will be discussed with the TREAT NMD Global Oversight Committee.

The members

Dr. Katherine Dianne Mathews

Katherine Dianne Mathews is Professor of Paediatrics and Neurology at the University of Iowa. She has served as Director of the Division of Pediatric Neurology since 2001 (expanded to the division of Neurology, Behavior and Development in 2008). She was involved in the early efforts to map the gene for FSHD, and was instrumental in setting up FSHD genetic testing at the University of Iowa. Her research activities are focused on clinical aspects of muscular dystrophies, with the goal of improving outcomes. Dr. Mathews runs an active clinical service, and has been director of the MDA clinic for the past 14 years. She is on the MDA Medical Advisory Committee and the FSH Society's Scientific Advisory Board.

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Profile Dr. Katherine Dianne Mathews

Dr. Anne Rutkowski

Dr. Anne Rutkowski, MD is co-founder and Chairman of Cure CMD. Dr. Rutkowski is a practicing board certified emergency medicine physician in Los Angeles. Dr. Rutkowski's daughter has congenital muscular dystrophy, subtype, dystroglycanopathy. Dr. Rutkowski graduated from the University of California Irvine Medical School, elected to Alpha Omega Alpha Honor Medical Society. She attended Bryn Mawr College as an undergraduate, graduating Magna cum laude with Honors in Biology. Prior to attending medical school, Dr. Rutkowski taught for 3 years in an inner city elementary school in Los Angeles, as part of Teach for America. She would like to shrink the diagnostic odyssey, see improvements and standardization of guidelines in medical care for all patients with CMD and a focused approach to identifying therapeutic targets. As a former educator, education and improved disease awareness are two further areas of focus for Cure CMD.

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Profile Dr. Anne Rutkowski

Dr. Volker Straub

Professor Volker Straub is joint co-ordinator of TREAT-NMD, executive board member of the World Muscle Society and executive board member of the Institute of

Human Genetics at Newcastle University. Together with Hanns Lochmüller, Volker was responsible for setting up the German muscular dystrophy network, MD-NET, of which he was joint coordinator until 2008. Within the neuromuscular research group at Newcastle, Volker has a long-standing interest in the pathogenesis of muscular dystrophies, with research using zebrafish and mouse models. His current research also involves the application of contrast enhanced MRI.

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Dr. John Vissing

John Vissing is Professor of Neurology at the University of Copenhagen, Denmark. John has been director of the Neuromuscular clinic and research unit since 2000. His main research interest is in exercise and the effect of training in muscle conditions such as Becker Muscular dystrophy and Limb Girdle Muscular dystrophy 2I. His other main interest is metabolic muscle disease such as McArdle disease and mitochondrial myopathies.

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Profile Dr. John Vissing

Dr. Maggie C. Walter

Maggie Walter is Assistant Professor of Neurology at the Ludwig-Maximilians-University of Munich. She has trained as a neurologist at the LMU Munich, and is working at the Friedrich-Baur-Institute, the neuromuscular department of the LMU, in leading position. Furthermore, she graduated with a master degree in management of social and health institutions.

Her main research interest are neuromuscular diseases, mainly muscular dystrophies, myofibrillar myopathies, inflammatory myopathies and clinical trials in neuromuscular patients. She is coordinator of the German Muscular Dystrophy Network (MD-NET), funded by the Federal Ministry of Education and Research (BMBF) since 2003, and member of TREAT-NMD, an European Network of Excellence in the 6th EU frame program for translational research in neuromuscular diseases. Since 1997, she is member of the Scientific Advisory Board of the Muscular Dystrophy Association of Germany (DGM), and ad hoc reviewer for several peer-reviewed journals.

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Profile Dr. Maggie C. Walter

Operator

Operator

This registry is operated by the Friedrich-Baur-Institut, Klinikum der Universität München (University of Munich hospital).

Head of the FKRP registry

Priv.-Doz. Dr. med. Maggie C. Walter M.A.

Maggie Walter is Assistant Professor of Neurology at the Ludwig-Maximilians-University of Munich. She is working at the Friedrich-Baur-Institute, the neuromuscular department of the LMU, in leading position. Her main research interests are neuromuscular diseases, mainly muscular dystrophies, myofibrillar myopathies, inflammatory myopathies and clinical trials in neuromuscular patients. She is coordinator of the German Muscular Dystrophy Network (MD-NET), funded by the Federal Ministry of Education and Research (BMBF) since 2003, and member of TREAT-NMD, an European Network of Excellence in the 6th EU frame program for translational research in neuromuscular diseases. Since 1997, she is member of the Scientific Advisory Board of the Muscular Dystrophy Association of Germany (DGM), and ad hoc reviewer for several peer-reviewed journals.
Profile Priv.-Doz. Dr. med. Maggie C. Walter M.A.

