Developing markers of neurological manifestations in Neuronopathic Gaucher Disease

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I, Elin Haf Davies, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

I dedicate this thesis to my parents for all their unconditional love and support in every aspect of my life. Diolch diffuant am bopeth.

And to make an end is to make a beginning,

The end is where we start from …

And the end of all our exploring

Will be to arrive where we started,

And to know the place for the first time.

T.S Elliot

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Statement of work and acknowledgment of others

Severity Scoring Tool

I had sole responsibility for the conception, development, validation and statistical analysis of the data collated while developing the Severity Scoring Tool. I conducted the assessment either individually or in collaboration with another clinician in at least 80% across all three centres. I was also responsible for identifying the world wide experts that collaborated to provide their expert opinion on the weighting of each domain and the identification of the Minimum Clinically Important Difference. Both of which were done using web-based nominal group technique, organised by myself.

Gait analysis

After training in the use of the equipment and analysis process by Dr Lucy Alderson and Michelle Wood, the recruitment of patients, assessment, analysis and interpretation of the data was all done by myself.

Magnetic Resonance Imaging

My involvement on this aspect of the study was to recruit and consent patients. Dr Kiran Seunarine processed the imaging analysis and we then collaborated to identify relevance in relation to pathology and clinical manifestations.

Each manuscript that has emerged from this work was written in the first place by myself, and revised according to comments from the co-authors in each case.

Abstract

Gaucher disease is a rare inherited lysosomal disorder caused by deficiency of the enzyme glucocerebrosidase. Classically, three forms of the disease are recognised: type I or nonneuronopathic, type 2 or acute neuronopathic, and type 3 or subacute or chronic neuronopathic. Neuronopathic Gaucher disease (NGD) is defined as a confirmed diagnosis of Gaucher disease in the presence of neurological symptoms and signs, for which there is no other cause. Horizontal gaze palsy is the clinical hallmark of NGD. Other neurological manifestations include seizures, cerebellar ataxia and pyramidal tract involvements. However, NGD is very heterogeneous and the neurological features vary greatly from patient to patient, not only in terms of manifestations involved but also in terms of severity. The emergence of enzyme replacement therapy has changed the *'natural history'* of the disease, and patients are now living longer where previously they would have succumbed to the visceral complications of the disease. New emerging therapies are being developed for NGD, however a suitable surrogate marker to monitor neurological disease is lacking.

In this study, three different assessment tools were explored to examine their value and sensitivity to assess neurological involvement in NGD. A Severity Scoring Tool developed specifically for NGD was modified and validated to offer a robust assessment tool, with demonstrated sensitivity to track disease progression and distinguish between phenotypes. Additional assessments utilised were gait analysis and diffusion tensor imaging, both of which were sensitive enough to distinguish between the NGD and Type I cohort studied. This is the largest cohort of NGD patients (recruited across three European countries) to be studied prospectively and systematically. It is also the first study to describe the gait pattern of NGD children, and to provide an *in-vivo* insight of the Gaucher brain utilising diffusion tensor imaging.

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Abbreviations

BOS – Base of Support

CNS - Central Nervous System

DTI – Diffusion Tensor Imaging

ERT – Enzyme Replacement Therapy

EMA – European Medicine Agency

FDA - Food and Drug Agency

FA – Fractional Analysis

FPSS - Four-Point Scoring System

GAG - Glycosaminoglycan

GOSH – Great Ormond Street Hospital

HGP – Horizontal Gaze Palsy

ICARS – International Cooperative Ataxia Rating Scale

ICH - International Conference on Harmonisation

LINCL - Late infantile neuronal ceroid lipofuscinosis

LMS – Skewness, Median, Coefficient of Variation

LSD – Lysosomal Stroage Disorder

MD – Mean Diffusivity

MCID – Minimum Clinically Important Difference

MPS – Mucopolysaccharidosis

MRI – Magnetic Resonance Imaging

mSST – modified Severity Scoring Tool

NGD – Neuronopathic Gaucher Disease

SPM – Statistical Parametric Map

SRT – Substrate Reduction Therapy

SST – Severity Scoring Tool

TBSS – Tract-Based Spatial Statistics

VBM – Voxel Based Morphometric

6MWT – Six Minute Walk Test

Oral presentations

Davies, E.H., Seunarine, K., Clark, C., Vellodi, A. Diffusion Tensor Imaging: Study of white matter brain in paediatric patients with Gaucher Disease.
7th Annual WORLD Symposium for Lysosomal Diseases, February 16-18 2011, Las Vegas, USA.

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Proposal for the organization and structure of the EMEA Network (2009)
Davies, EH. 1st EMEA European Paediatric Network, London. UK 16th February

Quantifying neurology in Neuronpathic Gaucher Disease. **Davies, EH**
Neuronopathic Gaucher Disease, European Family Conference. Leicester
UK. 29th November 2008

How did the EMEA prepare for Paediatric Regulation (EC) No 1901/2006.
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Chapter 1

Introduction

Research is one percent inspiration and 99 percent perspiration

1 Introduction

1.1 Lysosomal Storage Disorders

Lysosomal Storage Disorders (LSDs) are individually rare but cumulatively have an incidence of 1:7700 (Meikle *et al.* 1999a; Meikle *et al.* 1999b). Each disorder results from the defective function of a specific hydrolase, which ultimately leads to progressive accumulation of either undegraded substrate(s) or catabolic products that are unable to escape from this organelle. Most, but not all, are inherited in an autosomal recessive manner. Defects in lysosomal enzymes, cofactors or transport proteins may all give rise to LSDs and these can be classed conveniently according to the type(s) of storage material accumulating (*sphingolipidoses, oligosacharidoses, mucopolysaccharidoses, glycogenoses,* and *neuronal ceroid lipofuscinoses*). The severity of the phenotype is closely related to the residual enzyme activity. For practically all LSDs, the gene has been cloned and disease mutations identified (Lyon *et al.* 2006).

The phenotypic spectrum of LSD manifestations can generally be divided into three groups as follows:

1. Central nervous involvement with visceral manifestations
2. Central nervous involvement without visceral manifestations
3. Visceral manifestations only

The first group account for multisystem diseases where both visceral and central nervous systems are involved (e.g Gaucher Type III, some forms of Mucopolysaccharidosis (MPS) I and II). The second group is more commonly associated with the infantile forms of diseases, where neurological involvement

presents early in life, and is often progressive and unrelenting, with early death (e.g Tay-Sachs and infantile Krabbe). The third scenario appears when systemic disease progresses but the central nervous system remains intact (e.g MPS IV A, MPS VI). Lyon and colleagues categorised LSDs by their age of symptom onset and called attention to their phenotypic expression to offer a practical means of directing evaluation and diagnosis of patients (Lyon *et al.* 2006). The rate of progression, both somatic and neurological, is heterogeneous – not only across disorders but within each disease.

There are between forty and fifty different known LSDs; the majority involve the central nervous system (Wraith 2002; Vellodi 2005). A review of the literature identified at least twenty five that do so. This equates to around 50-60% of LSDs having neurological involvement. A wide range of neurological phenotypes have been reported. These include progressive psychomotor retardation, seizures and a number of neurological abnormalities, in both the central and peripheral system, sensorineural defects and psychiatric symptoms (Lyon *et al.* 2006). It is not fully understood how an accumulation of substrates accumulating in brain tissues gradually and irreparably damages nerve cells.

1.2 Gaucher Disease

Gaucher disease is an inherited lysosomal disorder caused by deficiency of the enzyme glucocerebrosidase, which is necessary for the catabolism of glucocerebroside. It is a multi-system disease that was first described by the French

physician Philippe Gaucher in 1882. Exactly a century later the gene for glucocerebrosidase (GBA) was first localised to 1q21 (Barneveld *et al.* 1983).

1.2.1 Classification of Gaucher disease

Classically, three forms of the disease are recognised: type I or nonneuronopathic, type 2 or acute neuronopathic, and type 3 or subacute or chronic neuronopathic (Fredrickson & Sloan 1972). Type I or nonneuronopathic Gaucher disease is diagnosed in childhood in 66% of Type I patients diagnosed, and an earlier onset is indicative of more severe visceral disease. Paediatric presentation is characterised by growth failure, hepatosplenomegaly, anaemia, skeletal involvement (Weinreb *et al.* 2002).

Neuronopathic Gaucher disease (NGD) is defined as a confirmed diagnosis of Gaucher disease in the presence of neurological symptoms and signs, for which there is no other cause (Schiffmann & Vellodi 2007). Primary neurological involvement in Gaucher disease was first described in the early 20th century (Rusca 1921). Historically NGD was sub-divided into ‘type 2’ and ‘type 3’ based on the severity of the symptoms, and the rate of progression (Fredrickson & Sloan 1972). However our group proposed that the terms ‘type 2’ and ‘type 3’ be dropped as they fail to take account of the spectrum of NGD phenotypes. We proposed instead that the terms ‘acute’ and ‘chronic’ NGD be used; these are now incorporated in the revised guidelines for the management of NGD (Vellodi *et al.* 2009).

‘Acute NGD’ refers to the onset at ≤1 year of age of progressive bulbar involvement (stridor, squint, swallowing difficulty) with pyramidal involvement (opisthotonus, head retroflexion, spasticity, trismus) with death by the age of 2-4 years. ‘Chronic NGD’ refers to all patients with NGD who do not have ‘acute NGD’. Historically chronic NGD has been subdivided in types A, B and C. However, our group felt that this subdivision of chronic NGD was artificial and should be dropped, as the clinical spectrum is too heterogeneous, and patients with intermediate severity have been described (Goker-Alpan *et al.* 2003). By using the terms ‘acute’ and ‘chronic’ NGD as classification will allow for the necessary flexibility to accommodate them.

1.2.2 Epidemiology

Gaucher disease is the most prevalent LSD, with a particularly high prevalence in the Ashkenazi Jewish population. In most Caucasian populations, including Ashkenazi Jews, type I is clearly the most prevalent. NGD is panethnic. However a founder effect has been described in certain populations (Dreborg *et al.* 1980; Tylki-Szymanska *et al.* 1996). Although no systematic study exists, it is estimated that about 6% of Gaucher patients have NGD, 5% have the chronic form and 1% have the acute form of the disease (Charrow *et al.* 2000). The neuronopathic forms are collectively the rarest variant with an estimated incidence of <1:100,000 live births.

The first two mutations described in GBA - c.1448T>C (L444P) and c.1226A>G (N370S), were identified in the late 1980s and these alleles are also the most prevalent. Nomenclature mutations were recently changed however. Traditionally mutations in the GBA gene were referred to by one letter amino acid codes with

amino acid number 1 being the first of the processed protein. According to the Human Genome Variation Society (HGVS) www.hgvs.org/mutnomen guidelines, protein numbering with amino acid 1 is now the initiating methionine of the precursor protein. L444P protein nomenclature is now therefore p.Leu483Pro (exon 11) and N370S is p.Asn409Ser (exon 10). For purpose of this thesis, however, only the original nomenclature will be used.

There is poor genotype-phenotype correlation in Gaucher disease, and this has been complicated further by the ever-expanding phenotypic spectrum observed. To confound the issue, phenotypically similar patients have many different genotypes, even in unique subgroups of patients (Hruska *et al.* 2007), while individuals with the same genotype can have different phenotypes (Sidransky 2004). However some genotype-phenotype correlations have been made. The N370S (c.1226A>G) allele is typically associated with the non-neuronopathic form of the disease. Individuals homozygous for L444P (c.1448T>C) or F213I (c.754T>A) usually develop chronic NGD, but either mutation with a null allele is more likely to be associated with the acute phenotype. Homozygous mutation D409H (1342G>C Protein nomenclature p.Asp448His (exon 10)) is a phenotype that is associated with aortic valve calcification and only mild neurological disease (Abrahamov *et al.* 1995; Chabas *et al.* 1995; Chabas *et al.* 1996; Abrahamov *et al.* 2000; Bohlega *et al.* 2000).

1.2.3 Neuropathology

The neuropathological hallmark of Gaucher disease and one of the most consistently identified pathologic features is the perivascular and periadvential

accumulation of lipid-laden macrophages, called Gaucher cells (Lee 1982; Wong *et al.* 2004; Wong 2007). This has been reportd in all forms of Gaucher disease. However, acute and chronic NGD have certain features. An extracortical, discernible loss of neurons, sometimes associated with crumpled, shrunken-atrophic neurons has been reported involving the basal ganglia, nuclei of the midbrain, pons and medulla, cerebellum, dentate nucleus and hypothalamus. A severe neuronal loss and degeneration of pyramidal cell neurons of the hippocampus has also been described (Wong *et al.* 2004).

The main cell types affected are astrocytes and neurons, with the most common astrocytic change being perivascular astrogliosis of grey and white matter. In Gaucher disease *of all types* perivascular gliosis is present in white matter centrum ovale, white matter tracts and interspersed grey matter of the striatum, cerebellar white matter, and the brainstem interlaced with white matter tracts and interconnected brainstem nuclei (Wong 2007).

White and grey matter are therefore both affected. However, as highlighted in Chapter 4, the techniques available to investigate both white and grey matter *in vivo* are not as sophisticated.

Although by definition Type I Gaucher disease does not involve the brain, there are reports of neuropathological CNS involvement in patients who would otherwise be considered to have Type I Gaucher – these are patients who are asymptomatic until well into adulthood and the pathological CNS findings are much milder (Wong *et al.*

2004). Parkinsonian symptoms in patients diagnosed as having Type I Gaucher disease have been noted (Lee 1982). Clinical and pathologic Parkinsonism and dementia with Gaucher disease of all types have also been linked (Sidransky 2004; Wong *et al.* 2004; Sidransky 2005).

1.2.4 Diagnosis

The diagnosis of NGD is purely clinical. The clinical hallmark of NGD is an abnormality of horizontal gaze. This has been mistakenly described as oculomotor apraxia, but should more accurately be called supranuclear saccadic gaze palsy. Often this is the sole feature for many years. Furthermore, it can be difficult to detect clinically, especially in infancy. It is usually be observed when the child turns around while walking or when reading is associated with horizontal head jerks that represent an attempt to compensate for the saccadic deficit. Older children learn to compensate for their poor saccades by a combination of synkinetic blinking, looping and head thrusting. Vertical saccades may be affected as well, though always later. NGD should be suspected with early onset of disease, aggressive visceral disease or high-risk genotype (Schiffmann & Vellodi 2007).

1.2.5 Neurological features of Chronic Neuronopathic Gaucher Disease

Most patients with chronic NGD present in the first 5 years of life (Altarescu *et al.* 2001). Patients often present not with a neurological abnormality, but with hepatospenomegaly, anemia or failure to thrive. Historically, the severity of visceral involvement has overshadowed the neurological manifestations of chronic NGD, and the early demise from visceral complications has masked the “neurological natural history”. The first comprehensive account of the neurological manifestations

seen in the Norrbottnian cohort, typical of the pre-ERT era, was reported thirty years ago. The manifestations reported were convergent squint/ sixth nerve palsy; ataxic gait; low IQ; Cerebellar, Pyramidal and Extrapyramidal involvement; delayed gross and fine motor skills; seizures (including progressive myoclonus epilepsy) and dementia with a median age of death at 12 years of age (Dreborg *et al.* 1980).

The rarity of NGD renders large-scale, systematic studies difficult (Jardim *et al.* 2010). Most clinical reports are of small numbers of patients (maximum 22) from single ethnic or geographic locations (Dreborg *et al.* 1980; Tylki-Szymanska *et al.* 2006). Publications by our group, as part of a European collaboration across four countries was the largest cohort of NGD patients ever studied uniformly, and presented 52 patients (Davies *et al.* 2007a; Davies *et al.* 2007b).

The demographic and clinical features of NGD patients enrolled in an International Collaborative Gaucher Group Neurological Outcomes Subregistry were recently published (Tylki-Szymanska *et al.* 2010). This provides a great insight to the clinical presentation of patients in the ERT era despite the limitations associated with retrospective registries which are voluntary and observational, in particular missing data. Given that large prospective studies on the *natural history* are hampered by the rarity of these patients, registry data is valuable. Twenty three different neurological symptoms for 131 patients were reported. The neurological manifestation with the highest reported percentage is the ‘Ability to look to the extreme right or left’, at 71%. The heterogeneity of manifestations in NGD is also reflected in this cohort, as only three of the manifestations reported occurred in more

than 50% of the patients - Ability to look to the extreme right or left; 71%. Head movement rather than eye movement; 63% and Head thrusting; 55%. Head movement and head thrusting which are secondary manifestations to, horizontal gaze palsy (HGP) the clinical hallmark of the disease. Other cranial nerve characteristics reported were dysarthria (22%), swallowing difficulties (20%), chewing difficulties (11%) and stridor (11%). The most frequently reported motor abnormalities were muscle weakness (25%), extrapyramidal features (18%), spasticity (15%), intention tremor (24%), and tremor at rest (16%). Sixteen percent had suffered a seizure at the time their data was entered into the registry for the first time, with the median age of first seizure being 5.6 years. One limitation of this registry data however is that most symptoms are only accounted for as, present: yes or no. Defining a level of severity for patients was not attempted.

1.2.6 Treatment options

The emergence of enzyme replacement therapy (ERT) as a therapeutic option changed the outlook for Gaucher disease. Brady and colleagues developed the first purification method for human beta glucosidase (Furbish *et al.* 1977). Subsequently beta glucosidase from human placentae (alglucerase, Ceredase) was developed, and followed later by the recombinant form (imiglucerase) (Barton *et al.* 1990). ERT is now regarded as the treatment of choice for the visceral manifestations of Gaucher disease. It has completely modifying the clinical phenotype.

In the pre ERT era, splenomegaly was debilitating, particularly when respiratory function was compromised. Clinical management options at the time were limited to

splenectomy. However, splenectomy has been shown to result in increased skeletal and CNS morbidity (Svennerholm *et al.* 1991; Mistry *et al.* 2009).

Neurological outcomes in NGD patients on ERT have only been reported on in open label studies. The lack of control groups has prevented the results from being clearly conclusive, or to help understand the natural history (Aoki *et al.* 2001; Lonser *et al.* 2007; Davies *et al.* 2007a). Even with gaps in knowledge, it is estimated that life span of NGD patients has changed from the former 12 years of age (Dreborg *et al.* 1980) to the third or fourth decade after the advent of ERT (Jardim *et al.* 2010).

Bone marrow transplantation (BMT) was the treatment of choice before the efficacy and safety of imiglucerase was demonstrated. Successful engraftment reversed the manifestations of visceral disease, which are caused by enzyme-deficient macrophages. However BMT does not appear to have reversed neurological deficit or prevent continued deterioration (Schiffmann & Vellodi 2007). It is also not without risk, and requires a total splenectomy beforehand. In the event of graft rejection the patient is left asplenic and consequently at risk of rapidly progressive neurological deterioration (Erikson *et al.* 1990; Svennerholm *et al.* 1991). The clinical course of patients post-BMT is similar to the one commonly observed in NGD patients on ERT (Erikson *et al.* 1990; Altarescu *et al.* 2001).

Despite demonstration that ERT is associated with reduction of perivascular lipid-laden macrophages (Gaucher cells) in the brain (Schiffman *et al.* 1997). ERT does not seem to have any effect in patients with myoclonic seizures, supranuclear gaze

palsy or cognitive deficit (Altarescu *et al.* 2001). Whilst it was initially thought that NGD patients benefited from high dose ERT (Vellodi *et al.* 2001) there is now an increasing consensus that ERT has no measurable effect on the neurological manifestations of NGD patients, regardless of dose. To this end, it is now recommended that NGD patients are treated with doses sufficient to control the somatic manifestations (Vellodi *et al.* 2009).

The major obstacle thus far to halting the neurological manifestations is the blood-brain barrier, and the inability of the intravenously infused glucocerebrosidase to cross it in appreciable amounts (Schiffman *et al.* 1997). To overcome this limitation, an attempt has been made to directly infuse glucocerebrosidase into the brain of a patient with Type 2, acute NGD patient using convection-enhanced delivery (Migita *et al.* 2003; Lonser *et al.* 2007). Targeted perfusion of affected sites in the brain was achieved and well tolerated by the patient, without any evidence of toxicity. On this basis, Lonser *et al* (2007) concluded that convection-enhanced delivery may be a treatment paradigm that could be of benefit in the future. However, this approach presents significant challenges.

Substrate Reduction Therapy (SRT) is another treatment modality. Unlike ERT, SRT does not need to be disease specific. In this approach the enzymatic synthesis of glucocerebroside is blocked by an inhibitor of ceramide: UDP-glucoslytransferse. One of the principal substances employed in this regard is *N*-butyldeoxynojirimycin *N*B-DNJ. *N*B-DNJ is registered and marketed for Gaucher Type I disease as miglustat (Zavesca) (Cox *et al.* 2003).

Preliminary results indicated that SRT miglustat penetrated the blood-brain barrier, with cerebrospinal fluid concentrating reaching 20-40% of those achieved therapeutically in the plasma (Platt & Cox 2007). This led to a phase II, randomised, multicenter, open-label study recruiting 30 NGD patients. However, this study failed to demonstrate a measurable benefit for miglustat (Schiffmann *et al.* 2008).

Another future direction which holds hope for NGD patients is gene therapy. There has been extensive laboratory and limited clinical experience with glucocerebrosidase gene transfer by various techniques into animal and human hematopoietic stem cell. In the early 1990s, work by at least three groups demonstrated: 1) expression of human glucocerebrosidase following retroviral vector-mediated transduction of murine hematopoietic stem cells, 2) retroviral-mediated transfer of the human glucocerebrosidase gene into cultured Gaucher bone marrow, 3) transfer and sustained expression of the human glucocerebrosidase gene in mice and their macrophages after transplantation of retrovirally transduced bone marrow, and 4) high titer amphotrophic vectors containing the glucocerebrosidase gene capable of transducing at high efficiency (Peters & Krivit 2007).

Further laboratory work led to the first clinical trials of gene transfer in patients with Type I Gaucher disease (Barranger *et al.* 1997). While there was great promise initially, sustained and significant clinical benefit was not observed. Consequently

investigators redirected their focus to improving methods for gene transfer, in particular the use of viral vectors, particularly retroviral ones (Peters & Krivit 2007).

The history of treatment of Gaucher disease started with splenectomy, continued with BMT, and more recently focused primarily on ERT. Despite these major therapeutic advances, many questions and clinical management challenges remain (Erikson 2001). The cause of neurologic signs and how best to treat the CNS being one of the most complex. Combination therapeutic agents and modalities tailored to the patient's clinical condition and type of Gaucher may soon be warranted until new potential therapies on the horizon are a step closer. A parallel need to identify appropriate means of monitoring therapeutic interventions has also become evident.

1.2.7 Clinical monitoring

Clinical monitoring of NGD varies across centres, despite guidelines which were recently revised and published (Vellodi *et al.* 2009). The minimum specified clinical protocol for initial assessment include clinical neurological examination, neuro-opthalmological inestigation and peripheral hearing: Brain imaging (preferably magnetic resonance imaging (MRI), or computed tomography (CT) if MRI is unavailable): Neurophysiology and Neuropsychomtery (Vellodi *et al.* 2009). However due to age and cultural difference, and economic constraints it has not been possible to develop a standardised format for these assessments, and their sensitivity in demonstrating disease progression or improvement in response to new potential therapy is unknown.

As HGP is a clinical hallmark of NGD neuroopthalmologic evaluation is commonplace. Garbutt and Harris (2000) reported vertical eye movement involvement in NGD, and postulated that it could be used as a sign of progression of disease (Garbutt & Harris 2000). However the technical and practical difficulties in measuring saccades to detect change were made evident in a recent study in which our group participated (Schiffmann *et al.* 2008). This study highlighted the inherent problems with complex assessment for the paediatric population. Compliance with this assessment was not only difficult for the young, but also for those who had adopted blinking as a compensatory mechanism to deal with HGP. Furthermore the real value of saccades in reflecting clinical status, and its correlation with disease severity has not been established. It contributes little to determining clinical care.

In such a heterogeneous disease, identifying a common clinical manifestation with the potential for clinical monitoring is difficult. Encompassing all the neurological manifestations into one score has advantages. This was the rationale for development of a Severity Scoring Tool (SST) specifically for assessing and monitoring the neurological manifestations of NGD (Davies *et al.* 2007b).

However, exploration of individual clinical features as acceptable markers of the disease was also necessary. Selection of a feature that lends itself to be measured as a marker was challenging, and in particular selecting a manifestation that was present in a large enough incidence. In a study of 15 patients assessed at this centre identified ataxia (12/15) and pyramidal involvement (13/15) as the most common presenting feature after HGP (Davies *et al.* 2007b).

Table 1.1: Ataxic gait and pyramidal involvement as measured in two of the SST domains during assessment of 52 NGD patients

		Pyramidal			
		Normal tone with increased reflexes	Mildly to moderately increased tone and reflexes	Increased tone reflexes with sustained/ unsustained clonus	**Total**
Ataxia / Gait	Normal/ apparent only on tandem walking	22 (42.3%)	5 (9.6%)	1 (1.9%)	28 (53.8%)
	Ataxia on straight gait, able to walk without assistance	4 (7.7%)	7 (13.4%)	11 (21.1%)	22 (42.3%)
	Able to walk only with assistance	0 (0%)	1 (1.9%)	1 (1.9%)	2 (3.8%)
	Total	26(%)	13(%)	13(%)	52 (100%)

The presence of ataxia and pyramidal involvement was also identified to be common place in a cohort of 52 patients assessed during development of the SST, (Table 1.1) where a total of 38.3% presented with varying severity of both.

In the Neurological Outcomes Subregistry (Tylki-Szymanska *et al.* 2010), the reported incidence of 'walking ability requiring assistance/ non-ambulatory' is 15%, with 23% presenting with a 'wide base gait'. Combined, this is the commonest reported manifestation. Other frequently reported motor abnormalities were muscle weakness (25%) followed by intention tremor (24%), extrapyramidal features (18%) and spasticity (15%). All of these manifestations are likely to have an impact on gait. This suggests that measurement of gait is likely to reflect disease severity.

As previously highlighted, in such a heterogeneous disease, identifying a clinical manifestation that is present in all of the patients is difficult. Especially as the use of HGP, the disease hallmark has been identified to be unsuitable as a marker for monitoring disease progression. Encompassing all the neurological manifestations in one score, as proposed in the SST has clear advantages. However other manifestations which have an obvious impact on overall patient function warrant further exploration. Based on the percentage of patients presenting with manifestations that impact on gait (e.g ataxia, muscle weakness, pyramidal) it is hypothesised that gait could be a useful marker of neurology in NGD, and worthy of exploration.

1.3 Clinical trials qualifying for Marketing Authorisation Lysosomal Storage Disorders

Following the Orphan Drug regulation ((EC) No 141/2000), a number of therapeutic options have emerged for LSD over the last decade and more. Gaucher disease paved the way with enzyme replacement therapy (ERT) alglucerase (Ceredase) which was later replaced by imiglucerase (Cerezyme).

There are now eight ERT's and one Substrate Reduction Therapy (SRT) licensed centrally by the European community marketing authorisations: imiglucerase, velaglucerase alfa, agalsidase beta, agalsidase alfa, alglucosidase alfa, laronidase, idursulfase, galsulfase and miglustat for seven different LSDs, Gaucher, Fabry, Pompe, MPS I, MPS II, MPS VI and NP-C.

The selection of clinical trial endpoints that meet the requirements of the regulatory bodies is fraught with difficulties. Primary efficacy endpoints used in clinical trials for the management of Gaucher disease with imiglucerase (Cerezyme) for Gaucher disease included an increase in haemoglobin and platelet count and decrease in liver and spleen volume. These parameters are robust and validated markers which have clear correlation to clinical function.

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000157/human_med_000693.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d125

Studies of agalsidase beta (Fabrazyme) for Fabry disease showed that agalsidase beta could be administered safely and that it cleared glycosphingolipids accumulated in the vascular endothelium in all organs studied. The primary efficacy endpoint was a reduction of GL-3 accumulation from the capillary endothelium of the kidney, measured by three pathologists using a scale of 0-3. Secondary efficacy parameters included a reduction in pain as assessed by the Short Form McGill Pain Questionnaire.

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000370/human_med_000784.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d125

Studies of agalsidase alfa (Replagal) also for Fabry disease selected serious debilitating pain, as measured by the brief pain inventory (a quantitative, validated pain assessment scale) as the primary endpoint. The primary endpoint in the

second study was the effect of ERT on cardiac Gb3 levels as determined from cardiac biopsy samples.

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000369/human_med_001029.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d125

Efficacy of alglucosidase alfa (Myozyme) in the treatment of Pompe disease was studied in two main clinical studies recruiting 39 patients. An epidemiological study of the natural history of infantile Pompe examined the percentage of patients alive and ventilator-free at 12 months from birth, from the date of onset of first symptoms, and from the diagnosis date. “Percentage of patients alive and free of invasive ventilator support (endotracheal tube) at 12 months of age” was compared to a comparable historical untreated cohort derived from an epidemiological study. Other efficacy endpoints included 1) percentage of patients alive at 12 months of age, 2) percentage of patients with signs and symptoms of cardiac failure, 3) assessment of motor function by Alberta Infant Motor Scale (AIMS) from birth to the age of independent walking, 4) assessment of cognitive function by BSID II – modified Bayley Scales of Infant Development in children from birth to 42 months of age and 5) assessment of functional status and disability by Paediatric Evaluation of Disability Inventory (PEDI) and Pompe PEDI.

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000636/human_med_000917.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d125

The primary efficacy variables of laronidase (Aldurazyme) in patients with MPS I, were percent of predicted forced vital capacity (FVC) and absolute distance travelled (in metres) during the six-minute walk test. There were four secondary efficacy endpoints: apnea/hypopnea index, liver organ volume, disability score index of the Childhood Health Assessment Questionnaire/ Health Assessment Questionnaire (CHAQ/HAQ) and shoulder flexion of the Joint Range of Motion (ROM). Aldurazyme was only studied in children over 5 years of age, and conclusions about its efficacy on neurological manifestations not made.

http://www.emea.europa.eu/humandocs/Humans/EPAR/aldurazyme/aldurazyme.htm

Efficacy data of idursulfase (Elaprase) in MPS II, was also based on a 2-component composite variable of the sum of the ranks of the change from baseline in the total distance walked in the 6-minute walk test (6MWT) and % predicted FVC. Patients were stratified by age and disease severity score at baseline. The clinical variables of total distance walked in the 6MWT and % predicted FVC were also analysed separately. Secondary efficacy endpoints were passive joint range of motion, liver and spleen volume by MRI, urine glycosaminoglycan (GAG) levels and cardiac left ventricular mass (LVM) by echocardiogram. Tertiary exploratory efficacy endpoints growth velocity in prepubertal patients, radiological skeletal survey, CHAQ to measure physical function (disability and pain) and the Hunter Syndrome-Functional Outcomes in Clinical Understanding Scale (HS-FOCUS) questionnaire assessing physical disabilities (supplement to CHAQ), Quality of Life assessment using the Health Utilities Index (HUI) and the Childhood Health Questionnaire (CHQ). Again,

idursulfase is indicated to treat the non-neurological manifestations and therefore evaluations of the neurological manifestations have not been conducted. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000477/human_med_000636.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d125

The efficacy of galsulfase (Naglazyme) was evaluated in MPS VI patients based on the number of meters walked in 12 minutes, a three minute stair climb test and other assessments of systemic function: joint mobility, joint pain and stiffness, upper airway obstruction, manual dexterity and visual acuity. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000640/human_med_000918.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d125

A pivotal trial of miglustat (Zavesca) in Type I Gaucher disease was conducted in patients unable or unwilling to receive ERT. Efficacy variables were mean liver and spleen volume, haemoglobin concentration and platelet count. Other selected efficacy parameters were bone mineral density Z-scores at the lumbar spine and femoral neck and number of events of bone crisis, vascular necrosis or fractures during treatment period.

Miglustat (Zavesca) was also studied in 31 patients with Niemann-Pick type C (NP-C), 12 of whom were less than 12 years old. The primary endpoint was horizontal saccadic eye movement velocity, based on its correlation with disease progression.

Secondary efficacy endpoints were assessments of swallowing, auditory acuity, ambulatory ability (standard ambulation index), and for those 12 years and older - cognition (mini-mental status examination MMSE).

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000435/human_med_001171.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d125

Miglustat (Zavesca) was also studied in late-onset Tay-Sachs, but failed to demonstrate efficacy, much like in NGD. In this 12 month, randomised, multicenter, open-label study in late-onset Tay-Sachs change in eight measures of isometric muscle strength in the limbs and isometric grip strength was selected as primary efficacy endpoints. Secondary efficacy endpoints included gait, balance, overall disability. A timed walk test (nonwheelchair bound patients only) was used to measure gait speed, and the Standard Ambulation Index (range 0-9) was used to determine the time and degree of assistance required to a set distance (overall and for the nonwheelchair bound patients). The Modified Falls Efficacy Scale total score (0-140) was used to assess patients' fear of falling, and the Tinetti Scale balance (0-16) and gait (range 0-12) scores were determined to assess the risk of future falls. The overall functional disability throughout the study was measured using the Amyotrophic Lateral Sclerosis Functioning rating Scale (Shapiro *et al.* 2009).

In NGD, the selected the primary efficacy endpoint was vertical saccadic eye movement velocity as determined by the peak amplitude versus amplitude regression lone slope. Secondary endpoints included changes in neurological and

neuropsychological assessments - including Purdue Peg Board test, Wechsler Scale, Benton Visual Retention test, Rey auditory verbal learning test, D2 test of attention, continuous performance test, and Trail Making Test. Neurological assessments of metal state, cranial nerves, motor skills and brain auditory-evoked potentials. However, many of these assessments are not age-appropriate for the majority of the paediatric population that were recruited, resulting in a lot of missing data for this study.

Performing this overview of therapeutic options currently licensed for LSDs, and most importantly the endpoints used to evaluate their efficacy provides an insight into an approach that may be acceptable for neuronopathic Gaucher disease. Exploring and identifying valid and reliable means of measuring clinically meaningful neurological changes is an obstacle that must be addressed if the efficacy of new therapy is to be monitored effectively. To be truly valuable however this needs to be done in a way that will be accepted by the regulatory authorities, before evaluation of a drug therapy is commenced.

It is apparent from this review that very few of these drugs were studied for CNS involvement, or able to demonstrate CNS benefit within neurolysosomal. Guidance is therefore somewhat limited, however it is possible to deduct that a variety of clinical assessment tools (MPS II, Pompe, Tay-Sachs) and gait parameters (MPS I, MPS II, MPS VI, Tay-Sachs) have previously been used and accepted by the regulatory authorities. This indicates that exploratory and development work using a

clinical severity scoring tool of neurological manifestations and gait analysis is a worthwhile approach which can be justified in the context of this study.

1.4 Quantifying disease severity

The use of historical data to define the natural history of a disease and to demonstrate the efficacy of alglucosidase alfa (Myozyme) was used and approved by the Food and Drug Agency (FDA) for the licensing of ERT for Pompe disease (Hannerieke *et al.* 2003). This approach has significant limitations however. While it may be justified in a disease where mortality is the primary end point for efficacy, this approach is inappropriate in other outcomes, especially without a large number of published data to conduct a meta-analysis, particularly as the results are often irreproducible.

The emergence of new drugs in rare disease has identified a demand for clinical trials that incorporate adaptive designs. However, utilising a primary end point that has not been validated for the study subjects has practical, scientific and ethical implications. As an example, the use of nonstandardised outcome assessments has yielded in conclusive results, insufficient power from multiple end points, an inability to compare the results of different trials, and, overall, an absence of proven therapies for disorders in myositis, adult and juvenile rheumatoid arthritis (van Gestel *et al.* 1996; Giannini *et al.* 1997; Rider *et al.* 2004).

Paediatric drug development has experienced a complete revamp following the European Paediatric legislation (Regulation (EC) No 1901/2006) which came into

force in 2007. One of the big drivers for this initiative was the high occurrence of off-label prescribing occurring in the paediatric community. The legislation means that all products being developed need to be considered in terms of their potential use in the paediatric population. This is evaluated as part of a Paediatric Investigation Plan presented to the Paediatric Committee at a European level, resulting in a legally binding development plan between the European Medicine Agency and pharmaceutical companies. This development plan will include the design of all clinical trials, including the selection of the primary and secondary endpoints. The demand for primary endpoints that are disease and age appropriate and validated for the target population is therefore emerging as a critical necessity. In line with the International Conference on Harmonisation; harmonised tripartite guideline on statistical principles for clinical trials (E9) only surrogate markers with demonstrated correlation to disease progression will be accepted (International Conference on Harmonisation 1998).

In line with International Conference on Harmonisation guideline E9 a primary endpoint should be a reliable and validated variable measuring some clinically relevant and important benefit in the patient population. It is especially important to address factors such as content validity, inter- and intra-rater reliability and responsiveness for detecting changes in the severity of the disease (International Conference on Harmonisation 1998). Achieving validity and reliability of a measuring tool requires time and effort however, which is a powerful reason for using existing scales when available (Bowling 2001). The appropriateness of the instrument for the study population; and the acceptability of the instrument to the group under study

need to be evaluated and considered, this is particularly important for the paediatric population. Disease-specific scales have the aim of being more clinically significant in relation to specific conditions – of being able to discriminate more finely between patients' levels of severity of condition, and of being more sensitive to their clinical outcomes (Bowling 2001).

1.5 Surrogate Markers and Biomarkers

International Conference on Harmonisation guideline on 'Clinical Investigation of Medicinal Products in the Paediatric Population' (E11) (2000) clearly states that "Where efficacy studies are needed, it may be necessary to develop, validate and employ different endpoints for specific age and developmental age groups" (International Conference on Harmonisation 2000).

Furthermore, the Committee for Medicinal Products for Human Use at the European Medicine Agency (EMA) (2006) developed a Guideline on Clinical Trials in Small Populations, which discuss the choice of endpoints. The guideline states that; *Time to disease progression* is an endpoint of intermediate level and it requires a measure of disease severity or of disease progression. Ideally, this should be validated as a tool for use in clinical trials, but it is recognised that there might be too few patients to use some for validating endpoints and other for testing treatments. The choice of a primary endpoint may therefore pose considerable problems. In some cases, the 'most appropriate' clinical endpoint may not be known or widely agreed or a validated endpoint may not exist.

In the context of rare disorders; for a given clinical endpoint or validated surrogate endpoint, recruitment of a sufficient number of patients is difficult, or demonstration of change in this endpoint can take an unreasonable length of time. The use of other surrogate markers as substitutes for a clinical endpoint may be considered. However, selection of a surrogate marker as study endpoint requires it to be reasonably likely – based on epidemiologic, pathophysiologic, or other evidence – to predict benefit. Demonstrating that a surrogate endpoint adequately reflects the true clinical endpoint is difficult. Epidemiological data, and data from patient registries can provide some help. These data may be limited when there are very few patients, and data entry is incomplete.

Validated surrogate outcomes are those tests for which there is adequate evidence that a drug effect on the measure predicts the clinical benefit desired. Regulatory authorities have approved several treatments on the basis of their effects on validated surrogate measures e.g anti-hypertensives are approved on the basis of their effects on a blood pressure and not on any effect on a symptom that is detectable to a patient. These drugs are approved because evidence has demonstrated that lowering blood pressure in the long term has beneficial clinical effects.

Unvalidated surrogates, on the other hand, are measures for which evidence does not exist that a drug effect on the measure predicts the desired clinical outcome. In 1992 the FDA adopted a new regulation (Title 21 Code of Federal Regulations 314.500, Subpart H, Accelerated Approval of New Drugs for Serious or Life-

Threatening Illnesses, FDA) in which for the first time, approval of a treatment on the basis of its effect on an unvalidated surrogate was permitted.

This regulation permits approval of a drug on the basis of clinical trials (which must be adequate and well-controlled) in serious or life-threatening illnesses that establish that "… the drug product has an effect on a surrogate endpoint that is reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit…". This regulation only applies to treatments that offer a meaningful therapeutic benefit over that provided by available products (… "e.g., ability to treat patients unresponsive to, or intolerant of, available therapy, or improved patient response over available therapy"). The regulation require that the surrogate marker be validated in studies completed after marketing, and, if this is not accomplished, the FDA may remove the product from the market in an expedited manner.

Surrogate endpoints are defined as laboratory measures, imaging or other tests that have no direct or obvious relationship to how a patient feels, or to any clinical symptom, but on which a beneficial effect of a drug is presumed to predict a desired beneficial effect on such a clinical outcome. There is considerable interest currently in approval of treatments on the basis of their effects on endpoints for several reasons, with the view that studies that rely on surrogate measures as their primary outcome measures can be smaller (fewer patients) and shorter than studies that rely on an effect on a more traditional clinical outcome. Plasma levels of chitoriosidase in Gaucher and GL3 in Fabry disease can be regarded as surrogate endpoints.

However there are no laboratory measures that are currently available to evaluate CNS function. Chitotriosidase levels in the cerebrospinal fluid (CSF) may be one option to consider in the NGD cohort. However, the invasiveness of the lumbar punctures required to obtain CSF samples, and the unlikely possibility of obtaining ethical approval to conduct a study to explore its value for monitoring purposes makes this difficult.

The Paediatric legislation (2007) along with the Orphan Drug legislation (1990) can now result in 12 years exclusivity for pharmaceutical companies, which can be regarded as a real incentive to study new emerging therapies in LSD. This is an opportune time therefore to identify and develop disease specific clinical markers of NGD that are appropriate and validated for use in a paediatric population.

1.6 Aims of thesis

The aim of this work was to explore the potential use of three different assessment tools to monitor the neurological involvements in NGD. Horizontal gaze palsy is the clinical hallmark of NGD. Other neurological manifestations include seizures, cerebellar ataxia and pyramidal tract involvements. However, NGD is very heterogeneous and the neurological features vary greatly from patient to patient, not only in terms of manifestations involved but also in terms of the severity of the presentation. The emergence of enzyme replacement therapy has changed the *'natural history'* of the disease, and patients are now living longer where previously they would have succumbed to the visceral complications of the disease. New emerging therapies are being developed for NGD, however a suitable surrogate

marker to monitor neurological disease progress is lacking. Therefore, in a bid to identify suitable markers, three different tools were utilised to examine their value and sensitivity to assess neurological involvement in NGD.

Thus, the aims of this project were:

- To modify and complete validation of the Severity Scoring Tool (SST) - to ensure that it offers a robust tool for monitoring NGD patients.
- To describe the gait characteristics of NGD patients to explore the potential use of gait as a marker of disease severity.
- To use state of the art imaging (diffusion tensor imaging) to examine the NGD brain, and explore its value and sensitivity as a marker of disease severity.
- To examine the concordance of these assessments in defining disease severity.
- To consider the value of the three assessment tools for future clinical trials.

1.7 Ethics Approval

This study was approved in two parts.

1. By the Institute of Child Health Research Ethics Committee on 26 May 2006. Study Title: The Development of a Severity Scoring Tool for Neuronopathic Gaucher Disease. Reference Number: 06/Q0508/39

2. By Wandsworth Research Ethics Committee (Paediatric specialist) on 25 July 2007. Study Title: Quantifying neurological features in Lysosomal Storage Disorders. Reference Number: 07/H0803/126. With amendment submitted, and approved 06 January 2009.

1.8 Patient Recruitment

Patient recruitment for the validation and modification of the Severity Scoring Tool patients were from three different centres:

Villa Metabolica Children's Hospital, MC Universitatsmedizin Mainz, Langenbeckstr.2, Mainz, Germany

Clinic of Metabolic Diseases, Endocrinology and Diabetology, The Children's Memorial Health Institute, Warsaw, Poland

Great Ormond Street Hospital Children's NHS Trust, London.

Patient recruitment for gait and diffusion tensor imaging was performed only at:

Great Ormond Street Hospital Children's NHS Trust, London.

A detailed overview of all the patients recruited, and assessments performed by each patient are presented in Appendix 3.

Chapter 2

Severity Scoring Tool

Keep your fears to yourself, but share your aspirations with others.

- Robert Louis Stevenson

2. Severity Scoring Tool.

2.1 Introduction

Clinical rating scales are widely used in neurology (Masur 2007). The majority of these however are developed for diseases that are most commonly seen in adulthood, and rarely developed, or validated for disease that are predominately seen in paediatrics. Developing tools that are both disease-specific and age-appropriate is particularly challenging.

There are some exceptions. The 'International Cooperative Ataxia Rating Scale' (ICARS) is a disease specific instrument for Friedreich's ataxia, a disease that typically presents in childhood, with mean age at presentation being 10 years old. The scale is based upon 19 simple testing manoeuvres compartmentalised into postural and stance disorders, limb ataxia, dysarthria and oculomotor disorders (Trouillas *et al.* 1997). It is considered valid (Cano *et al.* 2005) with good inter-rater reliability (Storey *et al.* 2004). A second scale has also been developed, the Friedreich's Ataxia Rating Scale (FARS). The main component of the scale is a detailed neurological examination consisting of 25 manoeuvres along with three quantitative performance measures (Lynch *et al.* 2006). The scale has only provisionally been validated in one study which identified a larger variability compared to ICARS. This may have been caused by the fact that performing the FARS takes 45 minutes compared to 10 minutes for the ICARS. This may lead to patient and investigator fatigue (Lynch *et al.* 2006).

In response to the fact that there was no assessment tool available to plot the temporal course of mitochondrial disease, the Newcastle Paediatric Mitochondrial

Disease Scale (NPMDS) was devised for children in 2006 (Phoenix *et al.* 2006). It is a practical and semi-quantitative rating scale that covers the diverse clinical spectrum seen. It is also multi-dimensional and reproducible, offering a tool through which mitochondrial disease can be objectively monitored. It was designed according to several predefined objectives: (1) to accurately and objectively assess the natural history of mitochondrial disease throughout childhood and into adult life; (2) to encompass the multi-dimensional nature of mitochondrial disease through a process which required input from patient (where possible), carer, clinician and case notes; (3) to provide a quantifiable measure of the functional disability encountered and the impact this has on patients and their families; (4) to be a concise, pragmatic tool suitable for use in a clinic setting, yet able to provide a comprehensive assessment. The scale was developed based around four domains: Section I – current function: Section II – system-specific involvement; III – current clinical assessment and IV – quality of life. The list of items to be included in Section I-III was developed by three clinicians with expertise in mitochondrial disease. During this development, the experts acknowledged the difficult challenge in creating a tool that is relevant to patient's age and state of development. To address this, the scale was subdivided into three age ranges, infancy and early childhood (0-24 months), middle childhood (2-11 years) and adolescence (12-18 years). The scale was designed so that each subdivision of the scale merged seamlessly with the next as the children grew older, thereby moving from the adolescent rating scale to the adult scale (Phoenix *et al.* 2006).

The parallel between mitochondrial disease and LSD in terms of the multiplicity of clinical presentation, genetic mutations and biochemical deficiencies is clear. The heterogeneity of neurological involvement across all LSD also makes clinical evaluation and monitoring of disease status equally difficult. The rational of developing the NMPDS can therefore be applied to LSD, and in some part is reflected in the number of disease specific assessment tools already developed across the LSDs. Currently there are numerous tools available to monitor neurolysosomal disorders (Table 2.1.).

Table 2.1: Disease specific assessment scoring tools which account for neurological manifestations in Lysosomal Storage Disorders

Disease specific tool; Author	**Neurological domains**	**Development process**
Juvenile neuronal ceroid lipofuscinosis (JNCL) disease-specific scoring system (Kohlschutter *et al.* 1988)	Scores of 0 (maximal dysfunction) to 3 (normal) assigned to vision, intellect, language, motor function, and epilepsy.	Domains selected based on authors opinion. Not all neurological manifestations accounted for. No validation of tool published. No weighting of domains or MCID identified. Responsiveness to change demonstrated.
Late infantile neuronal ceroid lipofuscinosis (LINCL) Clinical scoring system (Steinfeld *et al.* 2002)	Assessment of four features; motor function, seizures, visual function and language (scored 0-3)	Domains selected based on authors opinion. Not all neurological manifestations accounted for. No validation of tool published. No weighting of domains or MCID identified. Responsiveness to change demonstrated.

Disease specific tool; Author	Neurological domains	Development process
Gaucher disease Severity Scoring System (Zimran *et al.* 1989)	Presence of CNS accounted in one total score of 20	Domains selected based on authors opinion. Neurological manifestations accounted for in one domain. No validation of tool published. Weighting of domains based on authors' opinion. Phenotypes defined according to score, but not MCID. Responsiveness to change demonstrated.
Neuronopathic Gaucher Disease Severity Scoring Tool (SST) (Davies *et al.* 2007b)	11 neurological domains; HGP, epilepsy, cognitive ability, ataxia/gait, cerebellar signs/ ataxia, pyramidal, extrapyramidal, swallowing, speech, ophthalmology, kyphosis	Domains selected based on literature review. Internal and content validity published following pilot use Concurrent validity and Feasibility demonstrated. Responsiveness to change demonstrated.
Gaucher Disease – Type I GauSSI-I (Di Rocco *et al.* 2008)	Neurological domain is divided into: No signs/symptoms = 0 Peripheral neuropathy = 1 Parkinson's disease/parkinsonism = 3	Domains selected based on literature review and opinions of experts. Delphi technique used to achieve consensus on severity total score. Weighted domains. Pilot use and concurrent validity published. Responsiveness to change demonstrated.
Niemann-Pick C Disability Scale (Iturriaga *et al.* 2006)	Assessment of four features; Ambulation, Language (0-5) Manipulation + Swallowing (0-4)	Domains selected based on authors opinion. Not all neurological manifestations accounted for. No validation of tool published. No weighting of domains or MCID identified. Responsiveness to change demonstrated.

Disease specific tool; Author	Neurological domains	Development process
Niemann-Pick C Clinical Severity Scale (Yanjanin *et al.* 2010a)	Eye movement, Ambulation, Speech, Swallow, Fine Motor Skill, Cognition, Hearing, Memory, Seizures (all 0-5). Modifiers – Gelastic cataplexy, Narcolepsy, Behaviour, Psychiatric, Hyperreflexia, Incontinence, Auditory Brainstem Response, Respiratory.	Inter-rater reliability, sensitivity and validity of scale published. Domains not weighted. Responsiveness to change demonstrated.
Fabry Disease DS3 (Giannini *et al.* 2010)	Peripheral Nervous System: Sweating (0-2) Gastrointestinal (0-5) Pain (0-5) Central Nervous System: White Matter Lesions (0-8) TIA/stroke (0-8).	Reliability and validity demonstrated through expert consensus formation and statistical techniques used to identify domains and weighting. MCID estimated. Responsiveness to change demonstrated.
Fabry Disease Mainz Severity Score (MSSI) (Whybra *et al.* 2004)	Neurological domains: Tinnitus, Vertigo, Acroparesthesia, Fever, Pain Crisis, Cerebrovascular, Psychiatric/ psychological	Domains selected and weighted based on authors opinion. Specificity of MSSI demonstrated. Phenotypes defined according to score, but not MCID. Responsiveness to change demonstrated.
MPS IIIA FPSS (Meyer *et al.* 2007) (modified based on LINCL scoring system)	Four-point scoring system assessing three clinical features: Motor function, Speech abilities and Cognitive function	Domains selected based on authors opinion. Not all neurological manifestations accounted for. No validation of tool published. No weighting of domains or MCID identified. Responsiveness to change demonstrated.

MCID - Minimum Clinically Important Difference

Scales developed for use in LSDs

Neuronal ceroid lipofuscinosis (NCL)

Clinical assessment scales developed for paediatric use include the one for JNCL and another for LINCL. During the initial development of the scoring system for LINCL Steinfeld et al (2002) examined 26 patients, and developed the clinical performance score by rating motor, visual, and verbal functions. Incidence of seizures in 3-month intervals had originally been included but later omitted as seizure scores fluctuated strongly and seemed to depend on treatment modalities. A Total Disability Score was derived by summing up the single scores for motor, visual and verbal functions – totalling in a normal score of 9, where a declining score indicates progression of disease. The authors say that the performance rating scale is able to clearly and quantitatively delineate the disease course of the LINCL patients, and hence offers a useful tool for clinical evaluation of therapeutic interventions. In the author's view the system can be applied to other types of neuronal ceroid lipofuscinoses and could be adapted to various other neurodegenerative diseases of childhood (Steinfeld *et al.* 2002). However proceeding with this approach would still require disease-specific validation.

Mucopolysaccaridosis type IIIA

The four-point scoring tool developed for JNCL and LINCL was adapted to develop a scoring tool for MPS IIIA based on the assessment of 71 patients (Meyer *et al.* 2007). The domains selected for this disease were changed to include motor function, speech abilities and cognitive function. In the same regard however, the

total score of 9 indicated normal function and a declining score reflects disease progression.

However, a failing of these tools is that they fail to include domains for all of the neurological manifestations that are present in both LINCL and MPSIIIA. Furthermore, important issues such as internal reliability, feasibility, face validity or content validity have not been addressed. These are important issues to be established in order to demonstrate the robustness of any tool.

Gaucher disease

There have been three tools developed for Gaucher disease, the Zimran Severity Scoring System, the Gaucher Disease Severity Scoring Index, and the Severity Scoring Tool. The first two were developed predominantly for type I. In the Zimran Severity Scoring System, the neurological manifestations were all grouped into one domain (Zimran *et al.* 1989). Based on the opinion of the authors, patients were assigned to one of three clinical phenotypes: mild (severity scoring index 0-10 points), moderate (11-25), or severe ($\geq$26). The neurological domain was attributed 20 points, for any neurological involvement, regardless of presentation. This does not allow for level of severity to be reflected, and there is no means of capturing change in presentation. It was for this reason that the SST was originally developed (Davies *et al.* 2007b). The Gaucher Disease Severity Score Index (GauSSI-I) (Di Rocco *et al.* 2008) which was developed later is specific for Type I Gaucher disease and mostly omitted neurological manifestations, particularly those seen in NGD. The Zimran et al (1989) Severity Scoring System failed to address most of the criteria for

demonstrating validity and reliability, while the GauSSI-I (Di Rocco *et al.* 2008) has established some validity criterions.

Niemann-Pick C

Two clinical severity scales to state the progression of Niemann-Pick C (NP-C) have also been developed. Iturriaga et al (2006) created a disability scale containing four domains, with the aim of recording the chronological evolution of the disease. Despite describing 10 neurological manifestations leading to diagnosis in the cohort studied, the authors do not say why only four manifestations were selected for the scale. They state that the disability scale showed, as expected, differing scores according to NP-C form and age. However no work was published on the validation process of the scale (Iturriaga *et al.* 2006).

The most recent scale includes 17 domains, which give comprehensive coverage of the neurological manifestations of NP-C. The severity scale was applied longitudinally, in prospective and retrospective cohorts of NP-C, and their results outlined the disease progression of late-infantile, juvenile and adult forms of NP-C. Scores increased linearly as function of time interval between visits, and nonlinearly as a function of age (Yanjanin *et al.* 2010b). The authors also examined inter-rater reliability, sensitivity and validity of scale, with good results.

Fabry Disease

The MSSI was developed to assess the severity of Fabry disease and to monitor patient progress while on enzyme replacement therapy. MSSI was demonstrated to

be highly specific for differentiating Fabry from other patients who presented with similar complaints. MSSI also correlated to increasing age, which is clinically viable in Fabry disease (Whybra *et al.* 2004).

The development of the DS3 severity scoring system for Fabry disease, Giannini et al (2010) demonstrate an excellent and comprehensive approach to tool development, in which sixteen experts from six countries collaborated. Reliability, validity and feasibility were tested using a combination of expert consensus formation and statistical techniques. Furthermore, the minimum clinically important difference (MCID) in each of the instruments was estimated and the DS3's quantitative content validity was judged.

These tools demonstrate that there is a clinical demand for disease specific tools in LSD. Existing tools are not applicable across the board for all LSD however, given the large heterogeneity of neurological manifestations seen across the disorders - hence the need to develop and validate new, disease-specific tools.

The assessment of neurological or cognitive status in some of the MPS disorders can be rendered difficult by co-existing visceral disease. MPS II, for example, is difficult, as somatic disease can confound the neurological presentation (Wraith *et al.* 2008). Fine motor skills of hand function diminish as a result of progressive flexion contractures in the fingers, and ultimately a 'claw hand' deformity. Factors such as limited hand function; carpal tunnel syndrome, decreased visual acuity or hearing

loss may interfere with communication and learning, and ultimately give an impression of developmental delay.

Wraith and colleagues (2008) further highlight the difficulties in monitoring MPS patients' response to treatment when they are too young to cooperate with clinical assessments, and states that identifying a suitable biomarker that would reflect the disease burden and respond promptly to therapy currently remains a significant clinical need. The same complexities when patients are too young to cooperate with complex clinical assessments are true for NGD, which is primarily a paediatric presenting disease.

Severity Scoring Tool for NGD – previous work and original development

The *'natural history'* of the neurological manifestations seen in NGD remains largely unknown in the enzyme replacement era, despite some follow up studies of cohorts in Europe (Tylki-Szymanska *et al.* 2006; Erikson *et al.* 2006a; Erikson *et al.* 2006b; Erikson *et al.* 2006c).

Clinical monitoring was found to vary across centres, despite published guidelines (Davies *et al.* 2007a). Critically, there appeared to be little or no means of monitoring patients objectively.

The SST (Appendix 1) was developed in response to this, and in the aftermath of the miglustat clinical trial (Schiffman *et al.* 2008). This work, led by myself, was supported by our group and several members of the European Task Force for NGD.

Its development was based on extensive review of the literature, which accounted for 102 patients. Based on the neurological manifestations reported in the publications the domains were identified, and categories of severity defined. This was followed with a pilot use on 47 patients across four European countries – equating to the largest cohort of NGD patients ever studied systematically (Davies *et al.* 2007b). The neurological domains included in the SST after evaluation of internal reliability and content validity were - Horizontal Gaze Palsy, Epilepsy, Cognitive Development, Ataxia/Gait, Cerebellar Ataxia, Pyramidal, Extrapyramidal, Swallowing, Speech, Ophthalmology and Kyphosis (Davies *et al.* 2007b).

The SST was recently incorporated in the revised guidelines for the management of NGD by The European Task Force (Vellodi *et al.* 2009). The guidelines attempted to utilise generally available, cost-effective technology, while at the same time yielding the most possible clinically relevant data. Apart from the SST, other recommended neurological examination include neuro-opthalmological investigation, brain imaging, electroencephalography, neuropsychological assessments and measurement of peripheral hearing (electro-acoustical emission in small children, pure tone audiometry in older patients) (Vellodi *et al.* 2009). However, due to age, cultural difference, and economic constraints it has not been possible to ascertain a standardised format for any of these individual assessments, and their sensitivity in capturing disease progression or improvement in response to new potential therapy is unknown. As horizontal gaze palsy is a clinical hallmark of NGD, neuro-opthalmologic evaluation is common place. However the value of using saccades in detecting change in disease status is now questioned following a recent study

(Schiffmann *et al.* 2008). The complexities of this study, in which our group recruited half of the patients identified many challenges. The biggest was that the evaluation of saccades was extremely difficult. The assessment requires complex cooperation for a prolonged period of time. Furthermore, each time the child blinks or head thrusts – the natural compensatory mechanisms adopted in NGD, an artefact corrupts the quality of the data captured. This made the assessment stressful and traumatic for many of the children, and highlighted the need to develop clinical tools that are acceptable to the target population.

Despite the demonstration of internal reliability, feasibility, face and content validity (consensual and quantitative) of the SST (Davies *et al.* 2007b) further work was necessary to ensure that it offered a robust and valid means to capture disease progression, and to serve as a potential marker to monitor response to any new emerging therapies. To be in line with the International Conference on Harmonisation (ICH) E9 Statistical Principles for Clinical Trials guideline; factors such as content validity, inter- and intra-rater reliability and responsiveness for detecting changes in the severity of the disease need to be addressed for all selected endpoints (International Conference on Harmonisation 1998).

Additional development and validation of the SST was therefore needed to address the ICH E9 specification, which is the basis for this part of this study. Evaluating inter-rater and intra-rater agreement; identifying the *'weighting'* for each domain; demonstrating the SSTs responsiveness to change and identifying the 'minimum

clinically important difference' (MCID) will allow for a scale that accurately reflects the neurological phenotype, while at the same time being robust and valid.

2.2 Methods

2.2.1 Inter-rater and intra-rater evaluation

Repeatability assessment indicates the extent of potential error present; this is referred to as 'measurement error'. For example, if an assessor measures a child's height to the nearest millimetre on two separate occasions, then it is very likely that the two readings will be different. If the assessor is inexperienced or using new equipment then these differences are likely to be larger. If the two measurements are taken by two different assessors, then these differences are likely to be larger still.

It is important to understand the terms inter-rater and intra-rater agreement. *Intra-rater agreement* refers to the reliability of the same rater's scores on the same subjects on different occasions. *Inter-rater agreement* is the concordance of scores achieved by different assessors on the same occasion.

Therefore, with any clinical tool, it is imperative to demonstrate inter-rater and intra-rater agreement. Correlation techniques are often used to compute an index of agreement. By calculating the correlation coefficient, the degree of correlation among each observer's scores is determined. The higher the coefficient of correlation, the more reliable the score is.

When considering the impact of measurement error, one needs to consider whether the total score is likely to change over time naturally and the possibility of fatigue or learning effect. These are particularly relevant in progressive diseases like NGD. Patient fatigue can impact on how well the child cooperates with the assessor's examination, and of course the assessors become more familiar with the examination and assessment prompts which may affect the result (learning effect).

It is important to demonstrate that any variability in the data is due to natural variability and not assessor error. Appropriate statistical tests to consider when one assessor produces several replicate scores, or when a series of patients are assessed by more than one assessor, are 'repeatability coefficient' and 'intra-class correlation coefficient'. There are several different types of intra-class correlation (reliability) coefficients; one of the more commonly used is the Pearson coefficient.

A situation that may occur is that the total score of the SST demonstrates good agreement, while individual domains within the SST do not. Individual domains yield *ordinal* data, which means that non-parametric tests must be used. Although the SST total score could be regarded as *interval* data, and therefore possibly suitable for parametric analysis this will ultimately be decided based on the data generated and whether or not it is normally distributed

When the categorical outcome measured is ordered (ordinal data), as in these domains, this must be taken into account and a non-parametric statistical test used. *Kendall tau* tests the strength of association of the cross tabulations when both

variables are measured at the ordinal level. It makes adjustments for ties and is most suitable for rectangular tables. The values range from −1 (100% negative association, or perfect inversion) to +1 (100% positive association, or perfect agreement). A value of zero indicates the absence of association.
The statistical value of performing inferential statistics is limited given that the number of patient required to power them sufficiently is near impossible in the context of an orphan disease, particularly when recruitment is restricted to one specialist centre. This needs to be considered during data analysis.

In a bid to examine intra-rater agreement nine patients were assessed sequentially. Due to the slow nature of disease progression in NGD, as documented by Erikson *et al* (2006) and Tylki-Szymanska *et al* (2006), it was hypothesised that that disease status would not progress in a three month period of time. Three months being the time period that patients attend routine clinic. To increase the power with the limited number of patients available as many assessments scores as possible were sought.

An assessment of the SST's inter-rater agreement was evaluated by having NGD patients assessed on the same day by two separate assessors. The assessors were medical clinicians within our group. Both had extensive experience in managing NGD patients, and were familiar with the SST having been involved in its original development.

2.2.2 'Weighting' of domains

When devising a scale, the easiest way of combining the individual items or domains is to simply attribute the same score to each domain, and then add all the scores into a total score. In fact this is the most widely approach; all scales used in LSD to date have adopted it. It is conceptually and arithmetically simple, and makes few assumptions about the individual domains; the only assumption is that all are equally important in contributing to the total score. In fact, during the initial development of the SST each domain was attributed equal scores (0-3), or '*weighting*', for mathematical ease. It was acknowledged throughout, however, that some neurological manifestations contribute more morbidity in NGD, than others. However, the problem with this approach is that some domains may be more important than others, and perhaps should make a larger contribution to the total score (Bowling 2001). The identification of domain *'weighting'* is a theoretical process that involves incorporating the opinion of experts within the field conducted using nominal group technique. This approach seeks the involvement of key experts in the field, and then attempts to achieve a group consensus.

To achieve this and reflect disease burden of each domain measured in the SST, twelve international experts (Appendix 2) from eight different countries were invited to take part in a nominal group technique. Nominal group technique is used as a means of achieving group consensus. Given that arranging a meeting would be costly and difficult to arrange, a web based programme which allows interactive involvement of the participants while based at their respective place was utilised. The programme software utilised was WebEx; (http://www.webex.co.uk) This is a

sophisticated programme which allows each expert to remain in their own country but participate in the discussion by conference call, while seeing the discussion and voting outcome displayed in real time, on their own computers.

2.2.3 Identifying the 'Minimum Clinically Important Difference'

A valuable tool is one that is sensitive to identify change seen as a result of disease progression or response to therapy. There is however a need to identify what is the minimal change that can be regarded as a clinically important difference in score. This is referred to as the minimum clinically important difference (MCID).

The aim in this instance is to identify the smallest change in SST scores, plus or minus, that would be considered as a MCID - using clinician's ratings as deciding reference. Immunology and Rheumatology have led the field in this area (Giannini *et al.* 1997) (Rider *et al.* 2004) and the development of the DS3 for Fabry disease incorporated this aspect. The approach in Immunology and Rheumatology has involved over 100 experts in the field, and analysed over 100 case notes. Identifying this number of experts and patients in NGD was not possible, though the same principle could be applied. Therefore the same approach, but on a smaller scale was implemented.

2.2.4 Evaluating the Responsiveness of the SST for Detecting Change

Initial development of the SST was based on a cross-sectional analysis of 55 patients across four different countries; Germany, Poland, Sweden, UK. This is the largest cohort of NGD patients ever studied uniformly (Davies *et al.* 2007b). In order to demonstrate the SSTs responsiveness for detecting change therefore, the original

cohort was reassessed at four year follow up. Through visiting each country the SST was used to reassess each patient in collaboration with each physician in Germany, Poland and the UK.

2.3 Results

2.3.1 Inter-rater agreement

Assessments of the SST's inter-rater agreement took place on consenting NGD patients at Great Ormond Street NHS Trust Children's Hospital over a period of eighteen months. Six patients (*n*=6) were assessed on the same day by two separate assessors. Both assessors were clinicians with extensive experience in managing NGD patients, and were familiar with the SST, having been involved in its original development. Time points for each individual assessment were determined by patients' attendance for routine clinic and the availability of both clinicians on those dates. Patients were not all assessed in the same order by the assessors to avoid any potential order effect. The mean age at assessment was 11.7 years (±4.82). The SST scores for both assessors were not normally distributed. The median score for both assessors was identical at 5.75. The mean SST score for the two assessors were 9.33 (7.65±) and 8.42 (7.10±). A descriptive overview of the data for each domain is presented in Tables 2.2 to 2.12.

Table 2.2: Overview of inter-rater agreement in Horizontal Gaze Palsy domain

Horizontal Gaze Palsy		**Assessor One**		
		0	1.5	3
Assessor Two	0			
	1.5		**4 (66.6%)**	
	3			**2 (33.3%)**

Table 2.3: Overview of inter-rater agreement in the Epilepsy domain

Epilepsy		Assessor One			
		0	1	2	3
Assessor Two	0	**4 (66.6%)**			
	1				
	2			**2 (33.3%)**	
	3				

Table 2.4: Overview of inter-rater agreement in the Cognitive Ability domain

Cognitive Ability		Assessor One			
		0	1	2	3
Assessor Two	0	**2 (33.3%)**			
	1		**2 (33.3%)**		
	2		2 (33.3%)		
	3				

Table 2.5: Overview of inter rater agreement in the Ataxia/ Gait domain

Ataxia/ Gait		Assessor One			
		0	1	2	3
Assessor Two	0	**4 (66.6%)**			
	1		**2 (33.3%)**		
	2				
	3				

Table 2.6: Overview of inter-rater agreement in the Cerebellar ataxia domain

Cerebellar ataxia		Assessor One		
		0	1.5	3
Assessor Two	0	**1 (16.6%)**	4 (66.6%)	
	1.5			
	3			**1 (16.6%)**

Table 2.7: Overview of the inter-rater agreement of the Pyramidal domain

Pyramidal		Assessor One			
		0	1	2	3
Assessor Two	0	**1 (16.6%)**			
	1	3 (50%)	**1 (16.6%)**		
	2		1 (16.6%)		
	3				

Table 2.8: Overview of inter-rater agreement in the Extrapyramidal domain

Extrapyramidal		Assessor One			
		0	1	2	3
Assessor Two	0	**4 (66.6%)**	1 (16.6%)		
	1		**1 (16.6%)**		
	2				
	3				

Table 2.9: Overview of inter-rater agreement in the Swallowing domain

Swallowing		Assessor One			
		0	1	2	3
Assessor Two	0	**5 (83.3%)** *			
	1	1 (16.6%)			
	2				
	3				

Table 2.10: Overview of inter-rater agreement in the Speech domain

Speech		Assessor One			
		0	1	2	3
Assessor Two	0	**5 (83.3%)**			
	1		**1 (16.6%)**		
	2				
	3				

Table 2.11: Overview of inter-rater agreement in the Ophthalmology domain

Ophthalmology		Assessor One		
		0	1.5	3
Assessor Two	0	**1 (16.6%)**	2 (33.3%)	
	1.5	1 (16.6%)	**2 (33.3%)**	
	3			

Table 2.12: Overview of inter-rater agreement in the Kyphosis domain

Kyphosis		Assessor One			
		0	1	2	3
Assessor Two	0		1 (16.6%)		
	1	2 (33.3%)	**1 (16.6%)**	1 (16.6%)	
	2		1 (16.6%)		
	3				

A descriptive analysis of this data indicates that the assessors were in 100% agreement for four of the eleven domains and in 83% agreement for another four. Poor agreement was noted in the Pyramidal, Ophthalmology and Kyphosis domains.