Prof. Volker Straub

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Profile Prof. Dr. med. Volker Straub

Medical contact person

Dr. Olivia Schreiber

Olivia Schreiber is currently working as MD at the Friedrich-Baur-Institute, the Neuromuscular Department of the Ludwig-Maximilians-Universtity of Munich, where she specializes in the field of neurology and neuromuscular diseases. Her research interest focuses on inherited neuromuscular disorders and clinical trials in order to improve diagnosis, health care and therapeutic options in patients with neuromuscular diseases. Within the European Network TREAT-NMD and the German Network MD-NET she is concerned with the patient registries for Duchenne Muscular Dystrophy, Spinal Muscular Atrophy as well as the FKRP registry.
Profile Dr. Olivia Schreiber

Dr Tracey Willis

Dr Tracey Willis is a Paediatric Neurologist with an interest in neuromuscular diseases. She completed her paediatric neurology training in Birmingham, UK and since then has worked as a paediatric neurologist in Birmingham, UK and Auckland, New Zealand. In 2008 she became the Muscular Dystrophy Campaign (MDC) fellow at the Institute of Genetic Medicine in Newcastle with Professor Straub and is studying for her doctorate which has involved coordinating a multicentre MRI/MRS study in LGMD2I. At the MRC Centre for Neuromuscular Diseases in Newcastle, which is the national referral centre for patients with limb girdle muscular dystrophy, she is involved in the diagnosis and care of a broad spectrum of patients with neuromuscular diseases.
Profile Dr Tracey Willis

Curator

Simone Thiele

Simone Thiele is a member of the European Network TREAT-NMD as well as the German Network MD-NET and Curator of the FKRP registry. Besides, she is involved with the patient registries for Duchenne Muscular Dystrophy and Spinal Muscular Atrophy which are organised by the Friedrich-Baur-Institute in Munich. Her other focus lies on the coordination of clinical trials.
Profile Simone Thiele

IT

Marcel Kiel

Marcel Kiel is the software engineer for this and the other patient registries for rare neuromuscular diseases which are organised by the Friedrich-Baur-Institute in Munich. Please contact him if you have any technical questions or interest in this software.
Profile Marcel Kiel

Registration

Registration

Please complete this form and click on the "Continue" button to start the registration.

If you have already registered and would like to update your data or add another patient, you don't have to fill in this form again. Just go to the login page and login with the e-mail address and password you previously registered with.

Personal data

Please enter your own details here, even if you are not the patient yourself. If you are registering a child as a patient, you will be able to enter his or her details in a later step. Please note that you must be the patient's parent or guardian to enter a patient other than yourself.

First name(s)

Surname

Date of birth as year-month-day, e.g. 1967-03-19

Sex

Male

Female

User account

With your e-mail address and the password you choose here, you can login at any time to view or edit your data. In order to protect your personal data against unauthorised access, please choose a password which is hard to guess and write it down in a safe place. Note that the password is case-sensitive. Your password must be at least 6 characters long.

E-mail address e.g. joe_bloggs@example.com

Password

Repeat Password

Continue Cancel

Login

Login

Please enter your e-mail address and the password you chose when you registered. If you haven't got a user account yet, you can create one on the registration page.

If you have forgotten your password, please see the page Forgot password.

Email address

Password

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Forgot password

If you have forgotten your password, please enter the e-mail address with which you registered. You will then receive an e-mail containing a link with which you can reset your password.

Email address

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Legal notice

Legal notice

Operator

The global FKRP Registry, accessible on the web at www.fkrp-registry.org, is operated by the Friedrich-Baur-Institut, Klinikum der Universität München.

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Webdesign, application design and illustrations by Ricarda Kiel / Butter&Fische Webdesign

Icons by Mark James / famfamfam

Disclaimer

All the information and advice on the global FKRP Registry website has been assembled to the best of our knowledge. However, in any individual case consultation with the attending doctor or other qualified health professional is strongly recommended. The registry website contents are for informational purposes only and not intended to be a substitute for independent professional medical advice, diagnosis or treatment. All users of the registry website are responsible for their own medical care, treatment and oversight.

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