Kendall tau-c is presented in Table 2.13. As previously noted, although interesting to note, the validity of the analysis is limited considering the sample size.

Kendall tau c directionally demonstrates positive association, above zero for seven of the domains, although only statistically significant in five. Perfect agreement is not demonstrated by *Kendall tau c* even when agreement is 100%. This is probably as a result of the small sample size. Ophthalmology and Kyphosis have a value of zero indicating a complete absence of association. This is consistent with the low percentage of total agreement.

Spearman's rho correlation of SST total score between the two assessors was *r*0.899 which is significant at *p*0.015. This suggests therefore that despite some variability in individual domains, that the total SST score has good inter-rater-reliability.

Table 2.13: Percentage of total agreement and Kendall *tau c* for the agreement between assessors in each individual domain.

	% of TOTAL agreement between assessors	***Kendall tau-c***	***Sig* level**
Horizontal Gaze Palsy	100	0.89	0.001**
Epilepsy	100	0.89	0.001**
Cognitive Ability	60	0.89	0.001**
Ataxia/ Gait	100	0.89	0.001**
Cerebellar ataxia	83	0.56	0.17
Pyramidal	83	0.56	0.065*
Extrapyramidal	83	0.44	0.19
Swallowing	83	0.44	0.19
Speech	100	0.89	0.001**
Ophthalmology	50	0.00	1.00
Kyphosis	17	0.00	1.00

*** Statistically significant. *Close to being statistically significant.*

2.3.2 Intra-rater agreement

To evaluate intra-rater agreement nine patients (3 boys, 6 girls) were assessed sequentially, by the same assessor, between 3 and 6 times. Mean age at first assessment was 10.6 years (±4.89) while mean age at last assessment was 11.1 years (±4.76). Time points for each individual assessment were determined by patients' attendance for routine clinic. Assessments ranged from 3 to 7 months (mean 4.55 ±1.43), with the total duration of follow up (mean ±SD) being 12.8 months (±4.7).

An interval of 3 months between assessments had been thought to be short enough to avoid potential disease progression, yet sufficiently long enough to avoid recall bias by the assessor. However it became apparent that the cohort naturally divided into two groups, one that progressed even within a short time and one that remained mostly clinically stable. The group that remained constant, with no or minimal change seen were the L444P homozygote. These are patients 2, 3, 4, 5 and 6 as seen in Figure 2.1. The heterozygote group demonstrate progression with an increase in SST score.

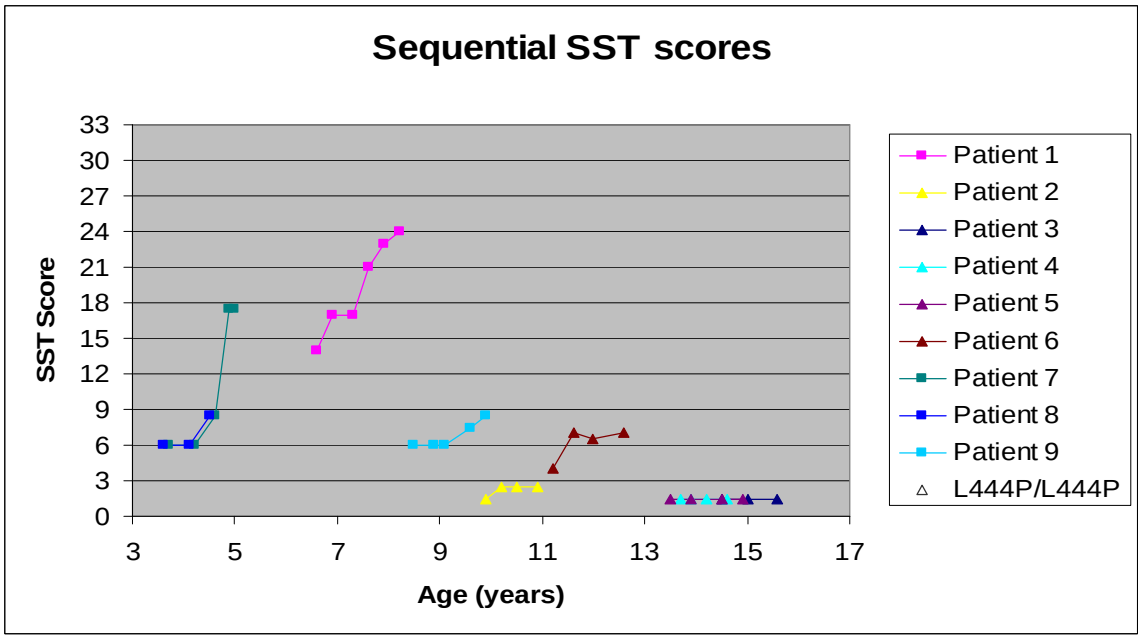


Figure 2.1: Severity Score Tool sequential data (n=9)

The overall heterozygosity within such a small cohort made inferential statistical analysis unfeasible. Descriptive observations, however, suggest that intra-rater reliability was evident in the stable patients with the SST score remaining consistent at 1.5 for three patients and consistent scores reported for four other patients on at least two of the occasions (Between time points 1 and 2 for patients 7, 8 and 9, and between time points 2 and 3 for patient 1).

These findings are encouraging, in that they not only demonstrate intra-rater agreement, but also the sensitivity of the SST in capturing and quantifying disease progression.

2.3.3 'Weighting' of domains

Four experts from three countries were able to participate in the nominal group discussion for '*weighting*' the domains. Prior to the WebEx meeting a power point

presentation was prepared detailing the background of the SST and the development to date. All experts were sent a presentation prior to the WebEx meeting. This was also briefly discussed at the beginning of the WebEx meeting. The meeting was chaired by an independent person (E. H. Giannini) who is a methodologist-biostatistician with experience in instrument development for Rheumatology and Fabry disease.

Firstly, the experts were asked to rank all eleven domains according to the amount of morbidity each contributes to NGD severity as perceived by themselves. A Likert-like scale, which is a psychometric scale used to specify level of agreement, was used for each domain, the range of which was selected by each individual participant. This provided an external criterion for the ratings of the clinicians' perceptions. Clinicians were then asked to rate on a scale of 0 to 10 where each level of domain would be placed in terms of its impact on disease - indicating the overall disease burden of that domain (e.g Cognitive Ability). Based upon these rankings the experts assigned the range of scores, representing the relative weights, for the clinical measurements within each domain. On both occasions participant's first votes were discussed in a 'round robin' type discussion – giving each expert an opportunity to explain their chosen score. Following this discussion, a second vote took place – providing the final results used for analysis. Results were calculated and made visible for all to see at the time of the WebEx meeting.

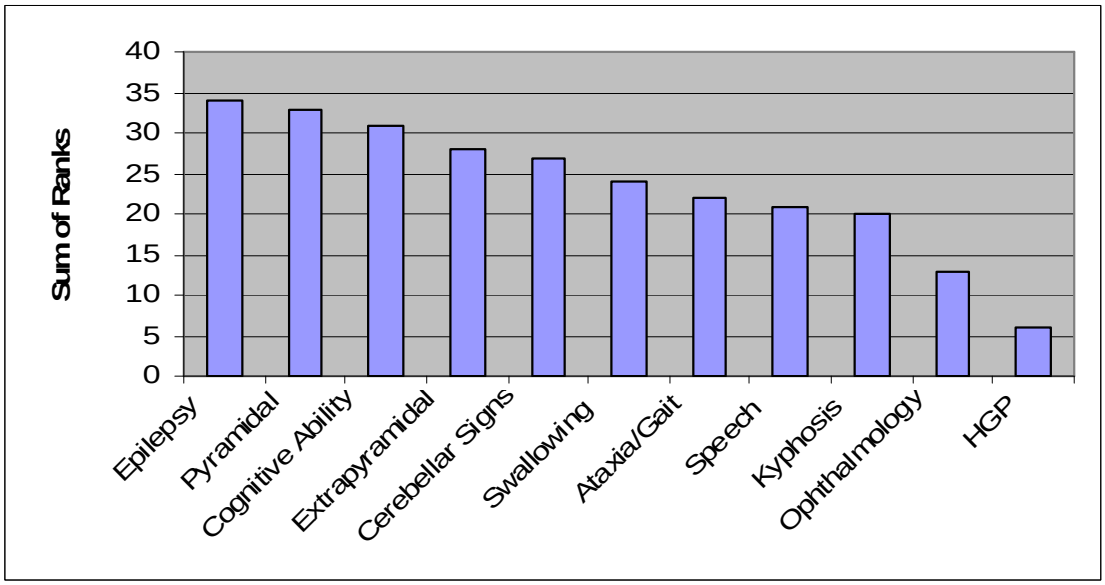


Figure 2.2: Total Sum of Ranks for each SST domain, indicating weighting order for perceived severity by international experts.
(HGP – Horizontal Gaze Palsy)

All the experts ranked epilepsy as contributing the most severity in NGD. This was closely followed by eight other domains (Pyramidal, Cognitive ability, Extrapyramidal, Cerebellar signs, Swallowing, Ataxia/Gait, Speech and Kyphosis). Ophthalmology scored much lower, along with Horizontal Gaze Palsy which scored the least. A graphic illustration of the rankings can be seen in Figure 2.2.

These ranking orders were later supported in the second part of the discussion: individual domain scoring. The lowest average score of 2.25 was attributed to the Horizontal Gaze Palsy domain and highest score of 9.25 attributed to both the Epilepsy and Pyramidal domains. Scores demonstrated overall consistency in order, with Epilepsy and Pyramidal domains again contributing the most to severity, followed by Swallowing (8), Cognitive Ability (7.5), Extrapyramidal (7.5), Ataxia/Gait (7.5), Speech (7.25), Kyphosis (6) and Cerebellar Signs (5.5), all of which were very closely ranked. Ophthalmology (4.5) then followed with Horizontal Gaze Palsy

scoring the least. Only Cerebellar Signs and Swallowing domains changed their ranking in the opinions of the experts when measured with two different methodologies.

Six domains, which were very closely ranked by the experts; Cognitive Development, Ataxia/Gait, Extrapyramidal, Swallowing, Speech, Kyphosis maintained the same 0-3 score. The two highest ranked domains, Epilepsy and Pyramidal were attributed higher scores of 0-5. Given the low ranking for Horizontal Gaze palsy (HGP), Cerebellar Signs and Ophthalmology they were attributed lower scores of 0-1, 0-2 and 0-2 respectively. Based on these results the domains that scored consistently higher by clinicians were allocated a higher 'weight' in each domain. The score allocated to each sub-section within each domain was not always linear, but based upon the rankings that the experts assigned for the clinical measurements within each domain. This was calculated for each domain based on the overall percentage and the original total severity score kept the same. This equates to a total severity score of 33, the original total score.

It is interesting to note that HGP was attributed the lowest score considering that the vertical component of the horizontal saccade was the chosen primary endpoint for the clinical trial of SRT in NGD. This choice was probably based on the fact that this clinical manifestation is ubiquitous in NGD patients and measurable. As previously stated, the selection of one clinical parameter which is present in all patients is difficult to identify in such a heterogeneous disorder as NGD. Given the high

prevalence of ataxia and the severity attributed to it during the weighting exercise, ataxia/ ataxic gait appears to be a promising option.

2.3.4 Identification of the Minimum Clinically Important Difference (MCID)

To identify the MCID, a second nominal group approach was organised, again with the aim of using WebEx. All experts were sent the clinical presentation of NGD patients as defined with SST scores prior to the WebEx. Due to technical difficulty however, it was not possible to use WebEx on the day. The meeting proceeded on a teleconference type basis, with the experts looking at the slides as previously sent to them. Scores were calculated manually. It was considered important to incorporate the opinion of as many experts as possible, therefore participants unable to join the meeting were asked to answer the questions in their own time and their opinions were subsequently added to the results for analysis. A total of five experts from five different countries participated, three on the day of the meeting and two subsequently.

SST scores generated from all nine consenting patients were presented to the experts (as previously presented in Figure 2.1). In order to reflect the full spectrum of the SST scale, the full range of neurological presentation was presented. Justification for the selection of the final four patients presented for discussion and scoring was based on the clinical phenotypes and the clinical changes seen using the SST. The patients selected for discussion not only varied in terms of baseline SST score, but also in terms of rate of progression.

The change in clinical presentation and the corresponding SST score over three to four different time points were presented for each patient. The time period between assessments ranged from 3-14 months, with the total follow up for all four patients ranging from 15-20 months.

The issue of whether the interval between assessments should be constant (e.g 12 months) for all patients was considered. This would allow experts to score each patient directly against each other in terms of level of severity and amount of progression. However it was felt that a change in SST score observed in a three month period may be viewed as a more important clinical difference compared to the same disease progression observed in a twelve month or two year period, as this would indicate a more rapid decline, and therefore more likely to be an important clinical difference. Therefore no fixed interval was chosen therefore.

The four patients selected for presentation were two boys and two girls, all with different genotypes: L444P/L444P, L279P/G243V, K198T/L444P, L444P/E233D. Mean age at first assessment was 7.5 years (±3.2) and mean age at last assessment was 10.2 years (±2.2). the SST scores ranged from 4 to 24 (mean SST 11.3 ±6.6) with changes in clinical score ranging from 0.5 to 9. All changes demonstrated disease progression (score increase) apart from one with a 0.5 improvement of SST score.

These patients were selected given their change in clinical presentation. The remaining five patients were not presented as their clinical state remained stable,

and their SST score remained constant over the time period of assessment. These five stable patients were L444P homozygote, with mild disease.

Participants were asked to vote on whether each SST score was considered to be ‘mild’, ‘moderate’ or ‘severe’. Participants were then asked to vote on whether, in their clinical opinion the change in the SST score could be regarded as “clinically meaningful” – “yes” or “no”. During the WebEx each participant were asked to justify or rationalise their answer, especially if it differed from the other participants. After discussion participants were given the opportunity to score again if they wanted to, however this did not happen. Answers received after the WebEx were incorporated for analysis, as seen in Table 2.14. Overall there was agreement amongst the experts on nearly all of the questions. The distribution of responses in the three categories (19=mild; 25=moderate, 22=severe) indicates that there was good coverage of the known clinical spectrum of NGD in the four patients discussed.

It became apparent during the WebEx discussion (subsequently supported by the follow up answers) however that the same score seen in different patients did not consistently fall within the same category of ‘mild’, ‘moderate’ or ‘severe’. The clearest example of this can be seen with Patient 3 and 4. Patient 3, an L444P/L444P female assessed at 11.2 years and 12.6 years, had SST score between 4 and 7. Patient 4, an L444P/E233D, who was 8.4 years at first assessment and 9.9 years at follow up, had SST scores between 6.5 and nine. Despite the overlapping of scores, Patient 3 was consistently scored as a ‘mild’ case while Patient 4 consistently scored as ‘moderate’.

Another illustration of a difference in opinion can be seen in Patient 2; a male K198T/L444P assessed between 3.7 years and 5 years of age, with SST scores progressing from 6 to 8.5 followed by a sharp increase to 17.5. In this case, experts were divided on whether a score of 6 for this patient was ‘mild’ or ‘moderate’ while a score of 8.5 was scored as scored as ‘mild’, ‘moderate’ and ‘severe’. Indeed an SST score of 17.5 was considered to be too low to account for the clinical presentation seen at this young age, by some experts.

Table 2.14: Experts feedback on the clinical presentation of NGD patients.

Patient	SST Score	Change in SST score	Mild	Moderate	Severe	Is Change Clinically Meaningful?	
						Yes	No
1	14			IIII	I		
	17			I	IIII		
		3				III	II
	23				IIIII		
		6				IIIII	
	24				IIIII		
		1					IIIII
2	6		II	III			
	8.5		I	I	III		
		2.5				II	III
	17.5				IIII		
		9		I		IIIII	
3	4		IIIII				
	7		IIIII				
		3				I	IIII
	6.5		IIIII				
		0.5				I	IIII
	7		IIIII				
		0.5				I	IIII
4	6.5			IIIII			
	8			IIIII			
		2.5				I	IIII
	9			IIIII			
		1					IIIII
Total			19	25	22		
Mean (SD)	11.29 (6.59)	2.90 (2.71)					

On discussion, and based on the comments received, the discrepancy in categorising similar scores arose from the significant differences in the child's age at assessment, not the interval between assessment. That is, the severity of a patient's disease was as much related to age as to individual domains. Age at assessment had not previously been factored into the analytical process. It was agreed by all that it needed to be addressed in order to ensure that the SST is as valuable as it possibly could.

To account for impact of age on disease severity a twelfth domain of 'Age 0-5 years =3. 6-10 years =2. 11-15 years =1. 16 years and over =0' was considered. However it was realised that this would not add to the identification of the severe patients. It was deemed necessary therefore to add 'Age at onset of an event' as a factor, and given the impact of epilepsy 'Age at first seizure' was decided upon as a new domain to the SST. Based on the cohort studied none of the patients assessed presented with seizures before the diagnosis of NGD was made. It was therefore considered that an accurate scoring could be made in the created categories, without having to rely on patient notes and/or patient/ carer memory. The twelfth domain was categorised in consultation with the experts and the *'Age at Onset of Seizure'* categorised into brackets as follows; 0-5 years =3. 6-10 years =2. 11-15 years =1. 16 years and over or seizure free =0. Seizures occurring for the first time in adulthood are not therefore given a score to attribute severity.

There was a difference of opinion on whether changes in SST scores of 2.5 or 3 were regarded as clinically meaningful. A change of SST score of 6 was unanimously considered to be clinically meaningful. As a middle-ground a 3 point change was proposed to the experts as being clinically meaningful, while changes of less than 3 would be regarded as individual variability not clinically meaningful. This is just over an 8% change in total scale score.

An increase of 3 points in the SST was therefore proposed to the experts as being the MCID for worsening, or progression of disease. The MCID for improvements could not be calculated as there was no patients who had improved sufficiently that could be discussed.

Based on the work done to identify a weighting for each domain, a score for MCID and an addition of a 12th domain the SST was renamed as a modified Severity Scoring Tool (mSST) and presented in Table 2.15.

Table 2.15: The modified Severity Scoring Tool (mSST)

HORIZONTAL GAZE PALSY	Normal (although not likely in diagnosis) Horizontal Saccades absent, Vertical Saccades present Horizontal Saccades and Vertical Saccades absent	0 0.5 1
EPILEPSY	No seizures. Seizures not requiring anticonvulsants Seizures controlled with anticonvulsants. Seizures requiring combination therapy or resistant to anticonvulsants	0 3 4 5
DEVELOPMENT/ COGNITIVE ABILITY	Normal Mildly impaired (IQ less than 85 or equivalent) Moderate (IQ between 50-57 or equivalent) Severe (More than half their chronological age)	0 1 2 3
NEUROLOGY PATTERN		
Ataxia/ Gait	Normal, apparent only on tandem walking Ataxia on straight gait, able to walk without assistance Able to walk only with assistance Unable to walk	0 1 2 3
Cerebellar signs/Ataxia	No intention tremor Intention tremor not affecting function Intention tremor with marked impact on function	0 0.5 2
Pyramidal	Normal tone with increased reflexes Mildly to moderately increased tone and reflexes Increased tone reflexes with sustained/unsustained clonus Severe spasticity with inability to walk	0 2 3 5
Extrapyramidal	Normal Variable tone and posturing not impairing function, with or without therapy. Variable tone and posturing impairing function, despite therapy Significant rigidity with no/minimal benefit from therapy	0 1 2 3
SWALLOWING DIFFICULTIES/ ORAL BULBAR FUNCTION	Normal Mild dysphagia (excess drooling) Moderate dysphagia (risk of aspiration, modification to diet required) Severe dysphagia (requiring non-oral feeding)	0 1 2 3
SPEECH	Normal (and those too young yet to speak) Mild to moderate dysarthia impairing intelligibility to unfamiliar listener Severe dysarthia with most speech unintelligible to familiar and unfamiliar listener Anarthria	0 1 2 3
NEUROLOGY FUNCTION		
OPHTHAMOLOGY	Normal Cranial Nerve Palsy (previously corrected or not) Cranial Nerve Palsy (reappearing despite surgical correction)	0 1 2
SPINAL ALIGNEMENT (Kyphosis)	Normal Mild kyphosis – but flexible Moderate kyphosis – partially corrected Severe kyphosis – fixed	0 1 2 3
AGE AT ONSET OF FIRST SEIZURE	Younger than 5 years 5 – 10 years 10 – 15 years 16 years or over, or seizure free	3 2 1 0
TOTAL		/36

2.3.5 Responsiveness for Detecting Change

During the intra-rater agreement analysis, it became apparent that the SST was sensitive enough to capture change. However analysis of this was required on a much larger scale to truly demonstrate this, and importantly to incorporate the modifications made to the SST. A separate piece of work was therefore conducted utilising the mSST.

Thirty nine (*n*=39) NGD patients from three European countries (Germany, Poland, UK) were assessed sequentially using the mSST. These were the same patients assessed in the original work performed during initial development of the SST. The Swedish cohort (*n=12*) could not be included in this review as the original managing physician had since retired and care of the patients had been dispersed. Out of the remaining 43 patients assessed in the original 2007 review, two patients had died and two patients had been lost to follow up. The time interval between assessments was 3.9 years (±0.55). The mean age at follow up was 18.5 years (±9.9). As per original publication (Davies *et al.* 2007a), the British cohort was the youngest while the Polish cohort was the eldest. 69.2% of the cohort was L444P homozygote, which is only slightly lower than that previously reported, and an indication of the missing Swedish cohort. Genotypes were divided into four categories. These categories were based on the original publication (Davies *et al.* 2007b), in order to allow for consistency in comparisons. Group 1 were all L444P homozygote. Group 2 had one allele of L444P with another allele, as follows F213I/L444P, L444P/G202R, L444P/E326K and L444P/E233D. Group 3 all had a D409H and L444P allele. Group 4 included all the remaining genotypes, as follows:

L279P/G243V, R433S/R433S, D409H/G202R, 1599AG/1603T. Demographics can be seen in Table 2.16

Table 2.16: Demographic data of European NGD cohort assessed

	Poland	**Germany**	**UK**	**Total**
Number	18	10	11	39
Mean Age (years) at baseline	19.2 (±11.1)	13.8 (±8.8)	9.7 (±4.35)	15.1 (±9.8)
Mean Age (years) at follow up	23.2 (±11.2)	17.9 (±8.8)	13.1 (±4.6)	18.5 (±9.9)
Median Age (years) at baseline	19.6	11.9	9.3	11.9
Median Age (years) at follow up	15.7	16.0	13.3	16.0
Genotype Group 1: L444P/ L444P	14	5	8	27 (69.2%)
Genotype Group 2: L444P and other	1	1	2	4 (10.2%)
Genotype Group 3: L444P and D409H	2	2	0	4 (10.2%)
Genotype Group 4: All Other Genotypes	1	2	1	4 (10.2%)
Total splenectomy	6	2	0	8 (20.5%)

In addition to mSST score, Chitoriosidase levels and current ERT dose were collated where possible. The Genotype and spleen status of each patient was already available from the original study. Genotype and whether the patient has had a total splenectomy have been reported to be correlate with disease severity, and therefore important to be considered for analysis. This was to offer a complete overview of the patients assessed, and allow for exploratory analysis of any patterns seen across the cohort.

Mean ERT dose was significantly lower at the time of follow up, and a reflection of the revised guidelines which states that given the lack of evidence, high dose ERT is no longer justified (Vellodi *et al.* 2009) and current worldwide shortage of Cerezyme.

Despite the reduced dose, the mean chitotriosidase was lower, although not quite reaching statistical significance, possibly indicating that time on ERT, rather than dose of ERT impacts chitotriosidase levels, as seen in Table 2.17.

The data was not normally distributed and therefore compared using a non-parametric related-samples Wilcoxon test.

Table 2.17 Median (Range) Enzyme Replacement Therapy and Chitotriosidase data for the European cohort

	Poland	**Germany**	**UK**	**Total**	**Sig. (2-tail)**
Median Baseline Dose of ERT (IU/kg per 2 weeks) *(n=34)*	30.0 (26-59)	87.2 (64-117)	117 (101-173)	70.4 (30-107	0.000 *
Median Follow Up Dose of ERT (IU/kg per 2 weeks) *(n=34)*	26.5 (16-44)	56.5 (44-69)	114 (102-1009)	51.0 (25-84)	
Median Baseline Chitotriosidase (nmol/hr/ ml) *(n=33)*	2260 (710–6128)	1185 (423-3575)	787 (520-2280)	1544 (542-3562)	0.020 *
Median Follow Up Chitotriosidase (nmol/hr/ ml) *(n=33)*	547 (330-2185)	1145 (265-3429)	557 (388-1700)	625 (340-2278)	

Severity Scoring Tool Follow up results

The original SST scores were transformed to the new mSST score – to incorporate the '*weighting'* of each domain and the addition of the 12th domain *'Age at onset of seizures'*.

The median original SST score for this cohort (*n*=39) was 5.75 (3.9-7.5) which reduced to 4.0 (2-6.5) when converted to mSST scores. This was expected, as the frequency of the domains now scoring five points (Epilepsy and Pyramidal) occurs in a relatively small percentage of the cohort, while HGP which present in all patients, including those who are mild in presentation, now has a total domain score of one. The true value of converting the SST to mSST scores is appreciated when one looks at the effect of converting the scores across genotypes.

The median baseline mSST score, generated by converting the original SST score, increases the difference in score across the various genotypes; reducing the D409H and L444P allele group the most, from 4.25 (3.6-5.6) to 2.75 (2.1-3.4) – also making the range slightly narrower. The L444P homozygote group reduced in median score from 5.75 (3.8-7.0) to 4.0 (2.0-6.5) while the L444P/other group increased marginally from 9.0 (3.75-14.3) to 9.5 (2.6-13.4), retaining it as the highest scoring genotype and increasing the difference between this group and the milder phenotype seen in the L444P homozygote cohort and those with D409H/L444P allele. The last group (Group 4) which included all the 'other genotypes' specified increased in score from 6.5 (6.5-12.8) to 8.0 (3.0-11.1) (Figure 2.3).

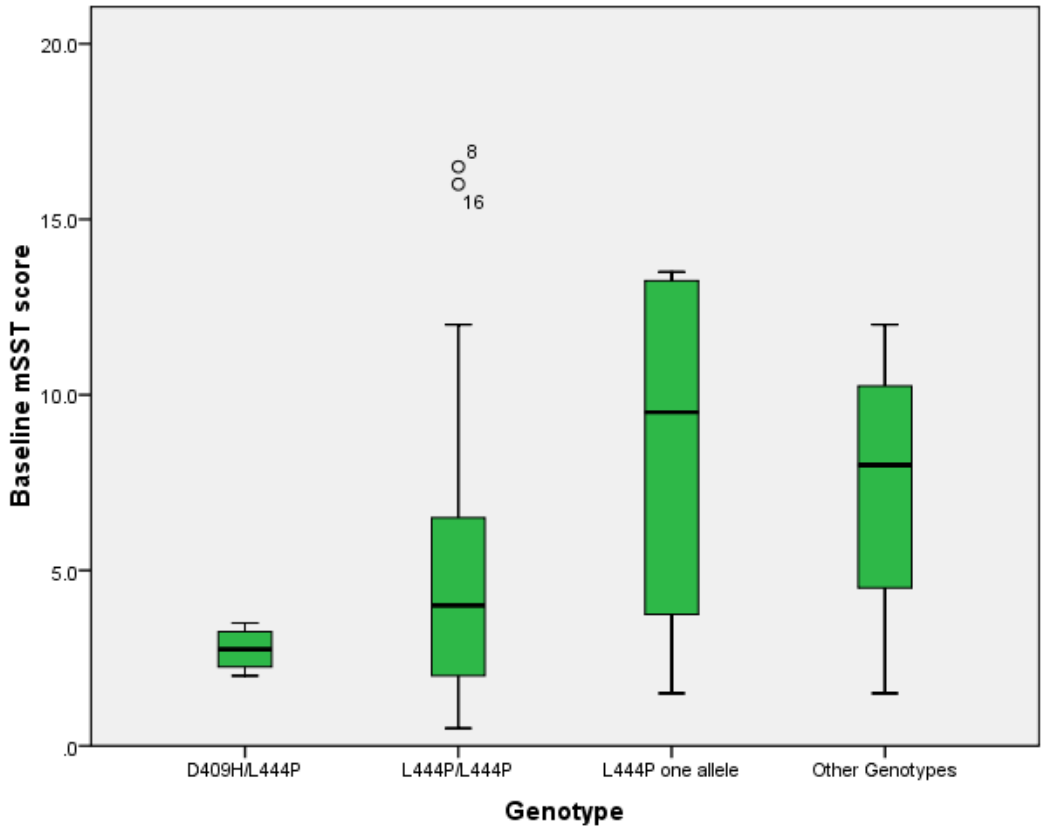


Figure 2.3: Median Baseline mSST score across genotypes (n=39)

The median follow up mSST score for the cohort collectively was 6.0 (2.0-10.0), an increase of 2.0 compared to baseline mSST score. Again this differed greatly across the various genotypes 3.0 (1.1-4.1) in those with a D409H and L444P allele; to 12.0 (3.5-19.4) in those with only one L444P allele; to 13.3 (3.6-23.6) in the 'other genotypes' cohort (Figure 2.4). The large inter-quartiles range is a reflection of the heterogeneity seen in these heterozygote patients.

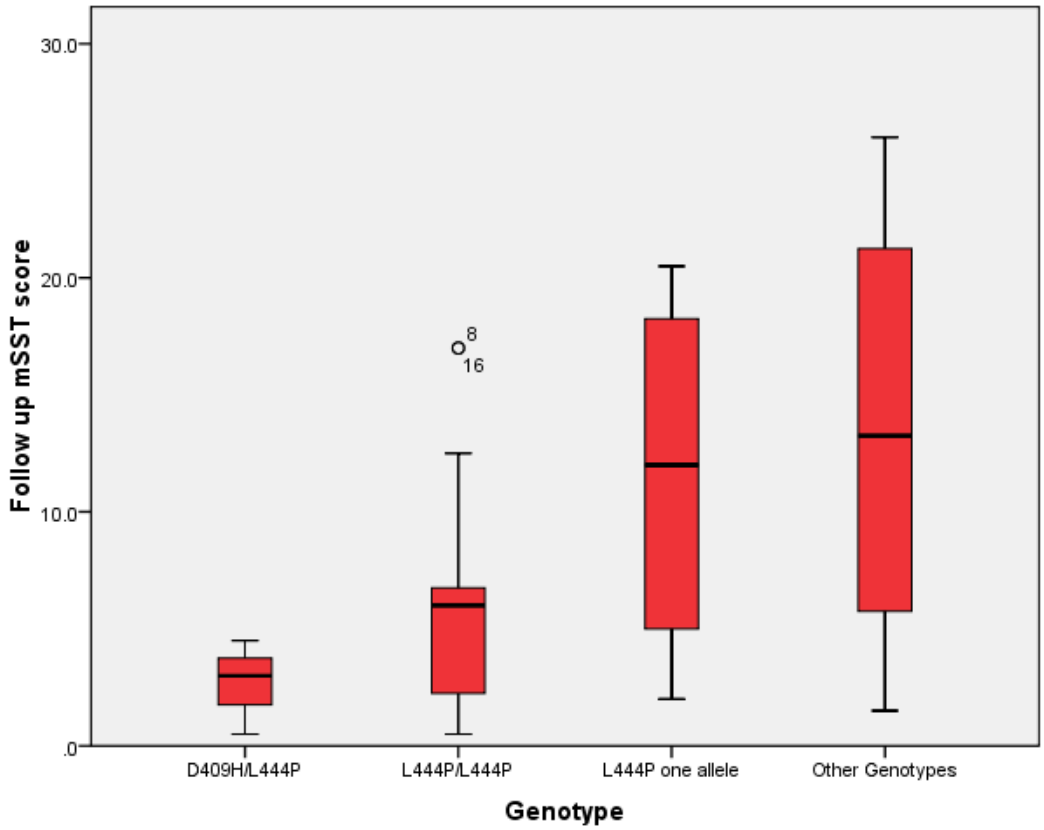


Figure 2.4: Median Follow up mSST score across genotypes (n=39)

Using a Wilcoxon paired test, to account for the non parametric data and small sample size, the median change in mSST score across the whole cohort is statistically significant *p* 0.007 (Table 2.18 and Figure 2.5). According to genotype. Only the L444P/L444P cohort is statistically significant in change from baseline to follow up (*p.* 0.032). The change in mSST score seen for all other genotype groups is not statistically significant, despite the large increase for two of the groups – possibly a reflection of the small sample size and large heterogeneity.

Despite the small sample size, a oneway ANOVA between groups was performed in a bid to explore if the differences seen across genotypes in baseline, follow-up and

mean change of mSST scores is statistical significant. The difference is only statistically significant however for the follow-up scores *p.*0.019.

Table 2.18: modified Severity Scoring Tool scores at Baseline and Follow-Up (Median and Q25/Q75)

	Baseline mSST (Q25/Q75)	**Follow Up mSST (Q25/Q75)**	**Sig. *p***
Poland *(n=18)*	3.5 (1.9-5.6)	5.0 (2-6.5)	0.152
Germany *(n=10)*	5.5 (2.3-9.8)	8.5 (2.6-16.1)	0.043*
UK *(n=11)*	6.0 (2-12)	6.0 (1.5-12.5)	1.77
Cumulative cohort *(n=39)*	4.0 (2-6.5)	6.0 (2-10)	**0.007***
Genotype Group 1: L444P/ L444P *(n=27)*	9.5 (2.6-13.4)	6.0 (2-7)	0.032*
Genotype Group 2: L444P on one allele *(n=4)*	2.8 (2.1-3.4)	12 (3.5-19.4)	0.465
Genotype Group 3: L444P and D409H *(n=4)*	8.0 (3-11.1)	3.0 (1.1-4.1)	1.000
Genotype Group 4: All Other Genotypes *(n=4)*	3.5 (2-6.5)	13.3 (3.6-23.6)	0.109
Not Splenectomised *(n=31)*			0.015*
Splenectomised (*n*=8)			0.259
Age at follow up =< 18 years (*n=24)*	3.5 (2-6.5)	6.3 (1.6-9.8	0.018*
Age at follow up => 18 years (*n=15)*	5.5 (2-8.5)	5.0 (3-12)	0.178

The UK cohort demonstrated the highest change in mSST score, closely followed by Germany (Table 2.18), which is a reflection on the heterogeneity of these cohorts and the younger age compared to the Polish, which is mainly L444P/L444P (78%). Given the fact that the UK cohort was consistently on the highest dose of ERT with the lowest Chitotriosidase levels it reaffirms that Chitotriosidase as a visceral marker does not reflect the severity of neurological manifestations. It also appears to

indicate, in keeping with the revised guidelines, that high dose ERT does not halt neurological progression in these severe cases.

As indicated in the introduction, NGD patients are reported to progress more rapidly neurologically following a complete splenectomy. The difference in mean mSST score between splenectomised and none splenectomised patients at baseline was statistically significant *p* 0.0062. However, at follow up the non-splenectomised patients had progressed more and this statistical difference between the two groups was lost. The difference in mean change of mSST score between the two groups is not statistically significant either; however the mean difference between baseline and follow up is statistically significant for those with an intact spleen *p.*0.015. This indicates that splenectomised patients has greater disease severity, but do not endure disease progression as the same rate as those with intact spleens. This is contradictory to that previously published, however these findings are biased by the fact that young more severe affected children, with more progressive disease are no longer splenectomised, while the splenectomised patients are still alive at an older age, and therefore indicating a milder rate of disease progression. Details of splenectomised patients are presented in more detail in Appendix 3.

The situation is similar when exploring the data according to age brackets (Younger than 18 years at time of follow up assessment vs Older than 18 years at time of follow up assessment), although without a statistically significant difference of scores at baseline. Those younger than 18 years at the time of follow up

assessment had an increase in mean mSST score of 2.3 (±4.6) which is statistically significant (*p* 0.26) compared to 0.8 (±2.2) for those over 18 years.

The similar pattern in the older patients and splenectomised patients is a reflection of the fact that the majority of splenectomised patients are the Polish older ones and that only one of the eight splenectomised patients was younger than 18 years of age at the time of follow up assessment.

Despite the overall increase in mSST score across the cohort there were 10 patients that reported an improved score. Five patients only improved by 0.5 which was based on improvement in Cerebellar signs/Ataxia. Four of the five were on SRT at the time of baseline assessment - a side effect of SRT treatment is intention tremor, which has resolved since stopping the SRT. Another patient was also on SRT at the time of baseline assessment, with a marked impairment of ataxia/gait, intention tremor and pyramidal involvement. These manifestations were improved at follow-up, with a correlating improvement in mSST score.

Individual domain analysis

An attempt was made to identify in which of the 11 domains the greatest change was seen between Baseline and Follow-Up. A basic overview of the manifestation seen (in %), regardless of level of severity is presented in Figure 2.5.

It can be seen that the presenting percentage has remained constant for HGP and Pyramidal. Involvement of Cognitive Ability, Ataxia/Gait, Speech and Extrapyramidal

has increased at a similar rate of 2.5%. Epilepsy and Kyphosis are increased by 5% while Cerebellar Signs/Ataxia involvement is nearly 13% higher. This is despite accounting for the fact that 5 patients had seen an overall improvement of 0.5 mSST score in relation to improved Cerebellar Signs/Ataxia. Two domains, Swallowing and Ophthalmology noted a 2.5% reduction in involvement. Given the poor inter-rater reliability for the Ophthalmology and Kyphosis domains, these results should be interpreted with caution.

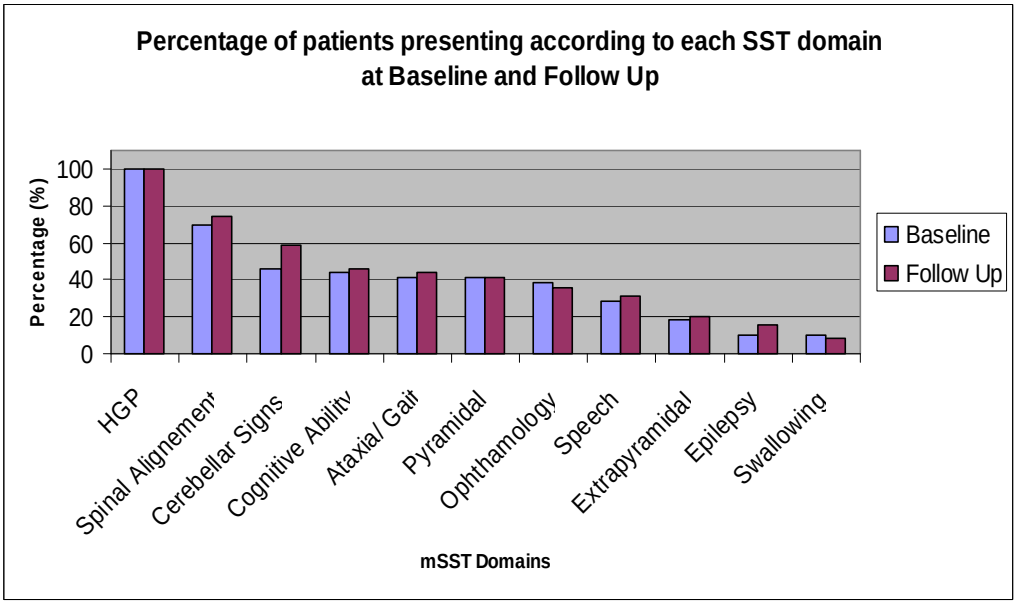


Figure 2.5: Percentage of patients (n=39) presenting with each individual domain at baseline and follow up.

Examining each individual domain in more depth identifies that the changes within a domain is statistically significant for nine out of the eleven domains. This is measured using a Pearson Chi-Square. The nine domains in question are HGP, Kyphosis, Epilepsy, Pyramidal, Speech, Ophthalmology, Ataxia/Gait, Swallowing and Extrapyramidal. These findings are presented in Table 2.19-2.27.

Table 2.19: Pearson Chi-Square and individual changes for HGP domain

		Follow Up Horizontal Gaze Palsy		
		Horizontal Saccades absent, Vertical Saccades present	Horizontal and Vertical Saccades absent	Total
Baseline Horizontal Gaze Palsy	Horizontal Saccades absent, Vertical Saccades present	20	10	30
	Horizontal and Vertical Saccades absent	2	7	9
	Total	22	17	39
Pearson Chi-Square 0.018				

This table indicates that the number of patients with “Horizontal and Vertical Saccades absent” had increased from nine to seventeen, a 20% increase. This increase in presentation is significant, however given the difficulty in assessing saccades without sophisticated equipment there may be some variability to this number.

Table 2.20: Pearson Chi-Square and individual changes for Epilepsy domain

		Follow Up Epilepsy			
		No seizures	Seizures controlled with AED	Seizures requiring combination therapy or resistant to AED	Total
Baseline Epilepsy	No seizures	31	3	1	35
	Seizures controlled with AED	0	0	3	3
	Seizures requiring combination therapy or resistant to AED	0	0	1	1
	Total	31	3	5	39
Pearson Chi-Square 0.000					

AED – Anti epileptic drugs.

The number of patients who are seizure free is reduced from 35 to 31 in this four year period. This equates to a doubling in the number of patients who experience seizures. At follow up this equates to 20% of the cohort, which is not to different to the 16% reported in the NGD registry publication (Tylki-Szymanska *et al.* 2010) . What is striking is that the number of patients with "Seizures requiring combination therapy or resistant to AED" has increased from one to five. This is in keeping with the known progressive nature of the seizures seen in NGD.

Table 2.21: Pearson Chi-Square and individual changes for Ataxia/Gait domain

		Follow Up Ataxia/ Gait			
		Normal/ apparent only on tandem walking	Ataxia on straight gait, able to walk without assistance	Able to walk only with assistance	Total
Baseline Ataxia/ Gait	Normal/ apparent only on tandem walking	19	4	0	23
	Ataxia on straight gait, able to walk without assistance	6	6	4	16
	Able to walk only with assistance	0	0	0	0
	Total	25	10	4	39
Pearson Chi-Square 0.006					

The total of number of patients with 'Normal/ apparent only on tandem walking' has increased from 23 to 25 during the follow up period, indicating that 2 patients have become symptom free in this domain. However the number of patients 'Able to walk only with assistance' has increased from 0 to 4 in the same period, indicating that it is more likely to be progressive for some patients.

Table 2.22: Pearson Chi-Square and individual changes for Pyramidal domain

		Follow Up Pyramidal				
		Normal tone with increased reflexes	Mildly to moderately increased tone and reflexes	Increased tone reflexes with sustained/ unsustained clonus	Severe spasticity with inability to walk	Total
Baseline Pyramidal	Normal tone with increased reflexes	19	4	0	0	23
	Mildly to moderately increased tone and reflexes	3	3	3	0	9
	Increased tone reflexes with sustained/ unsustained clonus	1	1	4	1	7
	Severe spasticity with inability to walk	0	0	0	0	0
	Total	23	8	7	1	39
Pearson Chi-Square 0.001						

Table 2.23: Pearson Chi-Square and individual changes for Extrapyramidal domain

		Follow Up Extrapyramidal			
		Normal	Variable tone and posturing not impairing function, with or without therapy	Variable tone and posturing impairing function, despite therapy	Total
Baseline Extrapyramidal	Normal	29	0	3	32
	Variable tone and posturing not impairing function, with or without therapy	1	1	1	3
	Variable tone and posturing impairing function, despite therapy	3	1	0	4
	Total	33	2	4	39
Pearson Chi-Square 0.014					

Overall there appears to be only minimal changes in the Pyramidal features but it does show a progressing trend – which are very statistically significant. A very similar pattern is also seen in the Extrapyramidal domain.

In the Swallowing domain it appears that one patient has improved, while two have remained symptomatic with mild symptoms, while a fourth has progressed to severe symptoms.

Table 2.24: Pearson Chi-Square and individual changes for Swallowing domain

		Follow Up Swallowing difficulties/ Oral bulbar function			
		Normal	Mild dysphagia (excess drooling)	Severe dysphagia (requiring non-oral feeding)	Total
Baseline Swallowing difficulties/ Oral bulbar function	Normal	33	2	0	35
	Mild dysphagia (excess drooling)	3	0	1	4
	Severe dysphagia (requiring non-oral feeding)	0	0	0	0
	Total	36	2	1	39
Pearson Chi-Square 0.010					

Again there appears to be very little change in the Speech domain. However this change is statistically significant. At the time of follow up, it appears that one patient has progressed to having “Severe dysarthia with most speech unintelligible to familiar listener”.

Table 2.25: Pearson Chi-Square and individual changes for Speech domain

		Follow Up Speech			
		Normal (and those to young yet to speak)	Mild to moderate dysarthia impairing intelligibility to unfamiliar listener	Severe dysarthia with most speech unintelligible to familiar listener	Total
Baseline Speech	Normal (and those to young yet to speak)	24	4	0	28
	Mild to moderate dysarthia impairing intelligibility to unfamiliar listener	3	7	1	11
	Severe dysarthia with most speech unintelligible to familiar listener	0	0	0	0
	Total	27	11	1	39
Pearson Chi-Square 0.001					

Table 2.26: Pearson Chi-Square and individual changes for Ophthalmology domain

		Follow Up Ophthalmology			
		Normal	Cranial Nerve Palsy (previously corrected or not)	Cranial Nerve Palsy (reappearing despite surgical correction	Total
Baseline Ophthalmology	Normal	15	8	1	24
	Cranial Nerve Palsy (previously corrected or not)	7	5	1	13
	Cranial Nerve Palsy (reappearing despite surgical correction	0	0	2	2
	Total	22	13	4	39
Pearson Chi-Square 0.001					

Table 2.27: Pearson Chi-Square and individual changes for Kyphosis domain

		Follow Up Spinal Alignment (Kyphosis)				
		Normal	Mild kyphosis - but flexible	Moderate kyphosis - partially corrected	Severe kyphosis – fixed	Total
Baseline Spinal Alignment (Kyphosis)	Normal	8	4	0	0	12
	Mild kyphosis - but flexible	2	9	2	5	18
	Moderate kyphosis - partially corrected	0	0	2	3	5
	Severe kyphosis – fixed	0	0	0	4	4
	Total	10	13	4	12	39
Pearson Chi-Square 0.000						

The changes in the Ophthalmology domain indicate again that the number of symptomatic and the severity of the symptom progresses over time. At follow up assessment, four patients have a “Cranial Nerve Palsy; reappearing despite surgical correction”.

The Kyphosis domain clearly demonstrates not only an increase in the number of presenting patients, but also a progression of symptoms within the domain. The greatest increase is seen in the number of presenting patients with ‘severe’ kyphosis, which is three times higher at follow up. Even accounting for the fact that Kyphosis had poor inter-rater agreement, this amount of progression can be viewed as a clinically important aspect in the management of NGD patients.

The two domains that are not statistically significantly different are Cerebellar Ataxia and Cognitive Ability. As Cerebellar Ataxia had demonstrated nearly 13% increase in percentage of patients with this manifestation it is unexpected that significance

was not reached in the Chi-Squared at *p.*0.057. The following two domains with highest increase in percentage of involvement, Epilepsy and Kyphosis (both at 5%) demonstrate the highest statistical significance in Chi-Square at 0.000 for both.

Value of mSST in predicting disease course

Regression is a topic that considers using the relationship between two or more variables for prediction (Pagano 2004). In Multiple Regression the value of a dependent variable, in this case mSST score at follow up are estimated from those of other variables. This is achieved by the construction of a linear multiple regression equation of the general form:

$y' = b_0 + b_1(x_1) + b_2(x_2) + b_3(x_3) + b_4(x_4) + b_5(x_5)$

Where b_0 represents the intercept, or regression constant, while $b_1\ b_2\ b_3\ b_4\ b_5$ represents the partial regression coefficient, or the slope of the five variables. This equation is known as the multiple linear regression equation of y upon $x_1\ x_2\ x_3\ x_4\ x_5$

While the regression equation assumes that all variables are interval data it is also accepted that ordinal variables can be used. Categorical variables can only be entered into the equation when the have been transformed into ‘dummy variables’.

Pearson correlation (*r*) assesses the magnitude and direction of a relationship between two interval variables. A perfect correlation takes a value of ±1 (range from -1 to +1) when all the points in the scatter-plot lie on the regression line. Correlation, in this instance in primarily concerned with exploring if a relationship exists, with its

magnitude and direction. To enable the relationship to be used for prediction, a regression analysis is required.

The independent variables considered likely to predict Follow Up mSST were: Baseline mSST, Genotypes, Spleen status, Age at first assessment and FSIQ at baseline. ERT dose IU/kg/2 weeks and Chitotriosidase are other variables that may have an impact on disease progression, however given the fact that their direct link to neurological function remains contentious, they were not included.

There are two general rules of thumb regarding the number of predictors that can be used in a model depending on the sample size in question. Generally there should be no more predictors in the model than *n*/10 or alternatively Square Root (*n*). So for this sample size of *n*=39 four to six predictors can be used, depending on the rule used.

R represents the multiple correlation coefficients, which is 0.897. *R* Square (R^2) is an estimate of the proportion of variance accounted for by regression. This is a positively biased estimate of the proportion of the variance of Follow up mSST accounted for by the regression. This is corrected in the Adjusted R^2 at .727. Using the Adjusted R^2 which takes into account sample size and the number of variables, and therefore a better estimate of the population, indicates the effect size is 73%, which is a large effect size.

Using the multiple regression equation to predict the 'Follow up mSST score' for any given patient, the following model can be proposed:

Follow up mSST score' =

5.485 + -0.151 x (Age) + -0.037 x (FSIQ) + 0.999 x (Baseline mSST) + 7.712 x (D409H/L444P) + -0.492 x (Other Genotypes) + 2.077 x (Splenectomised).

The standard error of the slope is ±3.458 and the 95% Confidence Interval for the slops is -1.9-12.9.

Little can be gathered about the relative importance of the variables from the sizes of their regression coefficients because the values of the partial regression coefficients reflect the original units in which the variables were measured. The Standardised Coefficient (Beta) allows all the independent variables to be comparable, as they are all expressed in a standardised form, as seen in Table 2.28.

Table 2.28: Multiple Regression model to predict 'Follow up mSST score' for any given patient, demonstrating that only Baseline mSST significantly contributes statistically

	Unstandardised Coefficients		Standardised Coefficients			95% Confidence Interval for B	
	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
(Constant)	5.485	3.458		1.586	.134	-1.886	12.856
Age at first assessment	-.151	.110	-.352	-1.369	.191	-.387	.084
FSIQ at baseline	-.037	.034	-.161	-1.094	.291	-.110	.035
Baseline mSST	.999	.181	.865	5.518	.000**	.613	1.385
D409H and L444P	7.712	4.684	.393	1.646	.121	-2.273	17.696
Other Genotypes	-.492	2.338	-.025	-.210	.836	-5.476	4.492
Splenect-omised	2.077	1.935	.196	1.074	.300	-2.047	6.202

a. Dependent Variable: Follow up mSST score

The Beta coefficient is the change in ‘Follow up mSST score’; the dependent variable (expressed in standard deviation units) produced by a positive increment of one standard deviation in the independent variable. In this instance, mSST score at baseline makes the greatest contribution to predicting follow up mSST score - as a change of one standard deviation on that variable produces a change of 0.865 on follow up mSST score. Indeed, baseline mSST score is the only significant predicting variable. This seemed surprising considering the group difference seen between Genotypes in previous analysis of change in mSST score between baseline and follow up.

However this multivariable regression model is for each factor after adjusting for others. Genotype as a variable is therefore not significant after taking into account the other variables, primarily baseline mSST.

When Genotype alone is used as a predicting variable, the regression model is as follows:

Follow up mSST score’ = 6.130 + 5.495 x (L444P/Heterozygote) + -3.380 x (D409H/L444P) + 7.370 x (Other Genotype)

(Constant being L444P/L444P).

‘Other Genotype’ is the only Significant predicting variable (*p*0.018) , with L444P/Heterozygote closest there after, but not significant at *p*0.071.

However the Adjusted R^2 for the model summary where only Genotype alone is included accounts for only 18% of the change seen. In the same regard the Adjusted R^2 for a model summary where only baseline mSST score is included is 58%. This is 15% less than when all the variables are included in one model. This indicates that Genotype, Spleen status, FSIQ at baseline, Age at first assessment and baseline mSST score, collectively, contribute to the most predictive model and should therefore all be retained. This appears to be consistent with the clinical phenomenon seen as well.

2.4 Discussion on mSST development

The original aim in developing the SST was to create a tool that was feasible to use, easy and quick to apply, and without any cultural or economical constraint (Davies *et al.* 2007b). The modifications made to the SST based on this work means that the mSST has been validated in line with the E9 ICH guideline for the development of a tools (International Conference on Harmonisation 1998), and could be used as a primary endpoint in a clinical trial – offering a measurement of a clinically relevant and important benefit to the patient population.

During the inter-rater evaluation Ophthalmology and Kyphosis was noted to have poor agreement. A reason which may explain why Kyphosis has poor agreement is that this is one domain where the terms mild, moderate and severe were used with minimal additional information included to direct the assessor in making their selection. Agreement may have been better if classification was based on degree of curve according to spinal x-rays. However the SST was devised with the aim of

being completely self-standing, that is, without requiring any additional assessments to calculate score, therefore this approach was not pursued.

The reason for the lack of agreement in the Ophthalmology domain is less clear. One reason may be that the assessor relied on memory rather than medical notes to score if the child had undergone surgical correction or not. Furthermore, on reflection, the distinction between “Cranial Nerve Palsy (previously corrected or not)” and “Cranial Nerve Palsy (reappearing despite surgical correction or not)” may be ambiguous. The first intended to reflect that a cranial nerve palsy may not be visible following surgical correction, while the latter reflects a patients whose nerve palsy reappears and persistent despite surgical intervention, a reflection of increased severity.

This suggests that these domains would benefit from further guidance and possible training to improve the concordance of opinion. Producing a video clip of a patient being assessed might be the most effective approach in this regard, particularly if the tool was to be used for clinical trial purposes.

Intra-rater evaluation findings were particularly encouraging, in that not only was intra-rater agreement demonstrated, but also the sensitivity of the mSST in capturing and quantifying disease progression. This sensitivity to capture and quantify disease progression was demonstrated on a greater scale during the follow up assessment of the European cohort.

The twelfth domain *'Age at Onset of Seizure'* was categorised in consultation with the experts and categorised into the age brackets of 0-5 years =3. 6-10 years =2. 11-15 years =1. 16 years and over or seizure free =0. On reflection, this may not have been the best categorisation selected. The International Conference of Harmonisation, E11 guideline on Clinical Investigation of Medicinal Products in the Paediatric Population, paediatric patients are classified as preterm newborns, terms newborns, infants and toddlers (28 days to 23 months), Children (2 to 11 years) and Adolescents (over 12 years). The age epochs utilised in epilepsy are 0-2 years, 3-5 years, 5-12 years and 12-18 years. The choice of age categories may therefore be subject to some criticism for failing to follow one of these. In the registry data of 131 NGD patients reported (Tylki-Szymanska *et al.* 2010) the median age myoclonus seizures were first noted at 2.5 years, while the median age of first seizure noted was 5.4 years. Given the categories of 0-5 years and 5-10 years it allows for the presentation of myoclonus seizures and other types of seizures to be accounted for separately.

Although not formally tested, the credibility of the mSST can be assumed to be quite high as it was modified using consensus formation among leading experts from around the world.

The MCID for worsening calculated from our data, must be considered with caution, and may not be valid as the numbers are small. Furthermore it was not possible to discuss MCID for improvement. However it does provide a benchmark, which can be considered again during continued monitoring of NGD patients with the mSST.

By performing the follow up assessment the mSST has also been demonstrated to be sensitive enough to capture change. The rate of progression identified is reflective of the slow nature of disease progression seen in the majority of NGD patients. The ability to be responsive to change, no matter how small, is obviously one of the key most important aspect of an useful assessment tool.

Weighting the domains and adding a twelfth domain, based on the opinion of the experts, has ensured that the domains that contribute most morbidity to the disease is reflected appropriately, while increasing the difference in scores between mild, moderate and severe patients. The modifications made to the SST have improved the ability of the tool to distinguish between severely affected patients and mild patients, which is particularly evident when summarising mSST scores according to genotype.

This ability of the mSST to measure disease severity as opposed to measuring progression can be considered in terms of total mSST score generated and individual domain. Based on the fact that the total mSST score progressed with statistical significant in the follow up period, and was able to distinguish between genotypes according to phenotype severity is demonstrative that the mSST is capable of measuring both severity and progression. This is further supported by the regression analysis performed to examine the value of the mSST in predicting disease course.

It must be acknowledged that each domain is unable to monitor progression once the worst level of severity, according to the defined criterion, has been reached – this is commonly referred to as a 'ceiling affect'. For example, in the HGP domain when deterioration classified as both horizontal and vertical saccades absent has been reached. As none of the patients assessed presented with the most severe criterion defined in all the domains – that is, none scored maximum possible score, reaching a 'ceiling affect' that prohibits its value for on-going monitoring of disease progression is not considered to be a problem.

The cohort studied here represents the largest cohort of NGD patients every studied systematically. Four years follow up period can also be regarded as a sufficient period of time to capture real change. Through validating the mSST and using it in this context, this work offers a valuable insight to the *'natural history'* of NGD patients in the ERT era, not only in terms of the neurological domains involved, but also in terms of the rate of disease progression. This will be of particular interest in the selection of clinically meaningful end points in future clinical trials.

Chapter 3

GAIT analysis

If you don’t like the road that you’re walking, start paving another one

- Dolly Parton

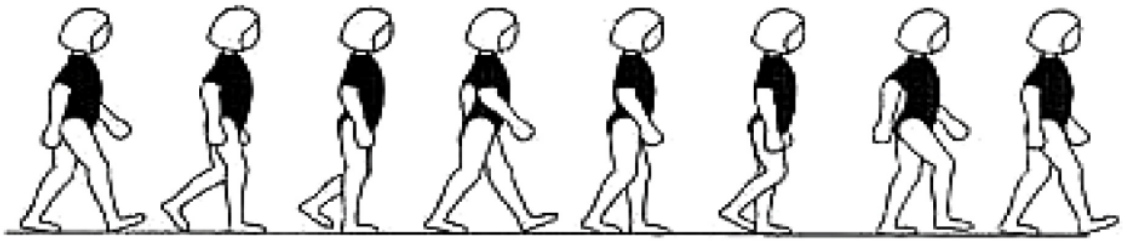
3 Gait Analysis

3.1 Introduction

Locomotion is the act of getting from one place to another. It is an action that involves the change of position of the body and limbs in space and time. **Gait** is the means of achieving the action of locomotion and is specified by the goal and environment in which the task is carried out. **Walking** is therefore a type of gait. Walking is a basic motor skill and the first locomotion pattern to appear in children in an upright position (Holm *et al.* 2009).

Walking measurement is typically simplified, and timed performance often used to describe walking and provide an impression of its efficiency. The assessment of gait involves more than simply measuring the distance walked however. As measures of walking alone may fail to identify subtle disorders or changes associated with postural control, measuring the components of the gait cycle has the potential to quantify clinical change. Gait analysis is the systematic study of human motion. This is an essential prerequisite for any clinical tool that purports to evaluate the effectiveness of therapy (Whittle 2007).

The normal gait cycle is shown in Figure 3.1. It is a fundamental unit to describe the gait during ambulation, which occurs from the time when the heel of one foot strikes the ground to the time at which the same foot contacts the ground again (heel strike to heel strike of the same foot). It has two phases, a *stance phase* comprising approximately 62% of the cycle and a *swing phase* comprising about 38%. The stance phase is the duration when the foot is in contact with the ground. The swing phase is when the foot is in the air.



Initial contact | Loading response | Mid stance | Terminal stance | Pre swing | Initial swing | Mid swing | Terminal swing

Figure 3.1 The normal gait cycle

Gait is made of *strides* and *steps*. A *stride* is measured from the heel strike of one foot to the next heel strike of the same foot. It is synonymous with the gait cycle. A *step* is measured from the heel strike of one foot to the heel strike of the opposite foot. One gait cycle equals one stride and two steps.

Gait assessment typically quantifies gait in temporal and distance terms, however the assessment of balance during walking is often overlooked. Gait parameters are primarily defined in terms of *temporal* and *spatial parameters.*

The temporal parameters of gait are as follows:

Cadence – the number of steps taken over a unit of time

Velocity – distance over an unit of time, or as *Step Length x Cadence/60.*

Stride time- the time taken to complete a full gait cycle.

Step time – the time taken to complete a right or left step. It is the reciprocal of Cadence for a symmetric gait.

Stance time – the duration when the foot is on the ground during a gait cycle, approximately 62% of a gait cycle.

Single Support time – the duration when only one foot is on the ground during a gait cycle.

Double Support Time the duration when both feet are on the ground simultaneously during a gait cycle. It is approximately 22% of the gait cycle, but decreases as walking speed increases.

Swing Time – the duration when the foot is in the air during a gait cycle. It compromises approximately 38% of the gait cycle and decreases as walking speed increases.

The spatial (distance) parameters of gait are as follows:

Stride Length – the distance between two successive heel contacts of the same foot. It increases as walking speed increases.

Step length is the distance from the heel of the trailing limb to the heel of the landing one, or the distance covered by a step in the direction of locomotion. It is measured in centimetres, and is usually between 80 and 90% of leg length. Symmetry (between the two legs) is usually the norm (Alderson 2007). Any asymmetry is often a predictor of some pathology present – asymmetrical range of movement, altered muscle power etc (Wood *et al.* 2009).

Base of Support (BoS) is the area between the feet in double support, and the area under the foot in single leg stance.

The *temporal-spatial parameters* (TSPs) of gait and their measurement form the basis of any gait. When one step of each (right and left) has occurred, the person has taken a *stride*, or performed a gait cycle, and the time it takes for this to occur is

called the gait cycle duration, or stride time. The gait cycle starts (0%) with the first contact (initial contact, often called heel contact in normal gait) of one foot, so that the end of the cycle (100%) occurs when the next contact of the same (ipsilateral) foot, which will be the initial contact of the next cycle. In normal, symmetrical walking, toe-off occurs at about 60-62% of the gait cycle, dividing the cycle into stance (when the foot is on the ground) and swing phases. Since there are two lower-limbs, the events on the opposite (contralateral) limb are offset by 50%. When one limb is in swing phase, the other is in stance. Since each stance phase is 60%, and 2 x 60 = 120, it follows that for 20% of the normal cycle both feet are on the ground (Kirtley 2006).

Other gait parameters measured are Double Support, Single Support and Base of Support. Double Support is the duration of time when both feet are in contact with the ground. There are two periods of double support in any gait cycle, which corresponds to the transfer from the first step to the second step. It is expressed in relative terms as a percentage of gait cycle rather than time in seconds. Single Support is the duration of time when only one foot is in contact with the ground, and again is expressed in percentage of gait cycle rather than time in seconds
Base of Support depends on the step width and the angle of foot placement relative to the line of progression. BoS is usually measured as the perpendicular distance between the heel centre of one foot and the line that joins the heel centres of the previous and following contralateral feet.

Stance phase is slightly longer while in bare feet compared to when wearing shoes (Eisenhardt 1996) as shoes provide a slightly increased base of support, which improves balance. This is an important consideration to remember when assessing gait. As balance is compromised, both stance and double support increase to provide an increased support time. This is an example of a compensation strategy which is seen in vestibular, cerebellar (ataxia) or non-specific instability. The step width (mediolateral distance between the heels in double support) also tends to increase with disequilibrium in order to increase base of support although this may only become evident at higher speeds (Krebs *et al.* 2002).

Gait velocity provides an overall measure of how quickly gait is achieved and it influences many other gait parameters. Cadence and Velocity have been used widely in the measurement of gait and are thought to reflect the overall ability to walk and balance. Freely selected walking speed is considered to be a good indicator of how well an individual walks (Alderson 2007). It has been used as an indicator of impairment and functional status, and of treatment efficacy in both clinical and research studies in numerous different clinical groups (Shore *et al.* 2005) (Bladen *et al.* 2007), (Rinehart *et al.* 2006), (Wondra *et al.* 2007), (Dusing *et al.* 2007), (Wood *et al.* 2009).

3.1.2 The development of gait

The gait pattern is a distinctive attribute of the individual that changes over the life span (Sutherland 1997). Gait performance is influenced by change in stature, and also maturation of postural control and coordination of movement. A working

knowledge of the developmental sequence of postural control and the development of mature walking in childhood is necessary to enable an informed clinical assessment.

Children use different walking strategies during development. The toddler has a fast Cadence (steps per minute) and uses Step Length to change speed. After about 5 months of walking, this pattern reverses and Cadence is used to drive Velocity change. The preferred Cadence rapidly decreases over the first two years the slowly decreases towards adults level (Sutherland 1997).

Selby-Silverstein and O'Reilly (2003) investigated the development of gait characteristics in children aged between one and five years. Velocity was also found to increase with age. The Step and Stride Lengths had high positive correlations to age and this relationship did not disappear when normalised to leg length (Selby-Silverstein & O'Reilly 2003).

Normalisation of data to account for growth is a widely disputed issue. It is applied in a number of different ways, often without mathematical justification or a firm basis in dynamics (Stansfield *et al.* 2003).

For scaling gait parameters of children Hof (1996) proposed the use of non-dimensional numbers, based on geometric scaling (Hof 1996). This was applied with success in a study of treadmill walking in children between 4 and 10 year of age

(Zijlstra *et al.* 1994). It was found that children over 7 years of age had essentially adult value gait parameters.

O'Malley J (1996) stated that preferred stride length divided by height keeps increasing with age, from 1 to 7 years (O'Malley 1996). The difference between O'Malley J (1996) and Hof (1996) is that while (Hof 1996) had normalised stride length and other distance parameters by leg length (measured in trochanteric height), O'Malley J (1996) had done so by total height (stature). Leg length increases more proportionally compared to the total body height in humans. Using O'Malley J (1996) data, Hof and Zijlstra (1997) demonstrated that preferred stride length when normalised by division by leg length, is essentially constant from the age of 3 ½ year up to adult (Hof & Zijlstra 1997). Scaling leads to a similar result as with stride length: in young children, normalised cadence increases up to age 3 ½ year and reaches approximately the adult level by then.

Hof and Zijlstra (1997) suggest that:

1. Geometric scaling is superior to raw data: leg length and *g* are the only factors necessary for an effective scaling. Size effects can thus be accounted for in a mechanically consistent way and separated from other effects, like age or pathology.
2. Leg length is a better scaling factor than stature.

However Alderson (2007) reports that as the "leg length to height ratio" is not linear, therefore this approach to normalisation has limitations. Based on this, Alderson (2007) developed percentile charts to graphically illustrate the developmental

changes of gait, according to age. The true value of these charts are in longitudinal measurement.

Alderson (2007) identified that development trends of Velocity continue beyond seven years of age, and were related to factors other than growth (leg length). A change in Velocity was influenced by increased Step Time with a reduced rate of stepping. Older children had less variation from step to step, which along with reduced rate helps to conserve and minimise energy expenditure and maximise efficiency (Alderson 2007).

Selby-Silverstein and O'Reilly (2003) proposed that factors, other than anthropometrical changes that occur with growth influence the maturation of gait. Possible explanations were the continued myelination of the central nervous system, or the refinement of the neuromuscular system. A decrease in coefficient of variation of gait parameters was proposed as an indicator of maturation in gait (Selby-Silverstein & O'Reilly 2003).

3.1.3 Abnormal gait

Different pathologies are believed to affect gait in different ways and research has been done to describe walking and balance ability in different patient groups. Musculoskeletal, cardio-respiratory and central nervous system involvement may impact on gait.

In relation to the central nervous system, peripheral inputs and proprioceptive reflexes processed in the spinal cord, the cerebellum, basal ganglia, and cortical mechanisms contribute to the motor control necessary for normal gait and balance. In addition to the spinal and central mechanisms, visual, vestibular and somatosensory inputs contribute to a stable posture (Thomas *et al.* 2004). Pathology impacting on any of these mechanisms has the possibility to affect balance and gait therefore.

Although the basal ganglia play a central role in the initiation and mediation of movement, the cerebellum is more involved in controlling and tempering end-stage movements. Cerebellar lesions may result in movements that are irregular and variable. A lesion in the cerebellum or spinocerebellar tracts may result in ataxia. Cerebellar ataxia is most apparent in the ambulatory child, where movements of the limbs are uncoordinated because of a lack of harmonious enlistment of all the muscles (dysynergia). The gait is not the only indication of ataxia however; rapid alternating movements are slowed and irregularly timed (dysdiadochokinesia). There may be tremor during voluntary movements that increases as the limb approaches its target resulting in an overshooting of the target (dysmetria), and occasionally there is nystagmus. Speech is typically altered, both slow and uneven in volume, varying from jerky or scanning speech to explosive and occasionally staccato. Muscle tone is generally unchanged or arguably reduced and tendon reflexes may be weak or pendular. Disorders of supranuclear gaze is also a frequent finding in disorders with cerebellar ataxia (Lyon *et al.* 2006). All of which impact on the child's gait.

Clinical descriptions of cerebellar gait typically include a widened base, unsteadiness and irregularity of steps, and lateral veering. The patients may compensate for these abnormalities by shortening steps and shuffling, spending more time on both feet (Adams *et al.* 1997).

Alderson (2007) in her study of normal and impaired walking in children was able to identify deviations from the normal developmental trajectory in numerous different pathologies – Peripheral Neuropathy, Muscular Dystrophy, Spinal Muscular Atrophy, Developmental Coordination Disorder, Cerebellar Pathology and Traumatic Brain Injury. Each group had significant differences from controls for specific outcomes. In addition there were also some significant differences between clinical groups. The Cerebellar Pathology tended to have a slower Normalised Velocity than the control groups. Cadence was also significantly reduced and Step Time increased relative to control. Step Length was significantly shorter, with an increased variation in Step Time and Length relative to controls. The Cerebellar Pathology group is unique in that the reduction in Velocity is related to both reduction in Step Length and an increase in Step Time. This suggested that one or both of these may be a strategy employed to reduce the balance requirements of walking. They also appeared to be the most likely to manifest signs of decreased balance control and reduced time spent in Single Support and increased Double Support.

Children with Peripheral Neuropathy in this study presented with a mixed picture; they appeared to walk more slowly with a limited Step Length, and an increased

Step Time relative to controls. Asymmetry was common place. Alderson (2007) proposed that the asymmetrical walking patterns were due to biomechanical alignments and musculoskeletal limitation – and highlights the importance of combining this information with clinical examination to assess muscle weakness, imbalance and possible loss of joint range.

3.1. 4 Methods for gait assessment

A comprehensive understanding of the normal gait, armed with a sophisticated measuring tool leads to the ability of to compare an abnormal gait in a methodical approach.

Apart from observational assessments armed with a stop-watch there are seven different types of tools available to measure walking balance: Force Platform, Pressure Platform, Pressure Walkway, Pressure Insoles, Accelerometer, Gyroscope, 3D Motion Analysis. Force Platform and Pressure Platform are unable to measure temporal-spatial gait data, while Pressure Insoles, Accelerometer, Gyroscope, 3D Motion Analysis are relatively not easy and quick to set up, and in most, to use. This, supported by the low-moderate cost (£10,000-20,000) makes Pressure Walkways an attractive option (Alderson *et al.* 2007).

Pressure sensitive walkways register multiple steps, but do not require insoles or footwear. Walkways have sensors embedded within the walkway which register the pressure exerted by the foot and record temporal-spatial gait data. They can be made of fixed plates that can be slotted together, the GaitMat, or in a continuous

flexible rubber walkway that rolls up, the GAITRIte. Both walkways automate the measurements that would otherwise be recorded manually. An electronic walkway is suited for use in a paediatric clinical setting due to ease of administration and portability.

The GAITRite system is the most commonly used walkway. It uses a pressure-sensing array arranged in 48 rows of 288 sensors to record the imprint of each footfall with six different levels of pressure (McDonough *et al.* 2001). It is only 3 mm thick and portable, generating data for immediate determination of gait parameters (Kirtley 2006). The most common mat, 4m long weighs 20kg in its case, although longer options are available. Concurrent validity and reliability of spatial and temporal measurements in adults have been supported. Validity and repeatability in children has also demonstrated, with within the same day reliability assessed in children aged one to eleven years as part of a larger normative study (Thorpe *et al.* 2005). The younger children (1-4 years) had the widest limits of agreement between measurements.

Normative data for children has been generated by three different authors in three different countries, (Dusing & Thorpe 2007; Alderson 2007; Holm *et al.* 2009) USA, UK, and Norway, respectively. These data sets provide the normal references ranges for children according to age, which is very important as gait evolves with age. These normative data, and the demonstrated validity and repeatability of the GAITRite make it an extremely useful tool for quantifying gait in children.

An illustration of the data as captured using the GAITRite can be seen in Figure 3.2. The computer software records in real time each foot print as it walks across the mat, and immediately calculates the parameters defined above. Data are therefore available for immediate assessment. A minimum of eight steps across the mat are required to provide this data. However numerous walks across the mat can be combined into one “test” data test, to provide an average for each walk on that test day.

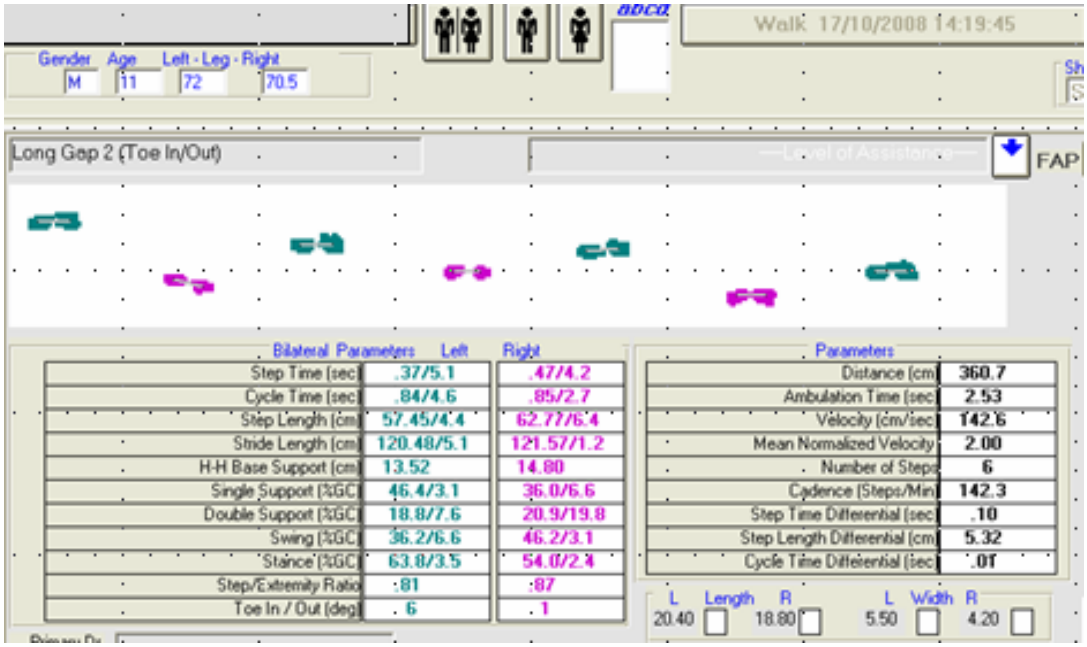


Figure 3.2: Graphic illustration of gait parameters captured using the GAITRite

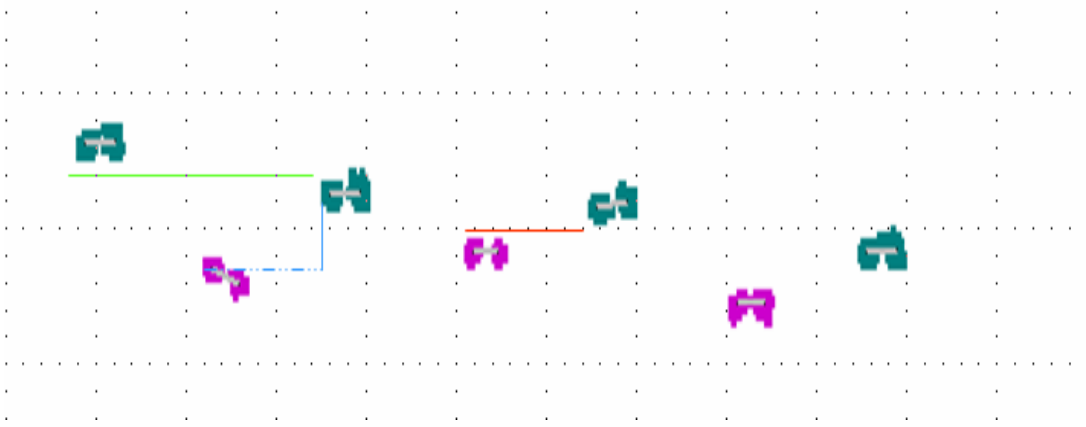


Figure 3.3: Illustration of Stride Length (green line), Step Length (red line) and Base of Support (blue lines) as measured on the GAITRite

Stride Length, Step Length and Base of Support as measured on the GAITRite are illustrated in Figure 3.3.

Utilising the normative data captured Alderson developed reference centile curves to show the distribution of measurements as each of the gait parameter change according to age. The gait parameters presented are: Velocity (cm/s), Cadence (steps/ minute), Step Length (cms), Step Time (seconds), Single Support (% of gait cycle), Double Support (% of gait cycle), and Base of Support (cms). The changes observed in gait parameters, as age increases are as follows:

- a consistent and gradual increase in Velocity,
- a large variability at 4 years, which decreases by 14 years in Cadence,
- a consistent increase across all ages in Step Length
- a large variability at 4 years, which narrows by 14 years in Step Time
- an overall stability in Double Support and Single Support
- a large variability at 4 years, which narrows gradually by 14 years in Base of Support.

These centiles were developed according to the LMS method, which summarises the changing distribution by three curves representing the median (M), coefficient of variation (S) and skewness (L), the latter expressed as a Box-Cox power. Using penalised likelihood the three curves are fitted as cubic splines by non-linear regression, and the extent of smoothing required can be expressed in terms of smoothing parameters or equivalent degrees of freedom, a method developed by Cole and Green (Cole & Green 1992). A big advantage of the LMS centiles is that is allows for a graphic illustration of where each child functions, according to their age.

Walking may also be compromised by pathologies of the musculoskeletal and cardio-respiratory systems. In this respect, Velocity and Cadence have been measured in an assessment known as the 6-minute walk test (6MWT) as a means of measuring cardiovascular endurance. Patients are asked to walk for 6 minutes in laps of 30-50m in length on flat, hard ground with a portable pulse oxymeter attached to the wrist to record pulse rate and oxygen saturation at baseline and then every minute during the test, and for three minutes after the test has been completed. The 6MWT allows the assessment of physiological changes during exercise performed in a given time, and was developed to predict functional capacity. The 6MWT has been demonstrated to be safe, easy to perform and highly acceptable (Geiger *et al.* 2010). However, children with cognitive deficits or behavioural challenges may be less likely to follow the instructions (e.g., they may run, skip or gallop). Lack of motivation and boredom may also affect 6MWT performance in children.

As noted in Chapter 1, the 6MWT was utilised as a primary outcome in registration-directed studies for MPS I, MPS II and Pompe disease. In MPS the 6MWT has been used to provide clinically relevant information about the global severity of the disease. In patients with MPS II however, an impaired 6MWT may be secondary to dysfunction of pulmonary, cardiovascular, musculoskeletal joint movement, and even neurological involvement. It would be very difficult to extract which of these pathologies impact the most on the 6MWT function. The impact of neurological

involvement, in relation to the other more prominent features would be particularly difficult to ascertain.

More recently however the value of using the 6MWT in neuromuscular diseases has also been explored. It was proved to be reliable and feasible in myotonic dystrophy type I (Kierkegaard & Tollback 2007) and more recently it was demonstrated to be feasible, safe and reproducible in documenting disease-related limitation on ambulation; the authors propose it as a new out-come measure for Duchenne Muscular Dystrophy (DMD) natural history and therapeutic trials (McDonald *et al.* 2010). In this study, the findings demonstrated that walking distance and walking velocity were substantially lower in boys with DMD compared to healthy boys; on average, values were ~60% of the average stride length observed in healthy controls. The difference in walking distance, walking velocity and stride length between the groups were highly statistically significant (p<0.0001). Cadence was only slightly lower in subjects with DMD but was much more variable. Understanding this variability is important if the use of 6MWT in this condition is to be utilised further. That is, Cadence alone, may not be appropriate as a suitable parameter to monitor response to therapy (McDonald *et al.* 2010).

The 6MWT does have the advantage that is has already been accepted by the regulatory authorities as a valid end point in clinical trials. However the value of the 6MWT in clinical management of MPS II patients has been questioned by Wood *et al* (2007), where younger patients, and those with neurological involvement and the electronic walkway GAITRite proposed as a more valuable tool (Wood *et al.* 2009).

Due to the fact that the GAITRite assessment requires the child to comply with the assessment for a much shorter time it is an attractive alternative – particularly in some of the MPS diseases where behavioural issues can be problematic to manage. The GAITRIte has also been successfully used to monitor the response of MPS I patients to transplantation (Dusing *et al.* 2007). Furthermore, in a recent concept paper on the clinical investigations of medicinal products for the treatment of pulmonary arterial hypertension, expert opinion stated that the validity and utility of the 6MWT as an endpoint is limited in patients younger than 6 years, and that its predictive value on the long term improvement of the disease is not established in the adult population, questioning its usefulness in the paediatric population over 6 years (Committee for Medicinal Products for Human Use & (CHMP) 2009).

Given the clinical presentation of an ataxic gait as described above; widened base, shortening of steps and spending more time on both feet, gait parameter other than Cadence and Velocity alone are also hypothesised to be valuable in defining the gait of NGD patients. The 6MWT was not considered sensitive enough to fully define these parameters. Apart from the work of Alderson (2007) six other studies, have successfully used the GAITRite to assess gait abnormality of children with various conditions - Hydrocephalus (Shore *et al.* 2005), Haemophilia (Bladen *et al.* 2007), Autism (Rinehart *et al.* 2006), Cerebral Palsy, (Wondra *et al.* 2007), and MPS I post transplant (Dusing *et al.* 2007), MPS II on ERT (Wood *et al.* 2009). This suggested the value of the GAITRite as a worthwhile assessment tool. On the basis of the above, the availability of normative gait parameters as referenced, the economical advantages compared to other gait assessments and the ease of use, the GAITRite

was selected as the appropriate assessment to attempt to define the gait characteristics of NGD patients, and to explore its potential value in defining disease involvement.

3.2. Methods

3.2.1 GAITRite Protocol of use

The European GAITRite Network Group approved guidelines for clinicians implementing spatio-temporal gait analysis in a bid to enhance reproducibility of gait measures and improve comparability of outcomes (Kressig & Beauchet 2005). These guidelines, which were developed for adults, were used in the absence of any specific paediatric guidelines. However, gait analysis in association with simultaneous cognitive tasks (dual-tasking) was not performed, as this was not deemed appropriate.

For the purpose of this study the above guidelines were used. Subjects were measured walking at their preferred pace; in four consecutives walks. Occasionally, encouragement (e.g parents either end of the mat with treats saying “keep going”, “you are doing well”) were necessary to keep the children interested and motivated. Height and leg length (measured from the anterior superior iliac spine to medial malleolus) in centimetres was also measured.

As ataxia is more pronounced in bare feet assessments (Eisenhardt 1996) were conducted without shoes when safe for the child to do so. Only children that were able to safely comply were requested to walk on the mat.

Children with Type I Gaucher disease were recruited to act as an additional control group to the normative data already available. Despite the availability of published normative data (and centiles) the rationale for recruiting the Type I Gaucher patients was to demonstrate that any abnormalities of gait seen in the NGD cohort would be secondary to neurological involvement, and not visceral involvement. Bone pain and splenomegaly, for example would also impact on gait. As the Type I Gaucher patients endure the same visceral manifestations they were regarded as an appropriate cohort to make these distinctions.

3.3 Results

Each patient performed the walk four times, barefoot as per The European GAITRite Network guideline. All four walks were then combined, using the GAITRite software to provide one set of results. Data was examined to ensure symmetry between left and right leg, and then combined to provide one data set per parameter, per patient. Parameters captured were based on those studied by Alderson (2007): *Velocity* (cm/s), *Normalised Velocity*, *Cadence* (steps/ minute), *Step Length* (cms), Step *Time* (seconds), Single *Support* (% of Gait Cycle), *Double Support* (% of Gait Cycle), and *Base of Support* (cms).

Fourteen patients with Gaucher disease were recruited in total to walk on the GAITRite. The Control group consisted of five Type I Gaucher patients. The mean age at assessment was 10.6 years (±3.4). Their height was 142.3 cms (±21.4) and leg length 68.6 cms (±11.1). Four of the Type I patients were boys, with only one girl.

The remaining nine patients had NGD. There were three boys and six girls. Mean age at assessment for this cohort was 10.2 years (±4.1). Their height was 138.5 cms (±24.7) and leg length 67.8 cms (±15.2). A more detailed outline of these NGD patients are given in Appendix 3.

The groups were closely matched in terms of age, height and leg length (the slightly taller height seen in the Type I Gaucher group a likely reflection of the fact that the majority were boys). Therefore, it was possible to directly compare the parameters of each group. The data was normally distributed and compared using an independent sample t-test, to account for the two groups. The means and standard deviation for each parameter is presented in Table 3.1

Table 3.1: Gait parameters for the Type I and NGD cohort (mean, SD), as captured utilising the GAITRite.

	TYPE I PATIENTS (*N*=5)	**NGD PATIENTS (*N*=9)**	**SIG (95% CI)**
VELOCITY (CM/S)	122.9 (±7.7)	92.93 (±18.2)	0.005** (11.0 TO 48.8)
NORMALISED VELOCITY	1.83 (±0.026)	1.34 (±0.34)	0.016** (0.11 TO 0.87)
CADENCE (STEPS/ MINUTE)	138.4 (±22.8)	116.1 (±17.7)	0.064 (-1.52 TO 46.0)
STEP LENGTH (CMS)	57.0 (±8.0)	49.6 (±11.0)	0.213 (-4.87 TO 19.76)
STEP TIME (SECONDS)	0.47 (±0.4)	0.53 (±0.1)	0.044* (-0.13 TO -0.00)
SINGLE SUPPORT (% OF GAIT CYCLE)	41.2 (±2.7)	38.3 (±2.0)	0.044* (0.09 TO 5.68)
DOUBLE SUPPORT (% OF GAIT CYCLE)	17.9 (±3.8)	23.1 (±4.2)	0.040* (-10.14 TO -0.28)
BASE OF SUPPORT (CMS)	10.0 (±2.3)	10.2 (±3.1)	0.897 (-3.72 TO 3.30)

Out of the eight parameters measured (including Normalised Velocity), a statistically significant difference was noted in five. The difference in Velocity was particularly significant at *p*0.005. The variability for Velocity in the NGD cohort was more than twice that seen in the Type I cohort.

The gait pattern of the NGD cohort appear to differ from that of the Type I Gaucher cohort in the amount of time spent in double support, at 5.2% longer (*p*0.040). Conversely, the NGD cohort spends 2.9% less of the gait cycle in single support (*p* 0.044). Step time in seconds was also longer, and statistically significant (*p*0.044).

Despite the fact that the groups were closely matched in terms of height in this case, the impact of height on gait and balance needs to be considered.

Although the mean age for both cohorts in this study was 10 years, and therefore older than the 7 years cut off where essentially adult value gait parameters are reached (Zijlstra *et al.* 1994), for completion however velocity data was normalised according to the Alderson (2007) (Velocity / Leg Length). The mean Normalised Velocity for the Type I cohort was 1.83 (±0.03) compared to 1.34 (±0.03) in the NGD cohort. The statistical significance observed in Velocity without normalising is maintained with normalisation *p* 0.016**. This indicates that there is no benefit to be gained from normalising data in this cohort, particularly as the LMS centiles are available.

The Type I Gaucher cohort provides a very useful insight on how the gait profile of these two cohorts differs, and what could be secondary the musculoskeletal manifestations of Gaucher disease rather than neurological involvement. Comparing both these data sets to a normative sample is therefore also necessary to identify any deviations that may be present in both cohorts, and to ensure that deviations observed are neurologically driven.

Utilising the normative data generated by Alderson (2007), Dusing and Thorpe (2007), and Holm *et al* (2009), as presented in Table 3.2, it becomes apparent that for the parameters available, the Type I Gaucher patients fall within the mean ± standard deviation for the majority of parameters.

The distribution for Cadence is wider and on the higher side, particularly when compared to the Dusing & Thorpe (2007) data, but are within mean and standard deviation for the Holm et al (2009) data set. The larger distribution may of course be related to the fact that the normative data sets are all from 10-11 year old children, while the Type I patients ranged from 7 to 14 years. The mean Velocity, Step Length and Double Support for the Type I patients fit within the available sets of normative data.

This is not the same for the NGD patients, where the mean for Velocity, Cadence, Step Length and Double Support are consistently outside the standard deviation for all normative data sets. The distribution of data for the NGD and Type I patients is best illustrated in Figure 3.4.

Table 3.2: GAITRite normative data by Alderson (2007), Dusing & Thorpe (2007) and Holm et al (2009) for the matched age groups (10-11 yrs) compared to NGD and Type I Gaucher cohorts

	TYPE I PATIENTS	NGD PATIENTS	ALDERSON (2007)	DUSING & THORPE 2007	HOLM *ET AL* (2009)
VELOCITY	122.9 (±7.7)	92.93 (±18.2)	125.8 (±25.0)	129.85 (±18.35)	N/P
NORMALISED VELOCITY	1.83 (±0.03)	1.34 (±0.03)	1.74 (±0.36)	N/P	N/P
CADENCE	138.4 (±22.8)	116.1 (±17.7)	N/P	126.14 (±10.29)	M135(±10.7) F136 (±9.4)
STEP LENGTH	57.0 (±8.0)	49.6 (±11.0)	61.2 (±8.3)	61.21 (±6.66)	M66.3(±4.8) F65.8(±4.5)
STEP TIME	0.47 (±0.4)	0.53 (±0.1)	0.49 (±0.05)	N/P	N/P
SINGLE SUPPORT	41.2 (±2.7)	38.3 (±2.0)	40.4 (±2.0)	N/P	N/P
DOUBLE SUPPORT	17.9 (±3.8)	23.1 (±4.2)	19.3 (±3.1)	17.91 (±2.39)	N/P
BASE OF SUPPORT	10.0 (±2.3)	10.2 (±3.1)	7.4 (±3.5)	9.44 (±2.72)	M9.5(±2.0) F9.0(±1.8)

M=male. F=female. N/P= not provided

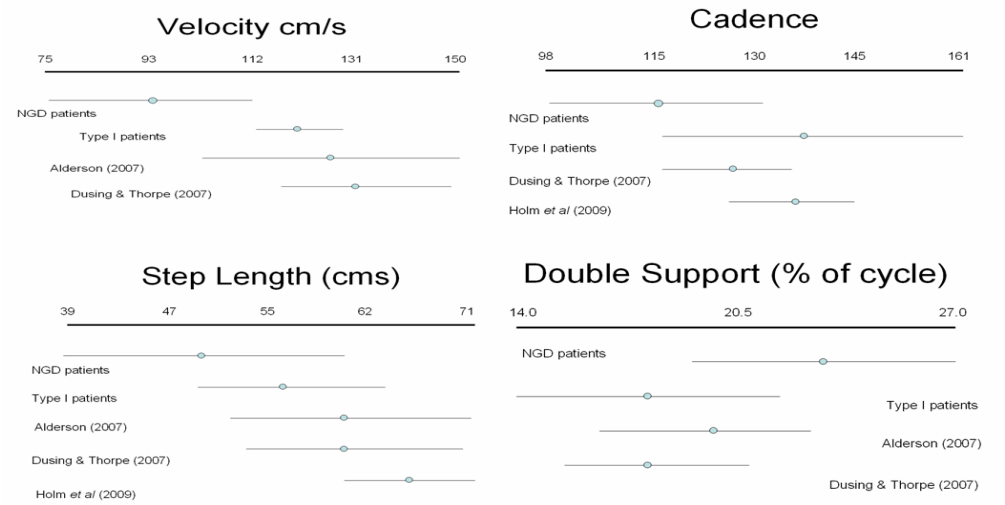


Figure 3.4: Distribution of gait parameters of NGD and Type I patients compared to normative data by Alderson (2007), Dusing & Thorpe (2007) and Holm et al (2009) for the matched age groups (10-11 yrs)

Both NGD and Type I patients are very similar when compared to all three sets of normative data for Base of Support (Figure 3.5). Even when accounting for Alderson's (2007) data set, which is 2cms shorter compared to the other two sets of normative data, all standard deviations overlap.

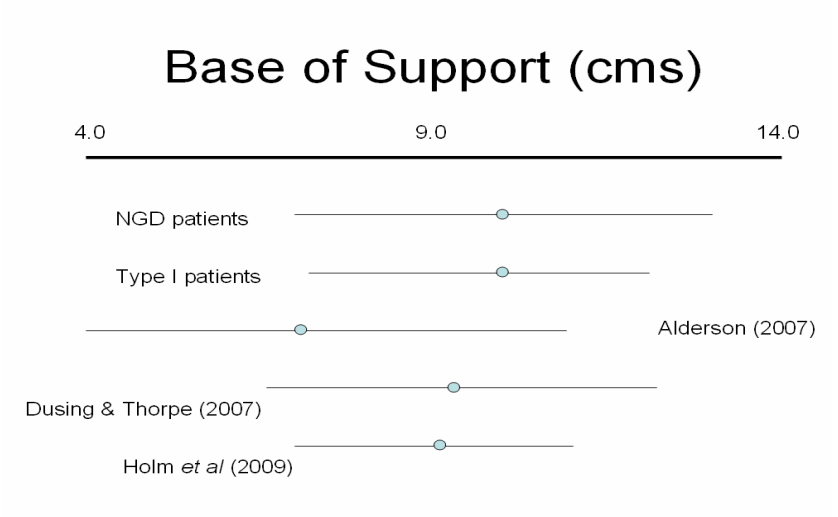


Figure 3.5 Distribution of normative Base of Support (Alderson (2007), Dusing & Thorpe (2007) and Holm et al (2009) and Type I and NGD patients

In this instance the NGD and Type I cohorts were closely matched, in terms of age, height and leg length, which allowed for a meaningful comparison between data. Although this was very fortuitous this may not occur in all studies, and therefore, in order to be a truly worthwhile marker of disease, a means of comparing each individual patient and cohort, regardless of age is needed. A means of monitoring individual gait parameters progress over time, while accounting for age, is therefore highly desirable. The LMS centiles developed by Alderson (2007) allows for a graphical illustration of this.

3.3.1 LMS gait centiles

Alderson (2007) studied 138 children between the ages of four and fourteen to establish normative data and developmental trends in a population of healthy children. There were at least 10 children for each year from 4 to 13 years, and 6 children recruited aged 14 years. Utilising this data Alderson (2007) developed gait parameter centiles. They provide a 3rd, 10th, 25th, Median, 75th, 90th and 97th centile for children from 4 to 14 years of age. The charts have the potential to have a valuable clinical application (Alderson 2007).

Plotting the Gaucher data on the LMS centiles provides a graphic illustration of how the Type 1 cohort (red squares) differentiate from the NGD cohort (blue triangles).

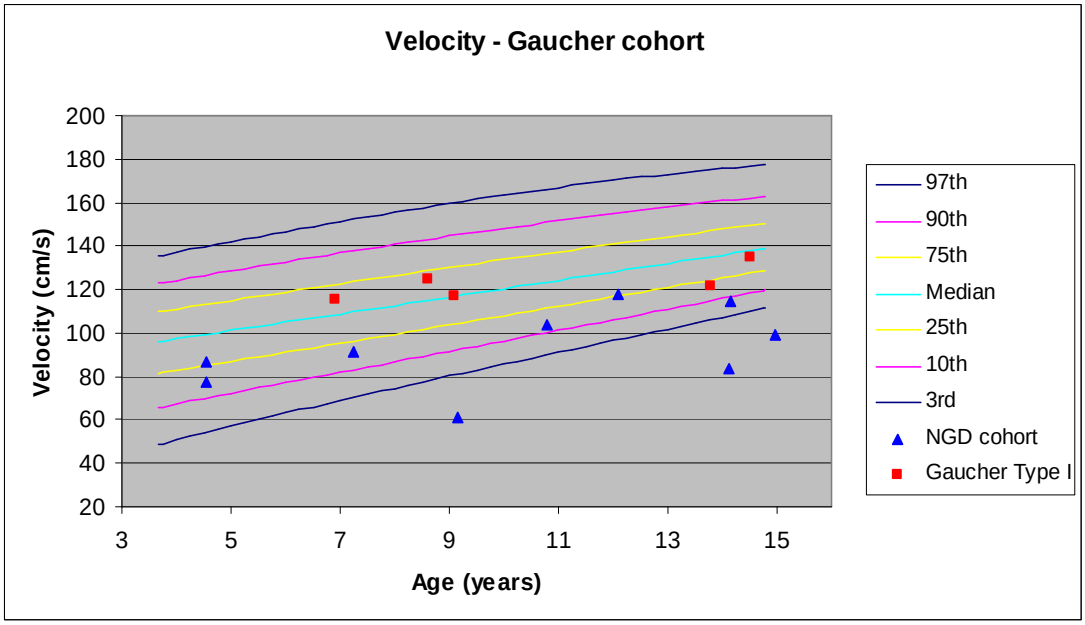


Figure 3.6: Velocity parameter of Gaucher children plotted on centile

In the Velocity parameter, it can be clearly seen that all the NGD cohort are well below the median line and seven are below the 25th centile. There is one Type I Gaucher patient also on the 25th centile line; this 13.8 year old boy had previously

suffered from knee joint pain which required use of steroids in clinical management. Although he did not report any pain on the day of assessment this may still have an impact on his gait. As expected, the illustration is the same for the Normalised Velocity data.

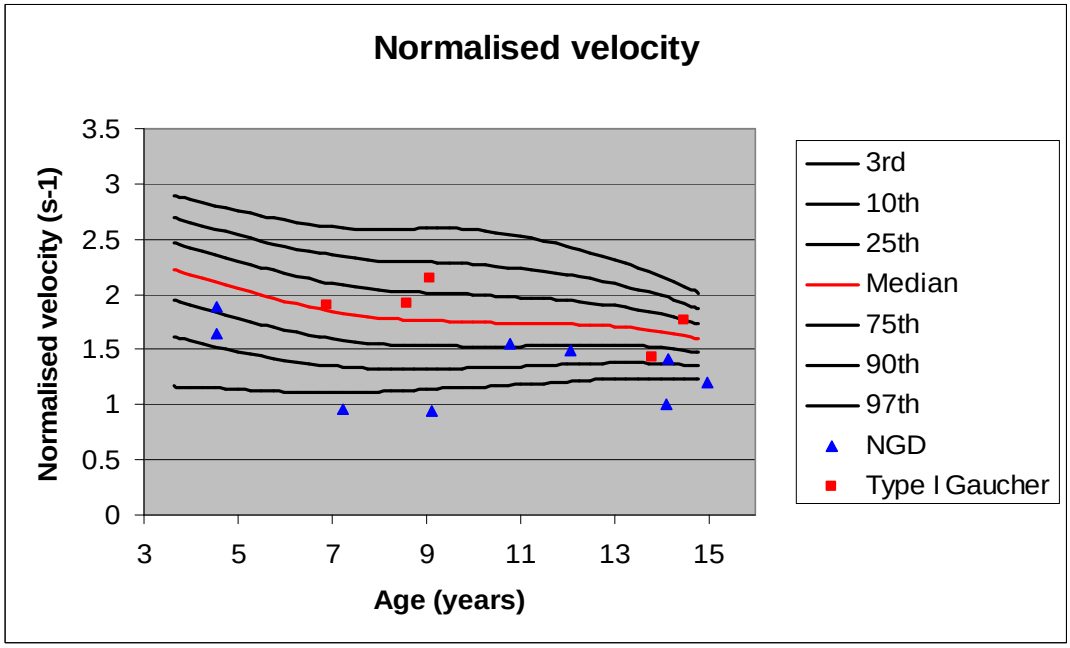


Figure 3.7: Normalised Velocity parameter of Gaucher children plotted on centile

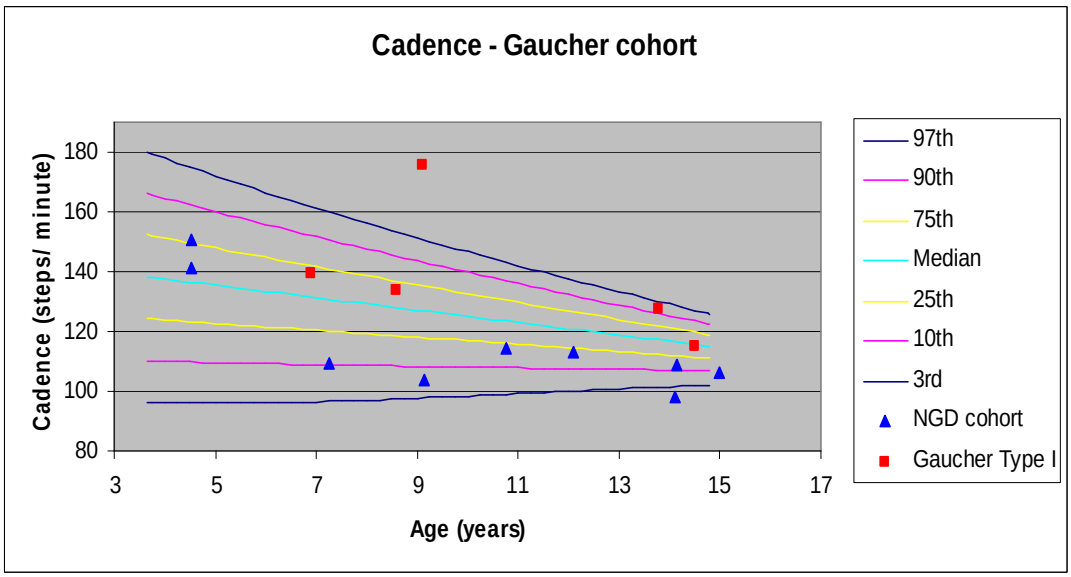


Figure 3.8: Cadence parameter of Gaucher children plotted on centile

While exploring the Gaucher data plotted on the Cadence centile it is apparent again that seven of the nine NGD patients fall below the 25th centiles. The two NGD patients that were above the median were the youngest two, male twins. The 9 year old Type I Gaucher, who evidently stands above the norm, took numerous short, fast steps. There was no apparent reason for this.

Before plotting the following gait parameters on the LMS centiles, left and right leg data were checked for any asymmetry. None of the patients were noted to have a significant asymmetry; therefore a mean number for left and right leg was calculated for ease of use.

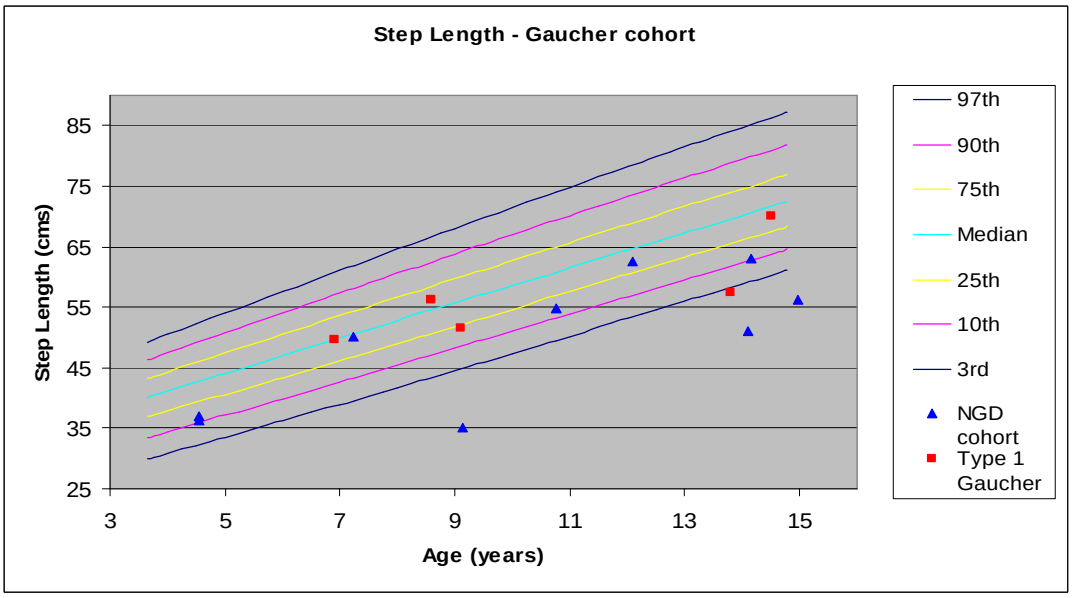


Figure 3.9: Step Length parameter of Gaucher children plotted on centile

Given that there was no statistical difference between the NGD and Type I cohort for Step Length it is not surprising that there is no apparent pattern of distribution on the Leg Length LMS centile.

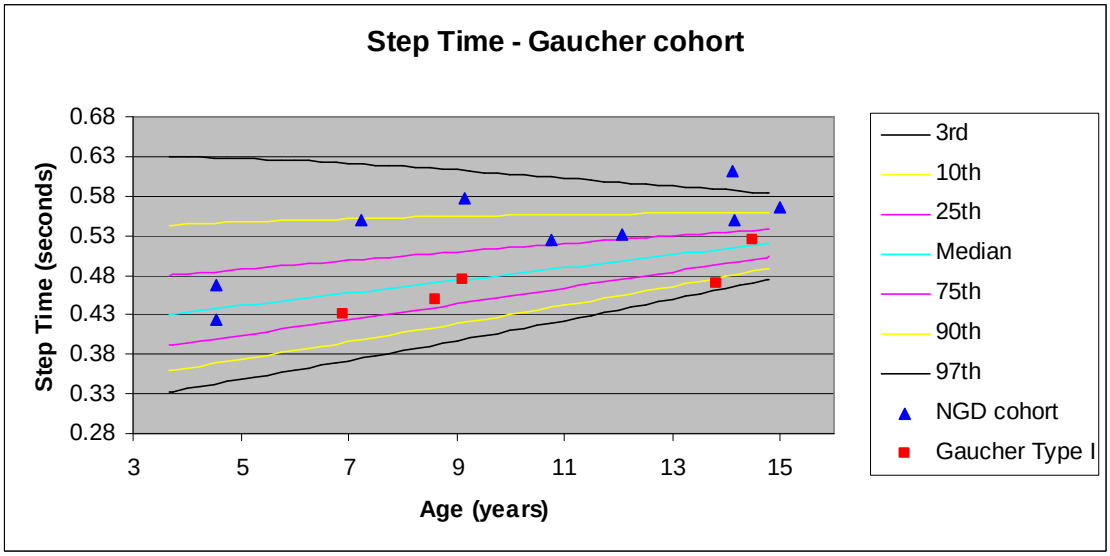


Figure 3.10: Step Time parameter of Gaucher children plotted on centile

The pattern observed on the Step Time centile is similar to that seen in Cadence, all be it that 8 out of the 9 NGD patients are clearly above the Median line.

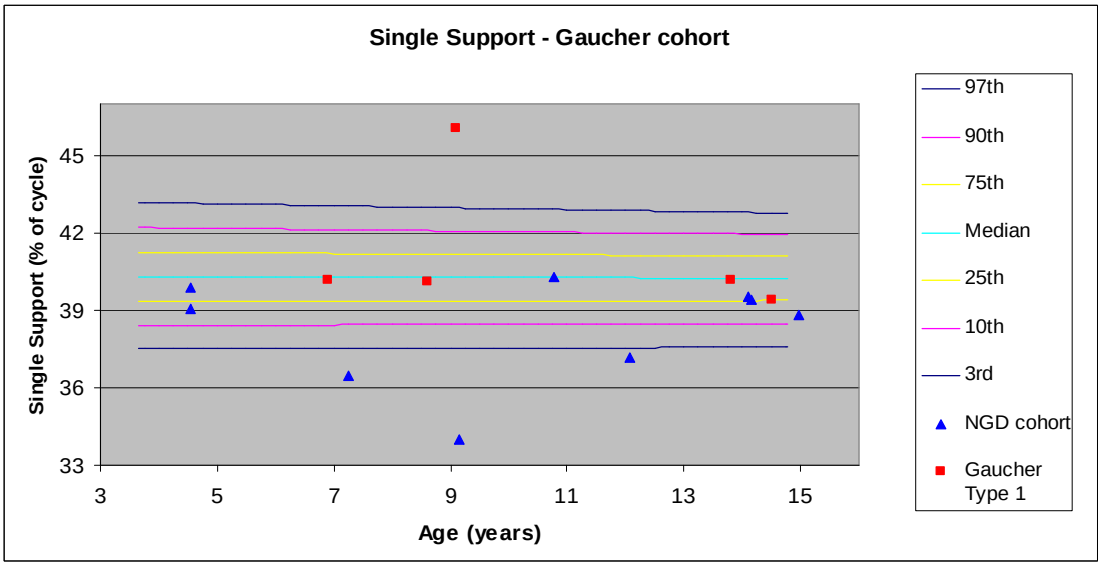


Figure 3.11: Single Support parameter of Gaucher children plotted on centile

As would be expected, the Single Support and Double Support centiles mirror each other. There is an evident distinction between the placement on the NGD and the Type I Gaucher children, however a total of seven overall are closely situated to the Median line.

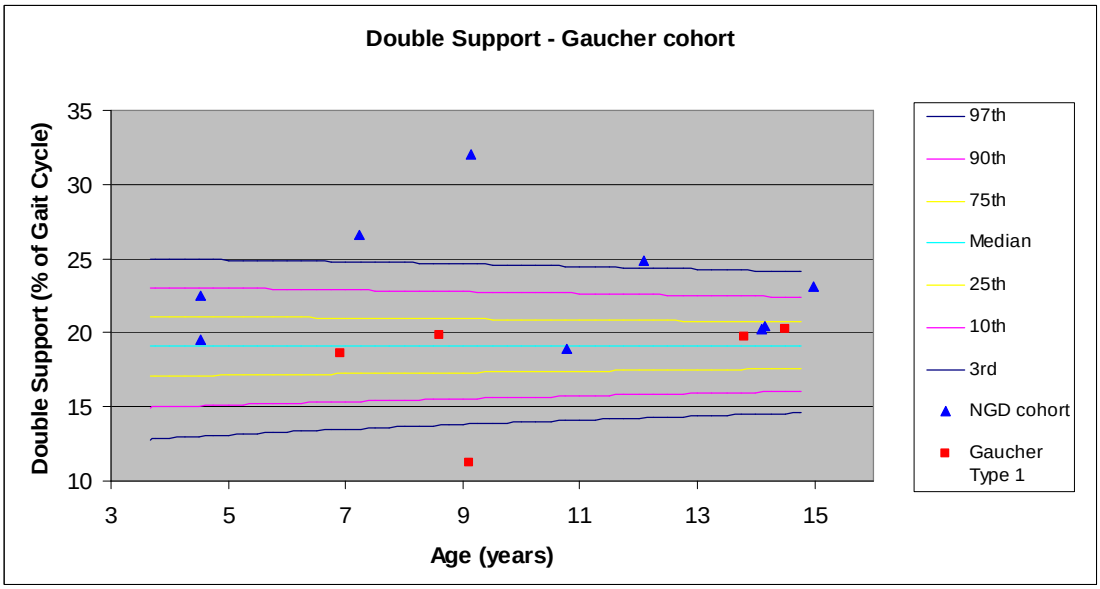


Figure 3.12: Double Support parameter of Gaucher children plotted on centile

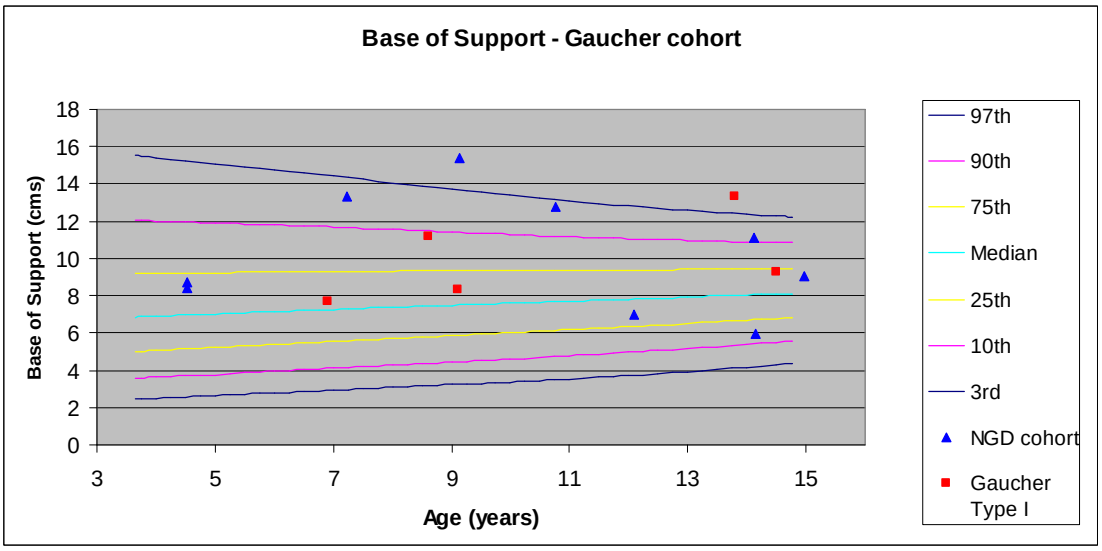


Figure 3.13: Base of Support parameter of Gaucher children plotted on centile

Again given that there is no statistical difference between the NGD and Type I group for Base of Support, there is no evident distinction of their placements on the centile.

3.3.2 LMS centile Z scores

Thanks to the work of Alderson (2007) these LMS centile charts of gait parameters provide an excellent graphical illustration of where each individual is placed, and

also how different grouping may appear between cohorts. However a numeric representation which maintains the relationship to age would be a better marker. Although this work had not been done by Alderson (2007) by obtaining the original data that created the LMS centiles, it was possible to generate this in the form of Z scores. Permission was obtained by Alderson to conduct this work.

Z scores or standard deviation scores as also known, of a child's measurement (*y*) are calculated from the L, M and S curves, using values appropriate for the child's age. Two formulae are relevant depending on the value of L (Huiqi & Cole 2005).

The formulae are:

$$Z = \frac{(y/M)^{L} - 1}{L \times S}, \text{ if } L \neq 0$$

$$Z = \frac{\log(y/M)^{L} - 1}{S}, \text{ if } L = 0$$

Using these formulae therefore an individual Z score has been calculated for each child, for each of the gait parameters studied. Scores range from -1 to 1. A minus score indicating that the child is performing below the median.

The calculated Z score can be seen in Table 3.3. The normal distribution that was present with the raw data is lost in conversion to Z scores, therefore only non-parametric tests were used to assess statistical significance.

It becomes apparent that the parameters which were statistically significant in the group comparison remain statistically significant when using the Z scores. However, when using the Z scores, the gait parameter Cadence becomes statistically significant (*p*0.028) while it had previously been borderline *p*0.064. The strength of the statistical significance for Step Time is also greatly improved while using the Z scores. This indicates that the Z scores may offer greater sensitivity compared to utilising the raw data alone, for these parameters in particular.

Table 3.3: Z score for all gait parameters assessed in the NGD and Type I Gaucher patients.

	Type I	**NGD**	**Sig. P.**
Velocity Z score	0.00243 (±0.011)	-0.04049 (±0.026)	0.004**
Normalised Velocity Z score	0.0107 (±0.02)	-0.0683 (±0.076)	0.009**
Cadence Z score	0.01289 (±0.02)	-0.00457 (±0.01)	0.028**
Step Length Z score	-0.00485 (±0.008)	-0.01736 (±0.014)	0.072
Step Time Z score	-0.0034 (±0.004)	0.00736 (±0.007)	0.006**
Single Support Z score	0.0008 (±0.0023)	-0.0016 (±0.0017)	0.033*
Double Support Z score	-0.0096 (±0.0287)	0.0286 (±0.0300)	0.039*
Base of Support Z score	0.1182 (±0.071)	0.0850 (±0.071)	0.739

*** highly significant * statistically significant*

3.3.3 Sequential data

As discussed in the development of the mSST, for an assessment tool to be truly valuable it needs to have the ability to be “responsive to change”. This is assessed through sequential assessments.

Four NGD patients consented to have sequential NGD assessment of gait, using the GAITRite. Unfortunately, the eldest three NGD patients are now too old for the LMS centiles, and therefore would not be useful. Sadly one patient passed away. This makes the number of patients suitable and available for sequential assessment small.

The four consenting patients that had sequential GAITRite assessments were three girls and one boy. The two eldest (Patients 3 and 4) are L444P homozygote. The other two were heterozygote with rare mutations.

Time interval between assessments ranged from 3 months to 15 months, again depending on the dates which the children were attending routine appointments at the hospital. Two patients were assessed on two occasions, and two assessed on three occasions. The children’s age ranged from 7 years 2 months to 12 years 1 month at first assessment, to 7 years 5 months to 13 years 5 months at the last assessment.

Through plotting the sequential gait parameters on the LMS centiles it becomes apparent that there is not specific pattern or trend to be seen. Indeed the data is

rather sporadic for some parameters in particular. Relating back to the individual domain analysis of Ataxia/Gait in the mSST follow up assessment, it was noted that two out of the 39 NGD patients assessed sequentially were symptom free for this domain, while others progressed. To expect a progression in all the gait parameters for all the patients over time may not therefore be realistic.

Velocity, the most statistically significant parameter between the NGD and Type I cohort, offers the most consistent in that all the data sets remain near or below the 25th centile. Patient 2 and Patient 3, both of whom had 3 assessments performed improved on the second and then declined slightly again on the third. Patient 1 and Patient 4 present a declining trend at second assessment, where Patient 1 falls from the 25th to the 3rd centile and Patient 4 falling from the 25th to the 10th centile.

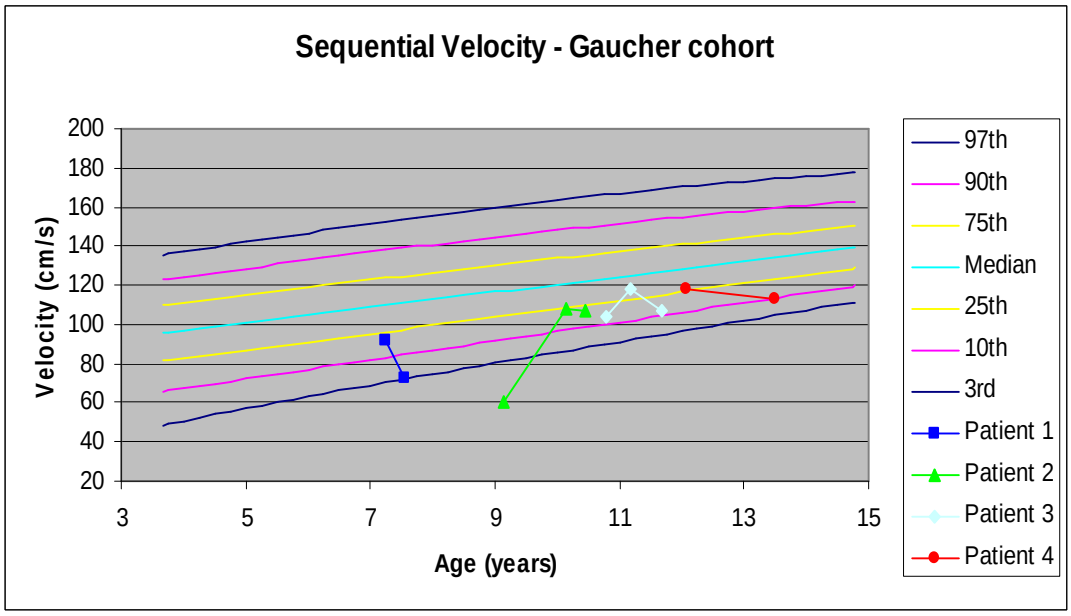


Figure 3.14: Velocity sequential NGD data plotted on centile

In the Cadence centile, Patient 1 declines slightly, while Patient 2 displays a very dramatic swap from near the 3rd centile to the 97th centile. Again Patient 3 improves

to the Median on the second assessment, but falls back to below the 25th centile on the third assessment. Patient 4 improves from just below the 25th to just above the Median.

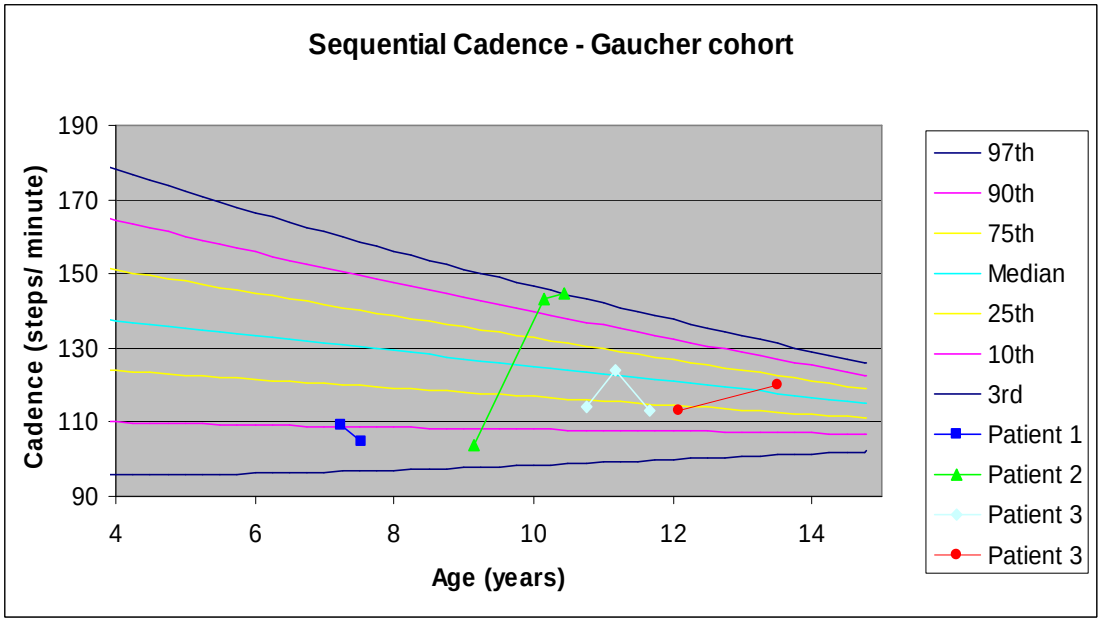


Figure 3.15: Cadence sequential NGD data plotted on centile

Only Patient 1 improves to a meaningful place on the centile for Step Length. A patient 2 improves, but remains consistently below the 3rd centile. Patient 3 remains relatively stable between the 25th and 10th while Patient 4 drops from just below the Median to below the 3rd centile.

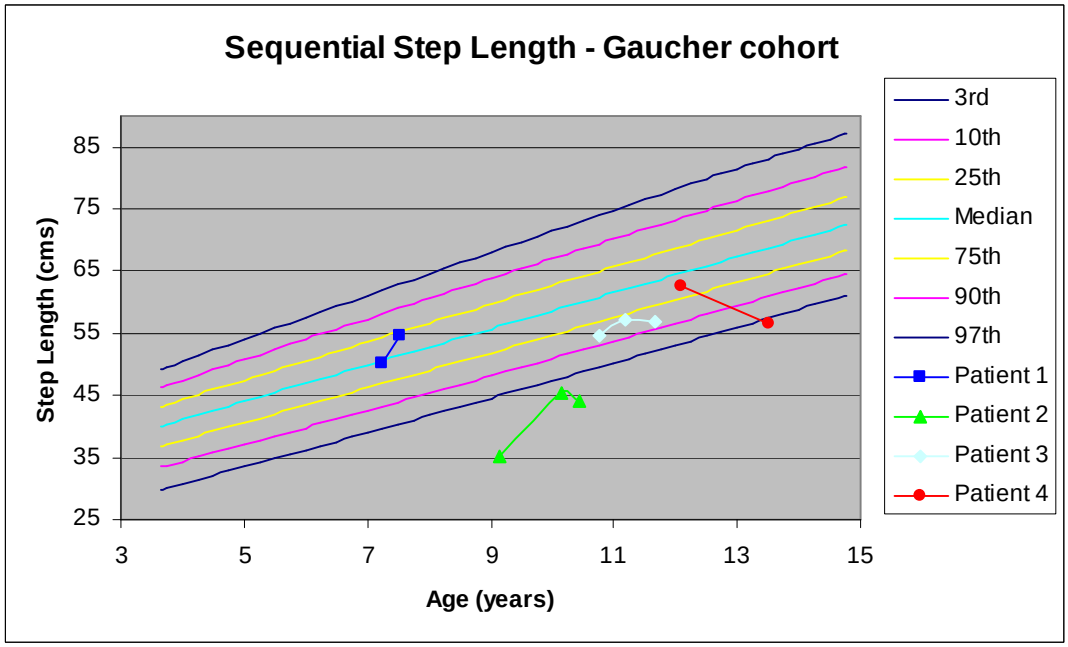


Figure 3.16: Step Length sequential NGD data plotted on centile

Step Time data is particularly erratic without any clear pattern seen, as 3 put of the 4 patients move across the Median line.

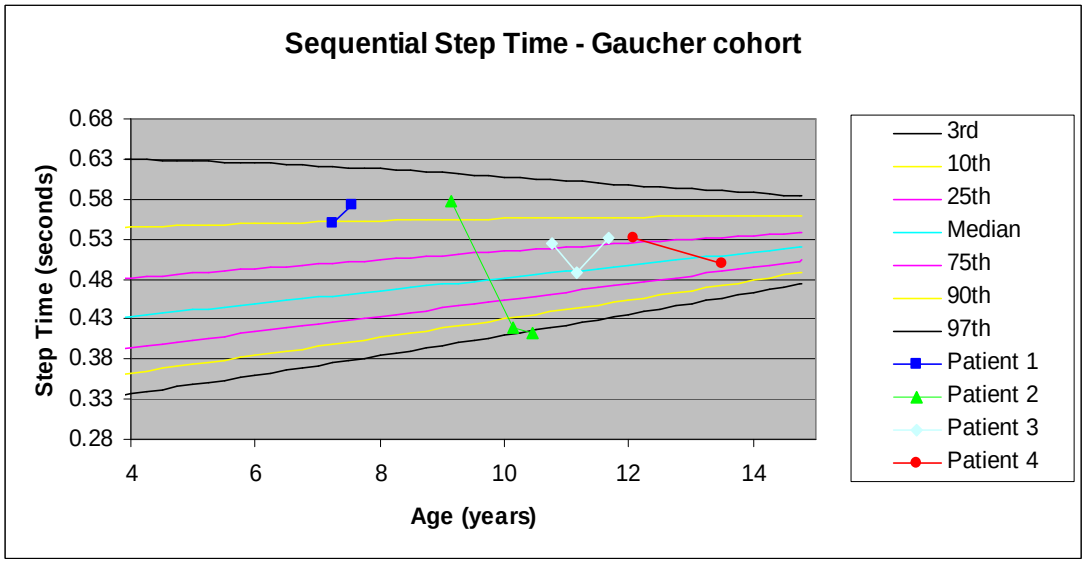


Figure 3.17: Step Time sequential NGD data plotted on centile

In Double Support Patient 1 and Patient 4 remain around the 97th centile. Patient 2 displays a dramatic improvement from 32.5%, which is completely outside the

centile, down to 20% which is just above the Median. Patient 3 remains relatively consistent.

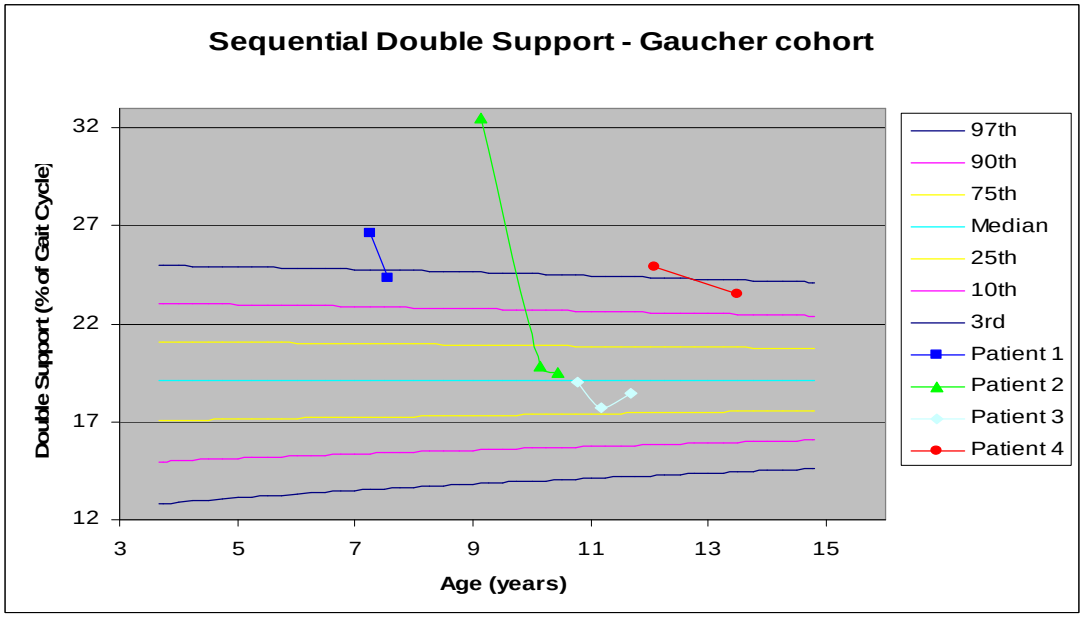


Figure 3.18: Double Support sequential NGD data plotted on centile

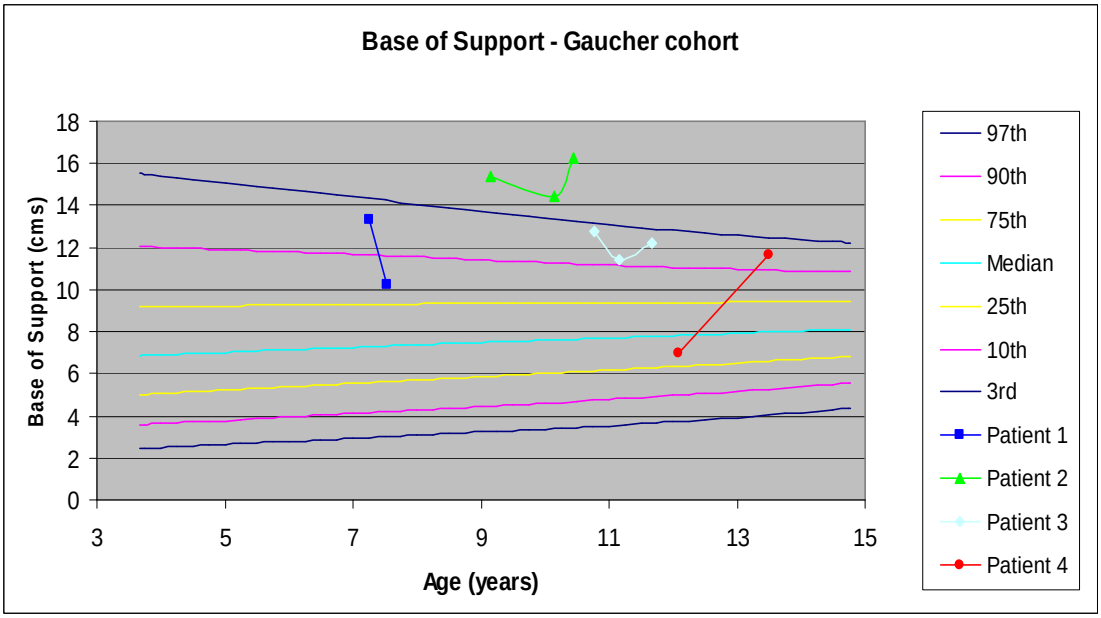


Figure 3.19: Base of Support sequential NGD data plotted on centile

Although there is no clear trend for Base of Support either, all four patients are above the 75th centile for their last assessment.

3.4 Discussion

The GAITRite was demonstrated to be a very user friendly assessment tool for the purpose of measuring gait parameters in Gaucher children. All the children were able to comply with the assessment easily, and all completed the assessment in less then 20 minutes time. Statistically significant differences were observed between the Type I and NGD patients in six of the gait parameters (Z scores). The NGD cohort also differed to the age-matched normative data of Holm, Alderson and Dusing & Thorpe (Dusing & Thorpe 2007; Alderson 2007; Holm *et al.* 2009).

The increased statistical significance observed using the Z score for five of the parameters, suggests that the Z scores offers greater sensitivity compared to utilising the raw data alone, particularly for Step Time. These parameters can be regarded as clinically viable too.

That is, the reduction in Velocity, and increased Step Time, decreases the postural control requirements during gait, which could be regarded as a compensatory mechanism, and may explain the altered gait cycle seen. While the shift towards longer periods in Double Support may be also be strategy to improve stability.

Cadence and Step Length was reduced in the NGD cohort, although not significant statistically for Step Length. This possibly indicates that the decreased Velocity observed is driven by the increased Step Time and time spent in Double Support.

Clinically it makes sense that children with an ataxic gait spend more time in Double Support to increase stability and balance. The trend towards a reduced Step Length and a statistically significant increase in Step Time are also consistent with this. All these are likely reflect a compromised ability to coordinate movement. Cadence and Velocity on their own could be a reflection of poor strength of muscle, or even poor fitness, therefore the incorporation of the other parameters offers a better means of truly understanding the gait profile of theses children.

In a study of gait disturbances in twelve adult patients with various cerebellar disorders, a significantly reduced step frequency with a prolonged stance and double limb support duration was found in patients with cerebellar disorders compared to age matched controls (Stolze *et al.* 2002). Stolze (2002) postulated that the coexistence of variable stride length with variable stride duration (time) may underpin the clinical features of cerebellar disease, in particular the deficiencies in adjusting the relative movement of multiple joints. Although this study utilised a different method for measuring gait, and only studied adults with cerebellar disorders the findings of prolonged double limb support duration and stride duration (time) are consistent with the increased double support and step time seen in the NGD cohort.

An interesting aspect of the data is that the Base of Support of NGD children was not different to compared to the normative data available and not statistically different when compared to the Type 1 cohort, with only 0.2cms difference between

the two. This is despite frequently documented ‘wide-base of support’ in clinical reports.

These findings were not expected based on the clinical picture observed. This may be because the *observing eye* interprets the increased Double Support time as a wide Base of Support? Or that the child’s raised arms, used to improve balance, gives the illusion that Base of Support is also increased.

Despite involving a slightly difference methodology, Stolze (2002) in his study of adults with cerebellar disorders reported an increased step width, indicating the need for stability. However, following a study comparing patients with vestibulopathy and people without any known neuromuscular pathology Krebs et al (2002) concluded that “wide-based gait alone cannot differentiate between subjects with and without balance impairments. Base of support and other whole-body kinematic variables are mechanical compensations of vestibulopathic instability” (Krebs *et al.* 2002).

On the other had, Alderson (2007) in her study of children peripheral neuropathy were identified to have a significantly narrower Base of Support than control at preferred speed. Alderson (2007) proposed that the reduced Base of Support in this case may reflect musculoskeletal limitations, such as balance imbalance and restrictions to joint range. Alderson (2007) further postulated that the increased Double Support, also seen in this cohort of children with peripheral neuropathy may have compensated for these restrictions, and introduced longer periods of stability

into the gait cycle. Overall their walk was slower, with a shorter Step Length. This reduced Velocity, shorter Step Length and increased Double Support time reported for the peripheral neuropathy group is similar to the NGD findings. Differently to the NGD cohort however, the peripheral neuropathy group were identified to have asymmetrical walking patterns

Alderson (2007) also studied four other different clinical groups in addition to peripheral neuropathy - muscular dystrophy; developmental coordination; traumatic brain injury and cerebellar pathology (Alderson 2007).

Children in the cerebellar pathology group showed the most significant alterations in walking and dynamic balance. Cadence in this group was significantly reduced and step time increased relative to control. Step Length was significantly shorter with an increased variation in Step Time relative to control. Single Support was reduced and Double Support was also increased. These findings are consistent with the gait pattern seen in the NGD group. Alderson (2007) reports that the cerebellar pathology group had a significant increase in Base of Support - measuring 9.4 cms (±4.9). This distance would however be regarded as normal in the Dusing and Thorpe (2007) and Holm (2009) data set.

The comparison of Type I children with NGD children indicates that the differences observed in the NGD gait is not secondary to the visceral manifestation of Gaucher disease, and are probably secondary to central nervous involvement.

It is important to consider however that CNS involvement other than those measured as part of mSST assessment may be contributing to the gait profile seen. Two likely important manifestations to be considered are peripheral neuropathy and vestibular involvement. Peripheral neuropathy is particularly important to consider given the similar findings identified in the group studied by Alderson (2007).

Table 3.4: Vestibular and Nerve Conduction Velocity assessments performed on the NGD cohort during study period

Patient	Vestibular testing	NCV testing
1	No AUDBAL assessment done	Mild sensory. Severe motor
2	11/02/2008 Negative on Romberg test, but on sharpened Romberg she fell to the left. Evidence of vestibular activity from both ears, although the values gave an apparent directional preponderance	Normal sensory and motor
3	No AUDBAL assessment done	Normal sensory and motor
4	No AUDBAL assessment done	Moderate sensory. Moderate severe.
5	No AUDBAL assessment done	Mild sensory. Normal motor.
6	11/02/2003 Vestibular function is most likely normal and her problems with balance are mainly due to ocular abnormalities	Mild sensory. Normal motor.
7	30/01/2008 evidence of bilateral vestibular function	Normal sensory and motor
8	30/01/2008 evidence of bilateral vestibular function. 13/01/2009 normal peripheral vestibular function bilaterally	Not done
9	No AUDBAL assessment done	Normal sensory and motor

AUDBAL – Audiology Balance

Neither peripheral neuropathy nor vestibular function was assessed systematically as part of this study – primarily because of the complexity of the assessment and the difficulty in getting the children to comply. However, some of the NGD patients had nerve conduction velocity (NCV) and vestibular testing performed as part of their routine clinical care during the same time period as this study. A basic

exploratory review of the data was therefore performed to consider whether of these could have a significant impact on the gait data (Table 3.4).

Ocular motor deficit (absence of saccades and pursuit) makes it difficult to interpret the results of vestibular testing, which are based on normal eye movement, however based on the findings of the limited number of children assessed, a link between vestibular and the gait profile seen can not be drawn.

More children were assessed with a NCV during the study. Based on the findings, Consultant Paediatric Neurophysiologist Dr Matthew Pitt was asked to classify neuropathy involvement (sensory and motor) as normal; mild; moderate and severe. Although this is a subjective overview it provided an indication of the involvement for this cohort. These findings indicate that peripheral neuropathy may be a contributing factor to the gait profile of four out of the eight assessed (three mild, one moderate). Two of these patients also have moderate and severe motor involvement, which may also impact on the gait profile seen.

This work has provided a valuable insight to the gait profile of the NGD cohort. Given that the gait profile of the Type I cohort was identified to be normal, it can be postulated that the gait deviations observed in NGD are driven by CNS, rather than somatic pathology. Whether the underlying CNS pathology driving this is cerebellar ataxia in isolation or in combination with neuropathy is unclear. However, given that both are progressive in nature, gait analysis remains a valuable assessment tool.

The variability and lack of a definite trend observed in the sequential assessments does make the value of using the GAITRite longitudinally or to assess clinical interventions or drug therapy difficult however. Variability of gait parameters, and lack of trend was also evident in the MPS II study previously discussed (Wood *et al.* 2009).

This lack of trend, as a progression in particular, may be because the duration between assessments may not be long enough. Follow Up assessment using the mSST was after a period of 4 years. Naturally, following a larger cohort would also be more informative. Assessing the Type I patients sequentially would also give an insight into whether this variability is seen in that cohort as well. Addressing these issues may enable the value of using GAITRite to monitor progress, rather than distinguish between cohorts.

Chapter 4

Diffusion Tensor Imaging

Exploration is the physical expression of the intellectual passion

- Apsley Cherry Garrad

4. Diffusion Tensor Imaging

4.1 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) uses the body's natural magnetic properties to produce detailed images from any part of the body. For imaging purposes the hydrogen nucleus (a single proton) is used because of its abundance in water and fat. Diseases can manifest themselves by a change in water signal. There are no known biological hazards of MRI because, unlike x ray and computed tomography, MRI uses radiation in the radiofrequency range, which does not damage tissue (Berger 2002).

MRI has become a virtually indispensable modality in the field of neurology (Lyon *et al.* 2006). Some lesions of the brain exist for some time without clinical signs, and certain lesions of the white matter (i.e., leukodystrophies) and basal ganglia necrosis may precede the emergence of the characteristic clinical syndrome. Conversely many neurological problems show no visible changes using MRI or CT scans, therefore neuroradiological imaging will never replace clinical examination, but the two can combine effectively to assess patients.

Lesions are now being visualised that previously were known only to pathologists; and in some diseases, still defined mainly by pathological criteria; in these cases, neuroradiologic imaging can enable diagnostic confirmation during life. The use of recently refined techniques, such as MR spectroscopy and diffusion tensor imaging, MR angiography, positron emission tomography (PET), and single proton emission computed tomography (SPECT), advances knowledge of the functional aspects of

brain pathology. These techniques have enabled the clinician to use living biopathology in formulating the disease process.

MRI is widely used in children and young adults with suspected brain disease and offers the opportunity to explore brain structure-function relationships. A number of MRI approaches offers ways of identifying subtle brain abnormalities that are not seen on conventional neuroradiological assessment (Rowan *et al.* 2007).

Brain imaging, preferably MRI is specified as one of the minimum assessment to be performed at initial assessment of primary neurological involvement in Gaucher disease. It is also included as part of the minimum follow-up clinical protocol if clinically indicated, taking into account the risk of anaesthesia if necessary.

Data generated from the MRI data provides objective, quantitative information (ensuring the same methodology is applied at all times in each individual analysis). In this respect it offers an advantage over the mSST and GAITRite which may be vulnerable to the subjective opinions of assessors and the compliance of children, respectively.

4.1.1 Brain MRI in Gaucher disease

There are few publications that have studied the paediatric Gaucher brain, despite its inclusion in the revised guidelines for the management of NGD patients (Vellodi *et al.* 2009). The literature available report non-specific unilateral cerebral atrophy

and dural thickening with contrast enhancement in routine MRI of Type II paediatric Gaucher brain (Chang *et al.* 2000).

A recent publication, by Abdel Razek *et al* (2009), utilised diffusion-weighted MR imaging to examine the apparent diffusion coefficient in thirteen patients, three Type II and ten NGD, with an age range of 8 months to 14 years (Abdel Razek *et al.* 2009). The study examined multiple regions of interest (ROI), which were selected and manually identified by one expert neuroradiologist who was blinded to the genotype and clinical presentation of the patient. The results demonstrated significant lower apparent diffusion coefficient value in the cortical frontal, cortical temporal, frontal subcortical white matter, corticospinal tract, medulla, midbrain, and cerebellum of Gaucher patients compared to the controls.

Abdel Razek *et al* (2009) in their study of apparent diffusion coefficient value in Gaucher patients using diffusion-weighted imaging, hypothesised that the lower apparent diffusion coefficient value identified in Gaucher patients compared to controls may be attributed to the accumulation of Gaucher's cells within the parenchyma with subsequent increasing cellularity of the brain parenchyma. Also, Gaucher cells are lipid-engorged macrophages that contain high amount of fat resulting in restricted diffusion and consequently lower apparent diffusion coefficient values.

4.1.2 Diffusion Tensor Imaging

Diffusion Tensor Imaging (DTI) is a technique that allows exploration of tissue microstructure, such as the white matter, as a non-invasive method for evaluating diffusivity and directionality of water molecules. It is more sensitive in quantifying structural brain alterations than conventional magnetic resonance techniques.

Pure water has *isotropic* diffusion properties i.e the same in all directions in space. The diffusion of water in brain tissue is affected by the local tissue microstructure; for example, water molecules diffuse more easily along the major axis of a white matter fibre bundle than perpendicular to it. This type of diffusion behaviour is called anisotropic. DTI is sensitive to *anisotropic diffusion* and has been developed as a tool for investigating the local properties of brain tissues such as white matter tracts (LeBihan *et al.* 2001). There has also been a great deal of interest in using scalar indices derived from the diffusion tensor as markers of white matter tract integrity for disease diagnosis and tracking disease progression.

DTI specifically probes the random-walk process, known as Brownian motion, which water molecules undergo. If there are no barriers to this random-walk (or no preferential direction to the barriers), then there is equal probability that a water molecule will displace by a given distance in any direction; the scatter pattern is isotropic (left diagram in Figure 4.1). However, if the water molecule is, inside a white-matter axon, for example, then the cell walls provide a barrier to diffusion. This means that the water molecules can travel along the fibre with less resistance than across it, so the scatter pattern becomes anisotropic (centre diagram in Figure

4.1). In diffusion MRI, there are often voxels which contain several fibre populations, in which case the scatter pattern contains a contribution from each population (right diagram in Figure 4.1).

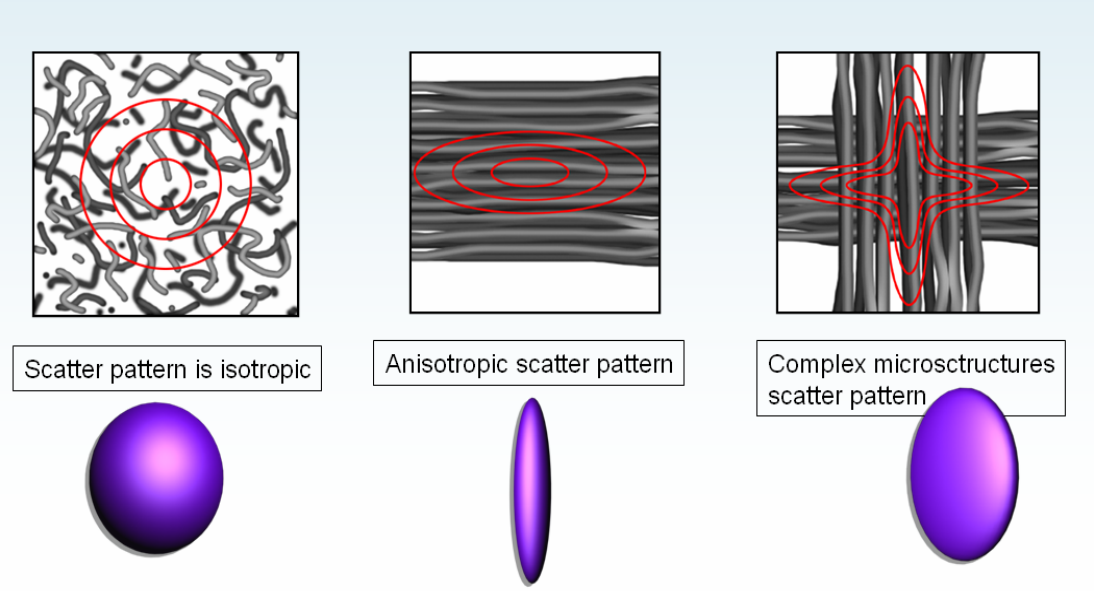


Figure 4.1: Water molecule movement

For anisotropic tissues, the physical orientation of the tissue (e.g fibre direction) in conjunction with the applied gradient direction will determine the signal intensity. If these two directions are the same, there is no problem in determining anisotropy, but usually this is not true. In this most general case the diffusion properties are described mathematically by a tensor. A tensor is a matrix of values.

To characterise the diffusion tensor for each voxel in the brain, measurement of diffusion are carried out in at least six independent directions (Basser *et al.* 1994) (Pierpaoli & Basser 1996; Pierpaoli *et al.* 1996).

The diffusion tensor is described by 3 eigenvalues (λ_1 λ_2 and λ_3, describing the magnitude of diffusion in three dimensions) and 3 eigenvectors (ε_1, ε_2 and ε_3,

describing the orientation of the three eigenvalues) and is insensitive to head orientation and fibre tract alignment in the scanner. From the eigenvalues, information can be derived about how far diffusion differs from being spherical (i.e. from the profile of isotropic diffusion). If the principal eigenvalue (by convention λ_1) is much greater than λ_2 or λ_3 then diffusion is highly non-spherical process (anisotropic) and the highest diffusion within this voxel will have an orientation along ε_1. Diffusion does occur along directions ε_2 and ε_3, but to a lesser degree than along ε_1 (Pierpaoli & Basser 1996).

From these data, images can be produced which represent at the voxel level how isotropic or anisotropic diffusion is. There are a number of parameters that can be used to describe the degree of anisotropy at the voxel level, which include *mean diffusivity* and *fractional anisotropy.* The most commonly used one however is fractional anisotropy (FA). This measure describes how much the diffusion tensor profile deviates from the sphere. The value of this parameter, ranges from 0 to 1, can be calculated for each voxel and the results displayed in the form of an FA map (Pierpaoli & Basser 1996). If the FA is zero, then the diffusion tensor is completely spherical; if it is one, then the diffusion tensor is a “spike” shape. In addition, principal eigenvector maps can be derived and colour coded to demonstrate whether the principle diffusion direction is in the anterior-posterior, left-right or inferior-superior direction with voxels.

The two popular choices of scalar indices therefore are *fractional anisotropy* (FA) and *mean diffusivity* (MD) (Pierpaoli & Basser 1996). Explained more simply, FA

describes the directionality of diffusion; while MD describes the amount of diffusion in a voxel, regardless of directionality. Both measures are independent of the local fibre orientation (and therefore a relatively objective and straightforward measure to compare across subjects). If high diffusion levels occur in white matter, then it is indicative of poorly developed, immature, or structurally compromised white matter. High levels of anisotropy are considered a reflection of coherently bundled, myelinated fibres orientated along the axis of the greatest diffusion (Cascio J.C *et al.* 2007). Other metrics, such as axial (λ_{axial}) and radial (λ_{radial}) diffusivity provide additional information on whether changes in FA and MD are caused by a change in diffusivity in the principal direction of diffusion or caused by a change in diffusivity in a perpendicular direction respectively.

As a marker for tract integrity, FA is a useful quantity to compare across subjects as it is computable voxelwise and is a scalar value that is independent of the local fibre orientation and therefore a relative objective and straightforward measure to compare across subjects (Smith *et al.* 2006).

To achieve this many studies have utilised a voxel-based morphometry (VBM) approach (Ashburner & Friston 2000). VBM has been used in many structural imaging studies, exploring differences in grey matter density once macroscopic differences in brain shape and size has been discounted.

In VBM-style FA analysis, each subjects FA image is registered into a standard space, and then voxelwise statistics are performed to identify areas which correlate to the covariate of interest (e.g., patients vs normals, disability score, age).

Traditionally voxel indices measured as VBM involves a number of imaging preprocessing steps and then subsequent analysis and anatomical localization of any detectable differences in the brains under study. The key steps involved in performing VBM are Normalisation, Segmentation, Smoothing and the Statistical analysis. These steps are available in more detail through the University College London / The Wellcome Department of Imaging Neuroscience website www.fil.ion.ucl.ac.uk./spm Results are displayed as an image where the voxel values are t-statistics, known as Statistical Parametric Map (SPM). A serious limitation of VBM-style approaches is the need for spatial smoothing, and the problem of arbitrarily choosing the spatial smoothing extent (Smith *et al.* 2006).

A method developed specifically for analysing FA maps is Tract-Based Spatial Statistics (TBSS) (Smith *et al.* 2006) . TBSS uses a slightly different approach to SPM. Instead of comparing all voxels of the brain, it only performs statistics for voxels on a white-matter skeleton. The procedure involves transforming the FA map of each subject into standard space and generating a white matter skeleton from the group mean FA map. To ensure that the FA measurements are from the centre of each individual's white-matter tracts, there is a projection step that searches for the locally maximal FA orthogonal to the skeleton. The same transforms can then be applied to the MD, λ_{axial} and λ_{radial} maps. The main limitation of this approach is that

large parts of the brain (grey matter) are omitted from analysis, however it has advantages in that it deals with alignment issues, has no need for smoothing and the processing is straightforward, by comparison.

4.1.3 Brain development and DTI

The structure of the brain continues to develop throughout childhood, and into adolescence and early adulthood. Although there is a clear consensus that anisotropy increases and diffusion decreases with age, there is conflicting data as to what trajectory those changes follow during development. Studies generally concur that stabilisation of diffusion properties is reached by 2 years of life (Schneider *et al.* 2004; Zhang *et al.* 2005). Following this steep pattern, is a slower process of change on through adolescence (Mukherjee *et al.* 2002). The slope of FA from 2 to 18 years of age is modest in magnitude (Schmithorst *et al.* 2002) although variable across various regions of the brain. It is mostly agreed that the most significant brain changes occur in children up to the age of 5 years old; however structures do continue to develop into adulthood. The rate of these changes is also known to differ between boys and girls. In the first TBSS study looking at sexual dimorphism in children aged 8 to 13 years, widespread white matter changes in boys were noted, but not in girls in this age range (Seunarine *et al.* 2010). These differences must be accounted for in any comparative analysis.

4.1.4 DTI in other LSD

DTI has already been used to investigate brain integrity in other lysosomal storage disorders with interesting results, most notably based on the findings in adults with Fabry disease performed in one German centre (Fellgiebel *et al.* 2006; Albrecht *et al.*

2007). These findings, which included 27 adult Fabry patients compared to 21 age-matched controls identified that global mean diffusivity (MD) was increased in Fabry patients whereas global fraction anisotropy (FA) did not differ significantly compared to controls (Fellgiebel *et al.* 2006; Albrecht *et al.* 2007). The authors recommended that diffusivity measurements was a more sensitive structural imaging tool to detect and quantify brain involvement in Fabry disease at an early stage of disease, even in patients without white matter lesions.

In paediatric patients DTI studies in two different LSD were identified, Krabbe disease and Cystinosis.

In 2001, Guo *et al* (2001) examined eight patients with Krabbe disease and eight age-matched control subjects (Guo *et al.* 2001b). Anisotropy maps were generated with diffusion tensor data, encoding in six directions. Loss of diffusion anisotropy appeared on anisotropy maps as areas of decreased hyperintensity in patients with Krabbe disease. Guo *et al* (2001) reported that diffusion tensor derived anisotropy maps provide a quantitative measure of abnormal white matter in patients with Krabbe disease, which is more sensitive than T2-weighted images for detecting white matter abnormality. The authors also stated that DTI may be a marker of treatment response (Guo *et al.* 2001a).

A later study applied DTI to 24 children with Cystinosis (age 3–7 years) and to 24 typically developing age-matched controls (Bava *et al.* 2010). Scalar diffusion indices, fractional anisotropy (FA) and mean diffusivity (MD) were examined in

manually defined regions of interest within the parietal and inferior temporal lobes. Bilaterally decreased FA and increased MD were evident in the inferior and superior parietal lobules in children with cystinosis, with comparable FA and MD to controls in inferior temporal white matter, and implicate a dissociation of the dorsal and ventral visual pathways. The strength of this study is that diffusion indices were correlated with performance on measures of visuospatial cognition and with white blood cell cystine levels. In older cystinosis children (age > 5), diminutions in visuospatial performance were associated with reduced FA in the right inferior parietal lobule. In addition, increased MD was found in the presence of high cystine levels in all children with cystinosis. This study demonstrated that the average diffusion properties in children with cystinosis deviate from typically developing children, suggesting the presence of early microstructural white matter changes in addition to a secondary effect of cystine accumulation.

4.2 Method

4.2.1 MR acquisition

Diffusion-weighted images were acquired on a Siemens Avanto 1.5T scanner using a twice refocused single-shot diffusion-weighted spin-echo echo-planner imaging sequence (TR 6300ms, TE 89ms, field of view 240mm, and acquisition matrix 96×96). A total of 45 transverse slices covering the whole brain were collected with slice thickness of 2.5mm, zero gap and an in-plane resolution of 2.5×2.5mm. Diffusion gradients were applied in 20 directions at b=1000 s mm^{-1}. This was repeated three times to improve signal-to-noise ratio. Three b=0 s mm^{-1} images were also acquired.

4.2.2 Image Processing

All images of Gaucher patients were inspected visually by an experienced Consultant Paediatric Neuroradiologist (Dr Kling Chong), who was blinded to the clinical presentation. Any lesions were described in terms of their location and graded 1-3 based on whether they were obvious or subtle.

Processing of the data involved removal of eddy-current distortion and skull-stripping of the brain volume using FSL (www.fmrib.ox.ac.uk/fsl). To improve alignment, all subjects were registered on a specific template. Diffusion tensors were then fitted to the data and the FA, MD, λ_{axial} and λ_{radial} maps were calculated utilising TBSS as previously described. All analysis was performed by Dr Kiran Seunarine (Institute of Child Health).

NGD patients were compared to a control group using a two sample t-test, where age and sex were added as covariates. The same procedure was used to compare Type I patients to a separate age-sex matched control group. The mean of each diffusion parameter over the white-matter skeleton was also calculated for each subject and plotted against age for a larger cohort of normal children to compare overall trend.

4.2.3 Analysis of DTI data

The pathology data presented in the Introduction suggests that the Gaucher pathology is likely to be found in both grey and white matter (neuronal cell bodies of grey matter and astrocytosis of cerebellar white matter). This has important

implications when considering MRI approach and analysis is the most appropriate to utilise.

To examine the grey matter in this context some exploratory VBM analysis was performed according to the methodology reported by Ashburner & Friston (2000). This initial analysis however failed to identify any areas of the brain that differed from Type I to the NGD cohort. This was believed to be partly attributed to the VBM analysis used and that possibly neuropathalogical findings in the grey matter are not as prominent as those in the white matter.

Based on this and the fact that extensive white matter lesion is a frequent finding in many metabolic diseases, and that there are currently no other robust methods for voxel-wise spatially normalised analysis of grey matter, Tract-Based Spatial Statistics (TBSS) was utilised. Given that TBSS only performs voxel-wise statistics on the main white-matter skeleton is clinically more meaningful that a voxel alone.

Type I Gaucher and NGD were compared to their own age-sex matched control group. This is to account for the fact that there was an age and sex differentiation between the NGD and Type I patients recruited. The rationale for including Type I Gaucher disease was again to be able to define the characteristic of the NGD brain compared to the Type I Gaucher brain, which may offer an insight into why Type I patients remain neurologically intact.

4.3 Results

4.3.1 Participants

A total of seven children had an MRI; three girls and one boy with NGD and three boys with Type I Gaucher disease. The oldest three NGD patients were L444P homozygote, girls, who were able to perform the scan unsedated. The youngest was a boy with L279P/G243V, who required a scan for clinical purposes under general anaesthetic. The four NGD children had a mean age of 12.2 years (±3.5) (range from 7.3 to 14.11) and the three Type I patients had a mean age of 9.5 years (±3.3) (range from 6.9 to 13.2 years). A more detailed outline of these NGD patients are given in Appendix 3. As these two groups were not age and sex matched two separate control groups were required. These were acquired from pre-existing normative data available at the institute.

From this set of data, an NGD control group was selected, with a mean age of 12.2 years (±2.2), while the Type I control group was 9.7 years (±2.7). In an attempt to improve power for this small study the control groups were made 2:1 to the Gaucher groups.

All the NGD children had the characteristic HGP. Neurological manifestations were relatively mild and stable in the eldest two girls, while the youngest girl had ataxia present on straight gait, with pyramidal involvement. The youngest child, a boy, presented with severe ataxia and progressive myoclonic seizures. The mean mSST score for the NGD was 7.5 (±8.1) while the mean mSST score for the Type I Gaucher group was 0.0 (±0.0). Despite the large difference in score there was no

statistical difference between the groups, possibly due to the small sample sizes and the large distribution in the NGD group.

4.3.2 TBSS Results

Figure 4.2 and 4.3 display TBSS results comparing NGD patients to their age-sex matched controls. The white-matter skeleton is shown in green, increases are shown as red-yellow and decreases are shown as blue-light blue. A decrease in FA and an increase in MD ($p<0.05$, uncorrected) is observed, which is still significant at a $p<0.01$ level. A slight increase in λ_{axial} and λ_{radial} (both $p<0.055$) is also seen, which suggests that the increase in MD and decrease in FA (seen in Figure 4.2) is driven by an increase in radial diffusivity, as opposed to a change in axial diffusivity. However, λ_{axial} is an inherently noisier measure than λ_{radial} and therefore may be less sensitive to change.

The most significant differences ($p<0.01$, uncorrected) between the two groups is in the right middle cerebellar peduncle, with some differences in the right superior cerebellar peduncle. In these regions we observe a decrease in FA (left) and an increase in λ_{radial} (right) and MD. There is also an increase in axial diffusivity; however the effect is much smaller than with other indices.

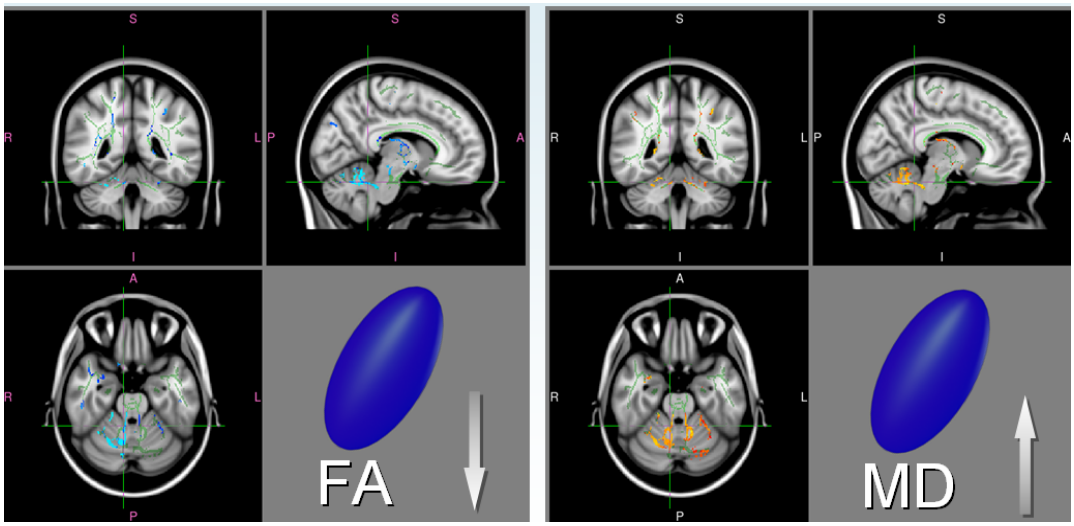


Figure 4.2: TBSS results (p<0.05, uncorrected) comparing NGD patients to controls using Fractional Anisotropy (left) and Mean Diffusivity (right). The white-matter skeleton is shown in green. Increases are shown as red-yellow and decreases are shown as blue-light blue. All results are overlaid onto T1-weighted template

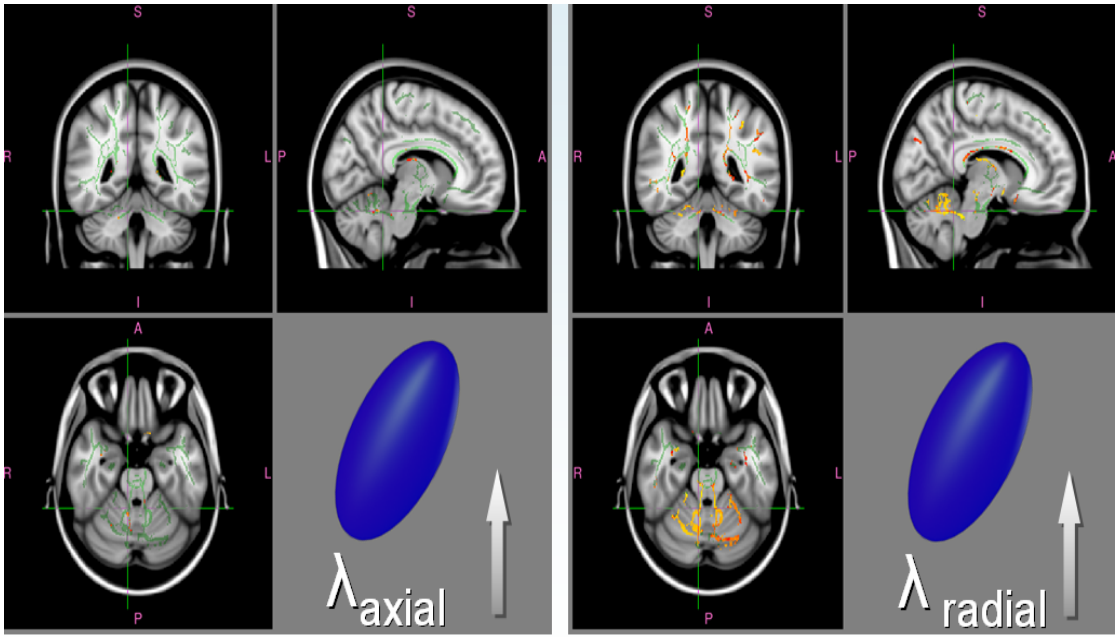


Figure 4.3: TBSS results (p<0.05, uncorrected) comparing NGD patients to controls using λ_{axial} (left) and λ_{radial} (right). The white-matter skeleton is shown in green. Increases are shown as red-yellow and decreases are shown as blue-light blue. All results are overlaid onto T1-weighted template

Exploring the TBSS results of the Type I patients compared to their age-sex matched controls (Figures 4.4 and 4.5) small, diffuse areas scattered across the brain are apparent, which are consistent to the NGD findings of a decrease in FA

and an increase in MD, also significant at $p<0.05$ (uncorrected). The increased pattern for λ_{axial} seen in the NGD group is not observed in the Type I cohort, furthermore there is no one specific area identified, and in particular no significant difference observed in the cerebellar peduncles.

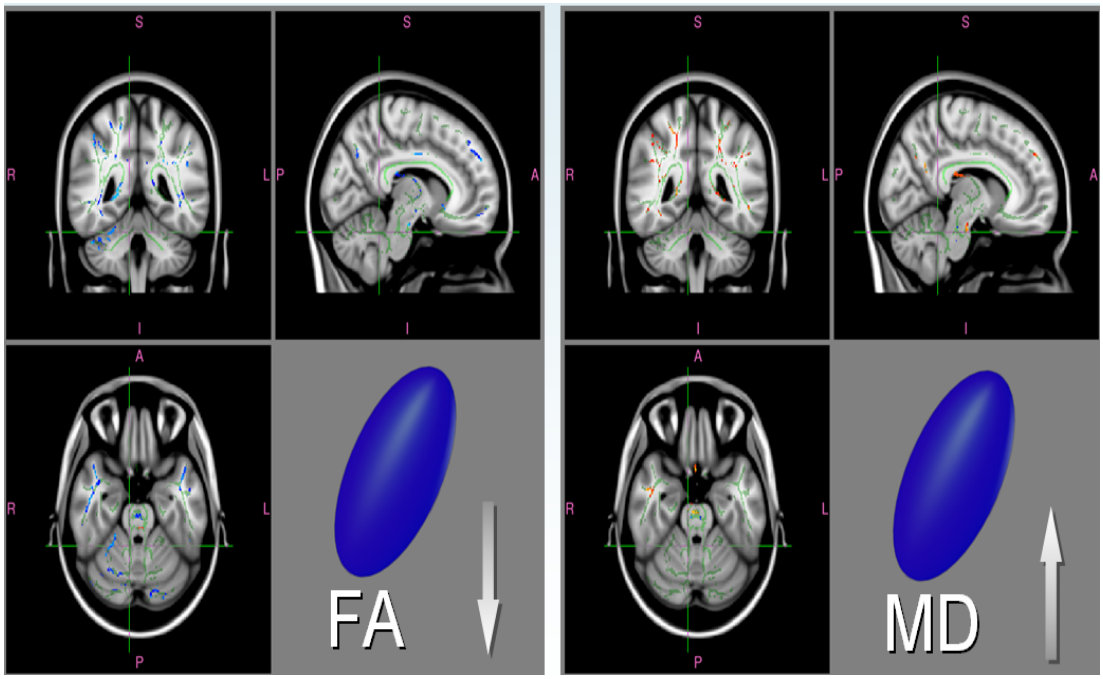


Figure 4.4: TBSS results (p<0.05, uncorrected) comparing Type I patients to controls using FA (left) and Mean Diffusivity (right). The white-matter skeleton is shown in green. Increases are shown as red-yellow and decreases are shown as blue-light blue. All results are overlaid onto T1-weighted template

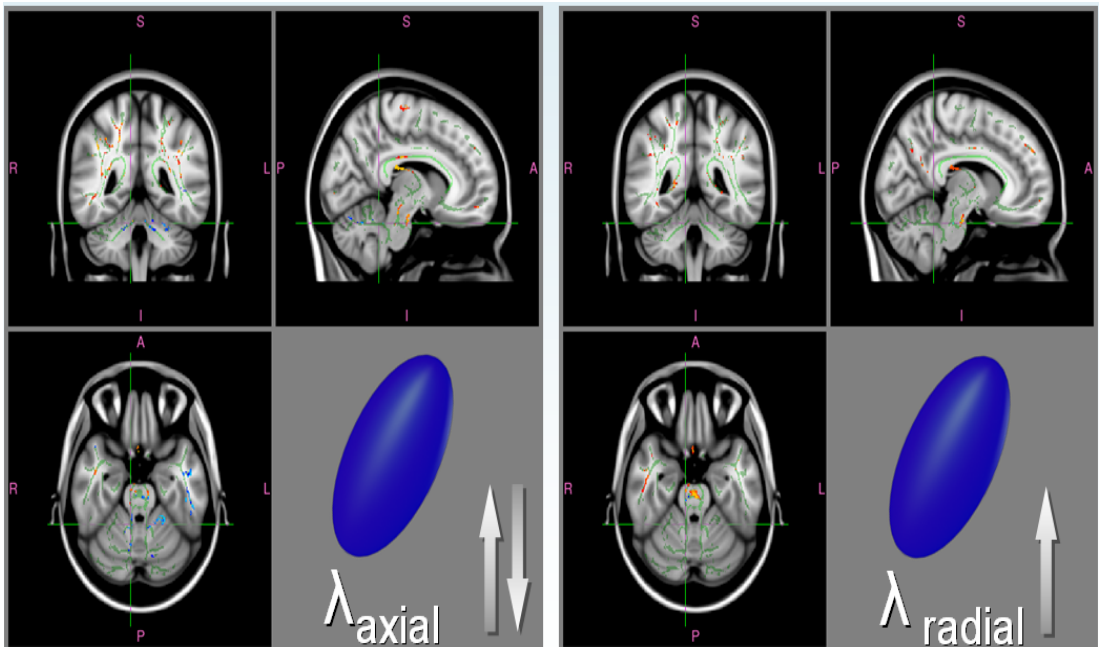


Figure 4.5: TBSS results (p<0.05, uncorrected) comparing Type I patients to controls using using λ_{axial} (left) and λ_{radial} (right). The white-matter skeleton is shown in green. Increases are shown as red-yellow and decreases are shown as blue-light blue. All results are overlaid onto T1-weighted template

These findings noted in the NGD cerebellar peduncle is consistent with the fact that all four children were right hand dominant.

Based on these areas of significant change noted in the cerebellar peduncle further analysis of this area was performed. This approach is known as selecting a Region of Interest (ROI) for individual analysis, and has been discussed previously. In this instance however the ROI was selected electronically and not generated manually. The ROI was selected from the significant cluster in the right cerebellum using computerised 'flood-fill' algorithm where the ROI is a point in the significant region and 8-neighbourhood fill operation is used to select the rest of the region. The ROI identified is shown in Figure 4.6.

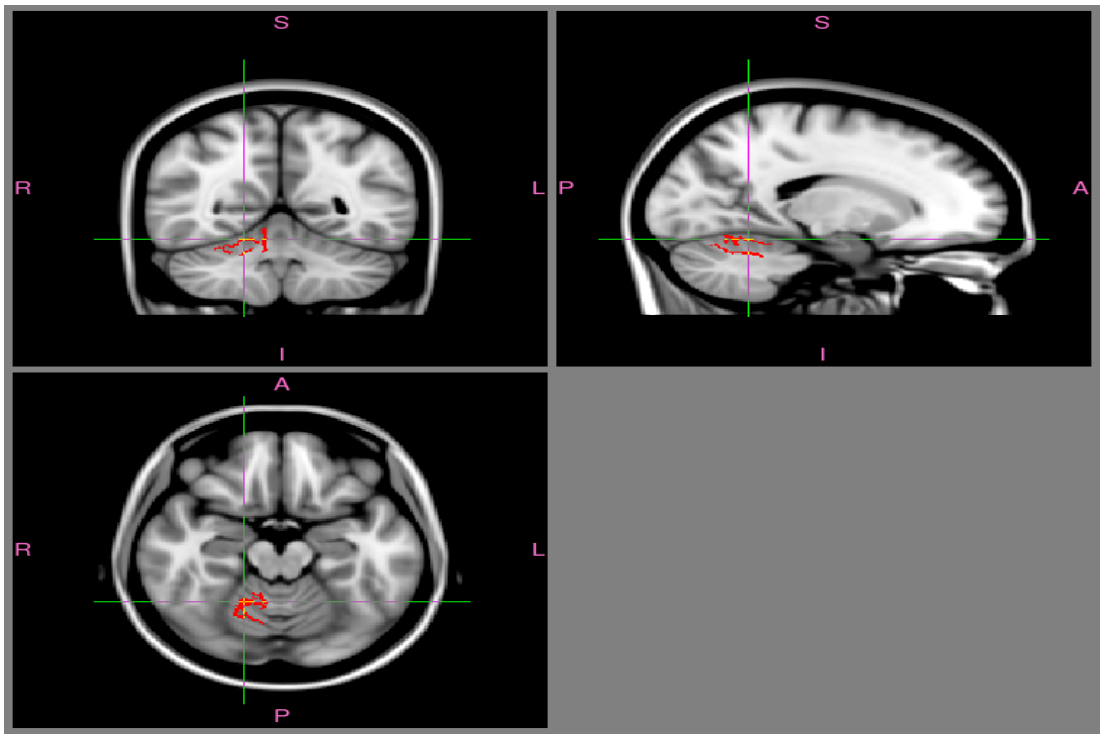


Figure 4.6: Selected Region of Interest (ROI) in the cerebellar peduncles

The mean FA and MD in the ROI was calculated and used to investigate group differences between the NGD group and their respective controls. These results were analysed with ANOVA to account for age and gender and are presented in Figure 4.7 and 4.8.

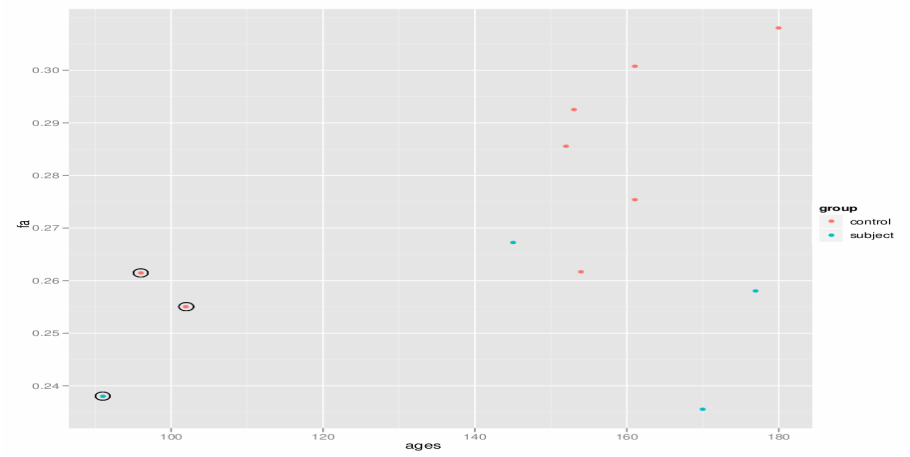


Figure 4.7: Plot of mean Fractional Anisotropy (FA) in the cerebellum ROI for NGD compared to their age-sex matched controls (circles indicating boys). P 0.026

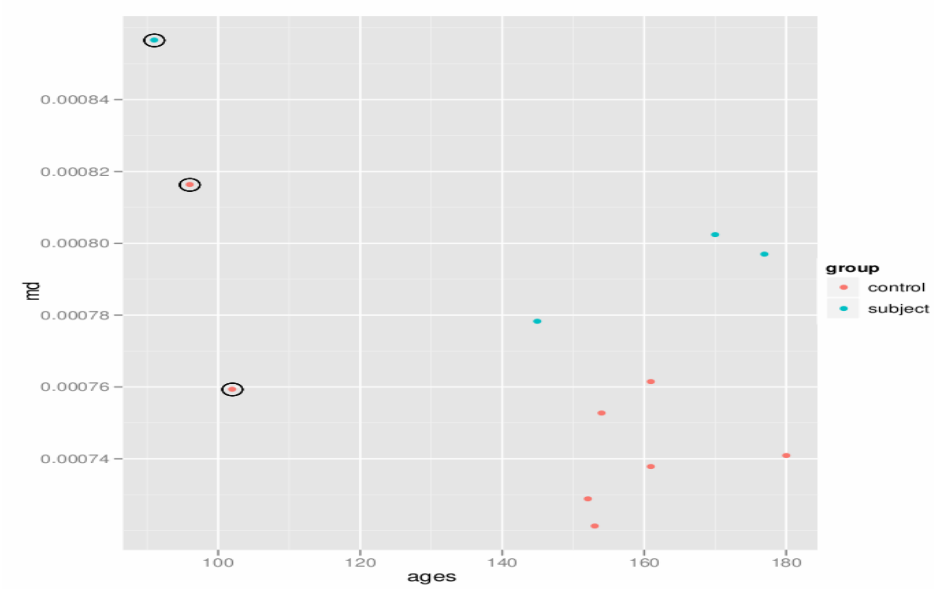


Figure 4.8: Plot of Mean Diffusivity (MD) in the cerebellum ROI for NGD compared to their age-sex matched controls (circles indicating boys). P 0.001

The ROI findings supports the initial findings in that NGD patient have evidently lower FA and higher MD compared to their age-sex matched controls.

4.4 Limitations

This data suggests that DTI may be a promising tool for the detection of ultrastructural cerebral changes in these patients; however it has certain limitation that may prohibit its immediate use as a biomarker in clinical studies. These limitations must be acknowledged, and addressed if its value is to be potentially considered in the future.

The sample size, while reflective of the rare incidence of Gaucher disease, and the challenge of children complying with an unsedated MRI, limits the statistical power of the study. It also precludes extensive meaningful analysis of relationship between the findings and mSST score, genotype or gait parameters.

Although the control group was selected to be as close as possible to be age and sex-matched for the Gaucher groups, the self-selection of control groups, from a limited source, is open to criticism.

Full Scale IQ for both Gaucher groups and control groups was not available to explore for difference. However, it is suspected that the groups were not IQ matched, as NGD patients are known to have lower IQ and the control group studied was reported to have a normal to high IQ. Although the issue of chronological age on DTI results were accounted for in the selection of an age-matched control group, any possible impact that developmental age may have is not. Indeed developmental changes may be more dependant on the data than chronological age.

A methodological limitation is that TBSS intentionally restricts the analysis to the small subset of voxels in the white-matter skeleton. This improves the statistics by reducing the multiple comparisons problem, although it also omits regions of potential interest, such as the basal ganglia and grey matter, from the analysis. Considering the advantages of TBSS in dealing with alignment issues, this approach is considered justified however, despite the loss of grey matter analysis.

4.5 Discussion

TBSS analysis of the white matter tracts identified decreased FA and increased MD in the cerebellum of Gaucher patients. Although statistically significant in both the Type I and NGD cohort the changes in the Type I cohort were diffuse, where as the

changes seen in the NGD were primarily in the middle cerebellar peduncles, with some differences also seen in the superior cerebellar peduncles.

Based on neuropathology findings, it is known that even patients with Type I have perivascular lipid laden macrophage, with astriogliosis identified in both non-neuronopathic and NGD. This may explain the diffuse changes identified in the Type I patients.

Neuropathological reports of neuronal loss in the dentate nucleus in a patient with myoclonus seizures (Verghese *et al.* 2000) is also consistent with our findings, particularly as one of the patients imaged in our study (youngest male patients) now presents with myoclonus seizures. The dentate nucleus is the most lateral and largest of the deep cerebellar nuclei, serving as a source of fibres composing the superior cerebellar peduncle, once again therefore consistent with these findings.

Our findings suggest the presence of microstructural white matter changes in the middle and superior cerebellar peduncles of NGD patients. Predominantly it's the cortico-ponto-cerebellar pathway that runs through the middle cerebellar peduncles. This is the main route by which the cortex communicates with the cerebellum, which naturally is very important for equilibrium.

Estimates based on light microscopy suggest that there are 20 million fibres in the peduncles and only one million fibres in the cortico-spinal tracts. The great majority of peduncle fibres end in the pontine nuclei. The possibility that cortico-spinal and

cortico-bulbar fibres also give off collaterals to pontine cells as the pass through the pons suggests that every fibre in the cerebral peduncles has a pontine target. One of the major circuits through the human brain is a route that originates in the cerebral cortex and connects to the cerebellum by the way of the pontine nuclei. The cerebellum, which is bilaterally symmetrical and placed in the posterior cranial fossa, influences the timing and force of contractions of voluntary muscles, through its afferent and efferent connections, resulting in smooth, coordinated movement (Young & Young 1997). Given the clinical presentation of ataxia seen in NGD patients, in the sense of gait and ataxia in the broader sense, which is not characteristically seen in Type I paediatric patients this finding is clinically viable. This finding would also be in line with the neuropathology seen in NGD patients as previously described.

Our findings of an increased MD (compared to age-sex matched controls) is consistent with findings reported for other LSDs, such as Fabry disease, Cystinosis and Krabbe disease, as previously discussed.

The findings of Abdel Razek *et al* (2009) however are at odds with all the other LSD publications utilising DTI, where increased diffusion coefficient, rather than lower diffusion is observed. A possible explanation for this discrepancy is that the patients and control group were not adequately matched for age. As stated, the patients studied by Abdel Razek *et al* (2009) were between 8 months and 14 years. Brain changes during this age group are significant. The authors do not provide the age ranges for the controls, but the presence of older subject in the control group would

manifest as apparent decrease in diffusion coefficient. Furthermore Abdel Razek *et al* (2009) do not specify if sex-matched controls are used. The developmental trajectory of boys and girls differ, which may contribute to the findings. One further consideration is that the DWI acquisition uses diffusion-weighted measurements acquired in 3 orthogonal directions and estimates the diffusivity from the measurements directly. DTI, on the other hand acquire 20 diffusion-weighting directions three times and the MD is calculated from the diffusion tensor, which offers higher precision.

Changes in the peduncles were also noted in neonates with infantile Krabbe disease (Escolar *et al.* 2009). Based on their DTI study of six neonates with infantile Krabbe disease identified through family history, Escalor *et al* (2009) claimed that FA differences identified in the superior and middle cerebellar peduncles may help to differentiate between babies who will develop the early-onset disease, those who will develop disease at a much later time and those who will never develop disease. This approach can provide very consistent FA values and detect regional differences and age-related changes in the first 2 years of life. For this reason the authors claimed that DTI with quantitative tractography may prove to be an excellent technology for studying infantile Krabbe disease and other demyelinating conditions of early childhood. A large sample of healthy controls and babies with low enzymes who do not develop early-infantile disease will need to be studied for an adequate standardisation and validation of this tool (Escolar *et al.* 2009).

In adults, there are many conditions that are associated with MR abnormalities in the middle cerebellar peduncle, the common one being multi-system atrophy, but also a rare genetic ataxic syndrome dentato-rubro-pallido-luysian atrophy (DRPLA) which causes a T2 high signal and restricted diffusion there, even cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) can cause a similar MR picture and progressive ataxia.

In a study examining white matter damage in adults with spinocerebellar ataxia type 1 and 2, TBSS revealed decreased FA in the inferior, middle and superior cerebellar peduncles (Della *et al.* 2008). Despite the different aetiology, spinocerebellar ataxia has clinical similarities to that of NGD - ataxia of gait, stance and limbs, dysarthia and oculomotor abnormalities, albeit progressing at a more rapid rate (Schulz *et al.* 2009).

In another study, apparent diffusion coefficient measurements of the middle cerebellar peduncle were identified to differentiate in the Parkinson variant of multiple system atrophy (Nicoletti *et al.* 2006). On the basis of symptoms at onset, multiple system atrophy (MSA) has been divided into two forms: MSA-C characterised by a predominance of cerebellar symptoms and MSA-P where Parkinsonism is prevalent. The clinical presentation of MSA-C is more comparable to that seen in NGD, however parkinsonian-like symptoms are increasingly being linked to Type I Gaucher disease (Sidransky 2005).

The value of DTI as a biomarker

There is great interest in developing objective biologically based markers that can be used to predict risk, diagnose, stage, or track the course and treatment of neurodegeneration and complement currently employed clinical measures. Fox and Growdon (2004) report that the ideal surrogate endpoint or outcome measure is a laboratory substitute for a clinically meaningful result, and should lie directly in the causal pathway linking disease to outcome. There are three different areas where imaging biomarkers may have important roles to play: as markers of trait, state and rate. A measure of disease trait is a marker such as a genetic mutation that predicts the likelihood of developing disease. A measure of disease trait may also indicate susceptibility to disease. A measure of disease state is, in essence, a diagnostic biomarker. A measure of disease rate or change is a marker that can be used to track progression of the pathophysiology of the disease, or to detect the effects of a therapeutic intervention. Promising markers in this regard, are measures of atrophy on quantitative magnetic resonance brain scans. It is theoretically possible that a single biomarker may fulfil all roles of measuring trait, state and rate in a particular disease. However, the requirements of a robust predictive marker, a sensitive and specific diagnostic marker to separate disease, and a validated marker of progression in trials in neurodegenerative brain disease may be quite different from each other, making this unlikely.

In practical terms, it is more likely that it will be necessary to use a number of different markers, either separately or in combination, to fulfil these three different roles (Fox & Growdon 2004). Recent advances in Alzheimer's disease illustrate the

use of neuroimaging as a biomarker for clinical trials (Dickerson & Sperling 2005) and increase the value their consideration in other neurodegendrative disorders, such as NGD.

Chapter 5

Discussion

“A p-value is no substitute for a brain.”

Anonymous

5. Discussion

Chapter 2-4 has demonstrated that the mSST, GAITRite and DTI are have all been able to quantifying disease severity across the Gaucher cohort studied. The relative value of each tool is considered to be variable however.

Through validation, the mSST has demonstrated its ability to monitor disease progression in the largest cohort of NGD patients ever studied systematically and prospectively. The GAITRite was able to distinguish between the gait profile of the NGD and Type I cohort, defining key parameters (Velocity, Step Time and Double Support in particular) that deviated from normal, however the lack of trend identified in the sequential data is a real limitation in its use. The DTI identified areas of statistically significant difference in NGD children compared to age-sex-matched controls. This difference was prominent in the middle cerebellar peduncle of the NGD cohort, which is consistent with the clinical finding of ataxia observed in this cohort, indicating a 'proof of concept' for this methodology.

It is important to consider however how the findings for each individual assessment correlate with each other, and what the advantages, disadvantages and potential use for all three assessments are.

5.1 Correlation of the three assessments.

Although each assessment tool has demonstrated to be useful in its own right, it is important to consider whether the findings correlate with each other, and whether the three assessment tools are in agreement in identifying and/or scoring severe patients.

The patients recruited for both gait analysis and mSST assessment demonstrated a statistical difference in the mSST score (*p*0.026) between the Type I and NGD patients, indicating a clear difference in the neurological profile, as would be expected, and an indication that a difference in gait profile identified using the GAITRIte was clinically expected. The mean mSST score for the NGD cohort was 6.3 (±5.3) and 0.2 (±0.4) for the Type I cohort.

Total mSST scores and those of five clinical domains within the mSST were correlated to gait parameter Z scores, as calculated using the LMS centiles (Table 5.1). The five chosen clinical domains within the mSST were: Ataxia/Gait; Cerebellar Ataxia; Pyramidal, Extrapyramidal and Kyphosis. These were selected as it was believed that these were the clinical domains most likely to impact on gait.

Table 5.1: Correlation of gait parameters and mSST score and domains

		Velocity	Cadence	Step Length	Step Time	Single Support	Double Support	Base of Support
Ataxia / Gait	*R*	-.588*	-.157	-.235	.510	-.510	.471	.510
	Sig.	.027	.592	.418	.063	.062	.089	.063
Cerebellar	*R*	-.172	-.447	.310	.447	-.379	.378	.378
Ataxia	Sig.	.557	.109	.281	.109	.182	.182	.182
Pyramidal	*R*	-.649*	-.277	-.362	.543*	-.757**	.690**	.106
	Sig.	.012	.337	.204	.045	.002	.006	.718
ExtraPyramidal	*R*	-.440	-.609*	-.061	.609*	-.603*	.602*	.602*
	Sig.	.115	.021	.835	.021	.022	.023	.023
Kyphosis	*R*	-.289	-.658*	-.052	.510	-.312	.312	-.021
	Sig.	.317	.011	.860	.063	.277	.277	.944
mSST	*R*	-.696**	-.464	-.287	.707**	-.750**	.678**	.202
	Sig.	.006	.094	.320	.005	.002	.008	.488

***. Correlation is significant at the 0.01 level (2-tailed).*

Utilising Spearmans Rho to accommodate for the ordinal data in the mSST domains, statistically significant correlation is observed between six of the seven gait parameters and the mSST domains. Cerebellar Ataxia did not correlate with any of the gait paramaters, and Base of Support only correlated with Extrapyramidal. Indeed, Extrapyramidal is the domain that correlates with the highest number of gait parameters. Velocity and Step Length are the only two gait parameters that did not correlate with Extrapyramidal. The strength of association was strongest between the Extrapyramidal domain and Step Time and Cadence, *r* 0.609 and -0.609 respectively. This negative correlation with Cadence possibly indicative that a significantly reduced number of steps correlates with moderate to severe Extrapyramidal involvement.

Given the number of gait parameters that correlate with Extrapyramidal, and the strength of correlation with Pyramidal, it would imply that these two domains have more impact on gait compared to the other domains measured within the mSST.

A reason why correlation is stronger in the Extrapyramidal domain compared to Ataxia/Gait is that the domain construct for Ataxia/Gait has “Normal, apparent only on tandem walking” as the first construct. This may therefore allow for a patient to have some pathology, which would not be scored within the realms of the mSST domain. The same is also true for the Pyramidal domain, in that ‘Normal tone with increased reflexes’ scores 0, but the presence of increased reflexes might be an indication of an underlying pathology.

Kyphosis only correlated with Cadence. This may suggest that the presence of Kyphosis has a limited impact on the gait profile as measured using the GAITRite.

The mSST total score had the strongest correlation with Step Time and Double Support, but also correlated with Velocity and Single Support. As previously discussed in the Chapter 3 Step Time and Double Support may be indicative of power and postural control, and are more subtle abnormalities compared to a change in Velocity, which in isolation could be just regarded as a indication of strength and power and/or none CNS manifestations. An increase in disease severity as measured utilising the mSST accounts for all the neurological manifestations seen in NGD, and this correlation with Step Time and Double Support indicates that these are important gait parameters which reflect a neurologically driven pathology.

When the FA and MD values obtained for both groups in the TBSS analysis of DTI are added to the correlation equation however, it is noted that neither FA nor MD correlates with any of the other mSST domains or gait parameters. One of the reason for this may be the small sample size that were able to consent to having an MRI performed, particularly as age and sex need to be considered as covariates. Furthermore, it was a disadvantage that the controls used for DTI analysis were not assessed the mSST or GAITRite.

5.2 The advantages, disadvantages and potential value of each assessment

The modified Severity Scoring Tool

In line with the ICH E9 guideline the SST has been modified and validated for measuring a clinically relevant and important benefit to the patient population. Factors such as content validity, inter- and intra-rater reliability and responsiveness for detecting changes have been addressed, which is advantageous compared to some other tools currently used clinically.

Based on a cohort covering three European countries the mSST has effectively distinguished disease severity and rate of progression across the genotypes, which strengthen its validity. Whether these findings can be extrapolated to account for the clinical presentation of patients in other geographical regions to Europe (e.g Asia and Africa) now needs to be established.

When considering the value of the mSST as a clinical tool, its potential as a primary end-point in a clinical trial is an obvious consideration. It is particularly advantageous that the mSST data generated this far can be used to calculate a sample size that may be required for any proposed clinical trial. This is hypothetical, and based on the assumption that the mode of action of drug “X” would be evaluated for halting disease progression rather than reversing clinical damage.

In the traditional, or classical approach to clinical trial design the mSST change observed in *n*=39 cases would be utilised to estimate the effect in any proposed untreated control group compared to a treated group. For instance, the treated

group whom would be anticipated to remain stable can be predicted based on the result for mildest group D409H and L444P, who did not change (i.e mean change = 0 with an estimated ± of 2.27), while the untreated group would be anticipated to progress at the same rate as observed during this follow-up assessment (i.e. mean change = 1.71 ±3.91). Based on this data, with a 5% significance level and 80% power 55 patients per treatment group would be necessary. Unfortunately this is too large a cohort to be recruited in such a rare disease. Furthermore this predicted change was observed over 4 years. Although this is reflective of the slow progressive nature of the disease, it may be difficult to persuade pharmaceutical companies to undertake such a long study.

Another way of calculating the sample size for the study would be to use the Minimum Clinically Important Difference (MCID). Based on discussions with the experts a change in mSST score of 3 was considered to be a MCID. Using a MCID of three to calculate the sample size can be done by utilising the mean change for the L444P/other genotype group (3.13 ±8.22) as a reference. However, assuming the same significance and power, 59 patients per group would still be needed. This is probably because of the large standard deviation observed in the L444P/other group.

These problems are frequently encountered when designing clinical trials for rare diseases, where patient recruitment is particularly problematic. Adaptive designs are therefore more appropriate. Regulators and industry alike now accept this (Committee for Medicinal Products for Human Use & CHMP 2007).

Variability (whether in terms of disease phenotype, underlying pathophysiology, pharmacodynamics or pharmacokinetics) is as an acknowledged threat to successful drug development. Efficient study design and analysis requires a clear understanding as possible of all these potential sources of variability. Stratifying for genotype may therefore be an appropriate consideration in this context, particularly given the differences in mSST scores seen across genotypes in terms of severity and rate of progression.

The mSST is a valuable clinical tool that offers a systematic way to monitor NGD patients in the clinical setting. Part of its value is its ease of use, which means that it could easily be incorporated into a registry or database format. This would allow data about cohorts across the world to be captured systematically, and in a way that can quantitatively measure change. Filling the gap about non-European cohorts, and increasing the number of patients with various genotypes would offer a better insight of whether stratifying according to genotype might be valuable in an adaptive study design. In an approach known as data mining - where pooled accessible data collection from well described phenotypes is used to build the foundation for a retrospective data mining. This can be used to qualify new tools as biomarkers and as possible markers for stratification.

An unavoidable limitation in the mSST use is the subjective aspect of evaluation. Despite the guidance of severity offered in each domain the assessing clinician still makes a subjective assessment on the presentation. Although this was not

considered to be an issue of concern in the small inter-reliability evaluation performed, and would be minimised by having on responsible clinician score sequentially, it is still vulnerable to this limitation, as is all clinical scores.

The GAITRite

The value of the GAITRite is that it has demonstrated itself to be an user-friendly and sensitive assessment tool for distinguishing between NGD and Type I Gaucher children. Four gait parameters in particular: Velocity, Cadence, Step Time and Double Support are clinically relevant and sensitive to detecting abnormalities in the gait profile of NGD children. Cadence became apparent while using the Z scores, which may have been missed exploring the raw data only. These can be regarded as meaningful markers of neurological disease in NGD.

A big disadvantage in proposing the GAITRite as a tool to monitor disease progression is the lack of a trend in sequential results - that is some patients actually appeared to have an improved gait pattern, which would not be expected in a progressive disease like NGD. Identifying a reason why there is no evident trend is difficult. Exploring the mSST data captured on the same assessment date, demonstrates that all patients remained stable according to mSST scores.

In a small recent study exploring the behavioural aspect of NGD, McPartlan *et al* (2010) identified that anxiety was a presenting feature in this cohort, with nearly 30% reporting emotions of fear and worry (McPartlan *et al.* 2010). Such psychological manifestations may also impact on the child's performance in a clinical assessment,

particularly on the first attempt. It was hypothesised that this anxiety and fear may have contributed to the child performing worse than could be explained by pathological disease alone. To explore this possibility further, the original data set for each walk, before it was combined into one mean test score was examined. If anxiety and fear was a contributing factor, it was anticipated that each child would improve slightly on each walk, and that this improvement would be consistent across each of assessment. A wide variation was predicted for Patients 2 and 3 where improvement was seen, with less for Patients 1 and 4, whom had shown more of a deterioration. However, this was not observed. There was great variability across the board, and no clear pattern emerged.

The value of the GAITRite at this time therefore remains unclear. The difference observed in the gait pattern of NGD patients compared to Type I and normative data indicated that it could be an useful marker, with proof of concept established. However the lack of trend in sequential assessment is clearly at odds with the progressive nature of the disease, and questions its value as a monitoring assessment for disease severity and/or progression.

Studying a larger cohort, such as that studied with the mSST, and following the patients over a longer period of time may provide a clearer indication of whether this large variation completely prohibits the use of the GAITRite as a worthwhile assessment.

These issues could again be addressed in a data mining exercise. Collating data from world wide cohorts in a data bank would allow for retrospective hypotheses to be performed and genotype-phenotype patterns of gait to be identified. This certainly seems warranted given that the GAITRIte is an attractive clinical tool given its ease of use, and the obvious impact of an impaired gait on the patient. This is regardless of whether the gait abnormality is driven by cerebellar ataxia or neuropathy, particularly as both are progressive in nature in NGD.

As previously mentioned, Velocity has been accepted as a primary endpoint in drug trials by regulatory bodies. McDonald *et al* (2010) has also proposed that Velocity can be utilised as a marker of disease status in neuromuscular disorders, not only as a surrogate marker but as a meaningful clinical endpoint (McDonald *et al.* 2010). Following their study comparing 21 ambulatory boys with Duchenne Muscular Dystrophy (DMD) (median age 8 years) to 34 healthy controls (median age 9 years), McDonald (2010) proposed that a change in performance on the 6MWT can be defined as an intermediate, non-ultimate endpoint that directly assesses how an ambulatory DMD patient functions.

McDonald and co-authors (2010) acknowledged that as their study was observational, and that information regarding the MCID is necessary as the targeted therapeutic difference in a clinical study is unknown. The necessity of predetermining a MCID for establishing substantial evidence of effectiveness in the therapy of a neurological disorder has been debated (Katz 2004). In the case of Velocity however precedent does exist from the 6MWT.

Results from placebo-controlled, registration directed, randomised studies in MPS I, MPS II and Pompe identified that Velocity in drug-treated patients increased performance by 8% to 13% relative to placebo controlled patients. Based on this McDonald *et al* (2010) suggest that a treatment effect of 30 to 40m (~8% to 11%) might reasonably be targeted in future therapeutic clinical studies in DMD.

They do not address the confounding problem of accounting for age-related increase, which could mistakenly be regarded as improvement in score. Based on the assumption of looking for an 8 to 11% improvement, Velocity in NGD would need to increase to between 100 and 103 cms/s. Based on the Alderson (2007) LMS centile data however, in a 12 month period an expected improvement of 3-4% would normally be expected with increasing age. 100-103cms/c would continue to be 20% lower than that expected for a 10-11 year old age group however. This highlights a complicated issue of incorporating the impact of growth on parameters, but one that could be addressed with the use of Z scores.

The 6MWT calculates Velocity based on meters walked in 6 minutes. The GAITRite calculates Velocity as centimetres walked per second. Transforming the data sets we see that the NGD cohort covered less distance, despite the slightly older age group (Table 5.2).

This may, of course be reflective of the different method of assessment. However, both groups are much less than the normal values identified in a study examining

normal values of 4-11 year olds (Lammers *et al.* 2008). Given that McDonald *et al* (2010) proposes the 6MWT as a useful marker in DMD, it supports the notion that if a more consistent pattern emerged in sequential assessments that the same may apply for NGD.

Table 5.2: Velocity of DMD and NGD patients, with normal values generated from 6MWT by Lammers et al (2008)

	Meters per Minute
Normal values for 8 year olds	80.5
Normal values for 10 year olds	84.3
DMD cohort (*n*=21)	60.6
NGD cohort (*n*=9)	55.7

Double Support may, however, be a more clinically relevant endpoint to consider in the NGD cohort, considering that Velocity may be more reflective of overall power and strength and not necessarily control and balance. More work would need to be done to justify this however; in particular demonstrating that Double Support is a gait parameter that is consistently increased and/or progressing in sequential assessments. This could be done in future work through refining the gait analysis, focusing purely on the parameters that appear to deviate: Double Support and Step Time.

This identification of a gait profile that deviates from the norm also has an impact on current clinical management. Utilising it as a clinical tool in day-to-day management may facilitate physiotherapists and occupational therapists to address care, and coping strategies designed specifically for that child.

Diffusion Tensor Imaging

DTI is a new and exciting assessment modality that has the potential to be developed into a truly objective marker of disease. Its ability to identify an area of statistical difference in a region of the brain that is in keeping with the clinical presentation observed in this NGD cohort studied offers a 'proof of concept' to its value, and makes it attractive for further exploratory work.

Although undergoing MR imaging is challenging for some children, its objectivity and relative non-invasiveness compared to some assessments certainly makes it worthy of further development. Notwithstanding their risks, complying with an MR can also be facilitated with sedation or general anaesthetic. Particularly given the lack of other *in vivo* alternatives.

A current disadvantage at this point is the lack of widespread use of the TBSS approach in other therapeutic areas, along with the lack of complete agreement of the trajectory of MD and FA changes during childhood. To enable larger number of healthy and diseases children needs to be imaged, along with performing sequential images. Addressing these issues will pave the way for encouraging regulatory bodies to approve it as a clinical tool with the potential to be a disease marker. Once again, this could be addressed in a global data mining exercise.

In summary, the mSST is believed to be the most valuable assessment tool out of the three examined. This is based on the extensive work done to develop and

validate it and the ability of the data generated not only to demonstrate progression, but also to demonstrate differentiation in scores according to genotype – indicating its value to capture phenotype-genotype correlation.

The GAITRite and the DTI both generated interesting data, which justifies the value of expanding the exploratory value of both. However, both needs further validation before they can be considered as outcome measures in either clinical care or in clinical trial use.

Chapter 6

Conclusion

“Commitment is doing the thing that you said you would do, long after the mood in which you said it in has left you.”

Ben Saunders

During this development of the mSST this work has demonstrated its use as a useful marker of disease severity in NGD. The sequential data generated in this study has indeed demonstrated that the mSST is effective for purpose . It was sensitive enough, not only to demonstrate its responsiveness to change, but to also distinguish between phenotypes. This is a crucially important distinction to allow for genotype-phenotype stratification in clinical trials, but also in providing counselling to patients, parents and families on the expected disease course trajectory. Based on that fact that it was used in three different European countries the findings are more representative than if the study had been limited to one geographical region only, which is a real strength in its validity and value.

The involvement of international experts in its development will encourage its use by world wide experts managing NGD patients – ideally in a registry or database format. Its inclusion in the revised guidelines (Vellodi *et al.* 2009) for the management of NGD patients will also facilitate this – assisting in a comprehensive understanding of the 'natural history' of the disease in the ERT era and the most frequently presenting domains which can be used for disease monitoring.

While the data generated from the GAITRite and DTI assessments are currently inconclusive for immediate use, the number of children recruited for each were small, despite being reflective of the rarity of NGD and the difficulty of a single centre study. However, the numbers studied can be considered as sufficient to justify that gait and the white matter tract as areas worthy of further exploration. Ideally this should be done in a larger multi centre study, encompassing the European cohort studied

during mSST development, would be valuable to corroborate these findings, and to make further progress in determining their value as disease markers.

This work has highlighted the benefit of systematically assessing the neurological manifestations of NGD. Incorporating the parameters of gait and DTI in databases or registries will allow for data mining and retrospective hypothesis testing. All of which will ultimately lead to an improved knowledge base that will facilitate the design of future clinical trials for NGD patients.

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Literature Cited

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Appendix 1

Severity Scoring Tool

HORIZONTAL GAZE PALSY	Normal (although not likely in diagnosis) Horizontal Saccades absent, Vertical Saccades present Horizontal Saccades and Vertical Saccades absent	0 0.5 1
EPILEPSY	No seizures. Seizures not requiring anticonvulsants Seizures controlled with anticonvulsants. Seizures requiring combination therapy or resistant to anticonvulsants	0 3 4 5
DEVELOPMENT/ COGNITIVE ABILITY	Normal Mildly impaired (IQ less than 85 or equivalent) Moderate (IQ between 50-57 or equivalent) Severe (More than half their chronological age)	0 1 2 3
NEUROLOGY PATTERN		
Ataxia/ Gait	Normal, apparent only on tandem walking Ataxia on straight gait, able to walk without assistance Able to walk only with assistance Unable to walk	0 1 2 3
Cerebellar signs/Ataxia	No intention tremor Intention tremor not affecting function Intention tremor with marked impact on function	0 0.5 2
Pyramidal	Normal tone with increased reflexes Mildly to moderately increased tone and reflexes Increased tone reflexes with sustained/unsustained clonus Severe spasticity with inability to walk	0 2 3 5
Extrapyramidal	Normal Variable tone and posturing not impairing function, with or without therapy. Variable tone and posturing impairing function, despite therapy Significant rigidity with no/minimal benefit from therapy	0 1 2 3
SWALLOWING DIFFICULTIES/ ORAL BULBAR FUNCTION	Normal Mild dysphagia (excess drooling) Moderate dysphagia (risk of aspiration, modification to diet required) Severe dysphagia (requiring non-oral feeding)	0 1 2 3
SPEECH	Normal (and those too young yet to speak)	0

	Mild to moderate dysarthia impairing intelligibility to unfamiliar listener Severe dysarthia with most speech unintelligible to familiar and unfamiliar listener Anarthria	1 2 3
NEUROLOGY FUNCTION		
OPHTHAMOLOGY	Normal Cranial Nerve Palsy (previously corrected or not) Cranial Nerve Palsy (reappearing despite surgical correction)	0 1 2
SPINAL ALIGNEMENT	Normal Mild kyphosis – but flexible Moderate kyphosis – partially corrected Severe kyphosis - fixed	0 1 2 3
	Other Neurological feature not captured	
TOTAL		/33

Appendix 2 List of International Experts

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Appendix 3 Overview of Patients

GERMAN PATIENTS

Patient ID	Splenectomy	Genotype	Date of Birth	Date of Follow Up Assessment	SST score	Baseline mSST score	Date of Follow Up Assessment	Follow Up mSST score
GERMANY								
1G	No	L444P/ L444P	22.06.1994	10.02.2006	6.5	4.5	30.03.2010	6.5
2G	No	L444P/ L444P	13.09.1991	10.02.2006	2.5	1.5	30.03.2010	1.5
3G	No	1599AG/ 1603T	14.07.1995	10.02.2006	7.5	7.5	30.03.2010	10
5G	yes at 8 years	L444P/ L444P	11.08.1982	10.02.2006	14.5	16.5	30.03.2010	17
7G	yes at 2 years	F213/ L444P	25.02.1994	10.02.2006	10.5	13.5	30.03.2010	16
8G	No	L444P/ L444P	26.11.1996	10.02.2006	7.5	6.5	30.03.2010	11.5
10G	No	D409H/ L444P	03.03.1993	10.02.2006	4.5	3.5	30.03.2010	0.5
11G	No	D409H/ L444P	18.03.1994	10.02.2006	3.5	2.5	30.03.2010	3
14G	No	L444P/ L444P	10.09.2003	10.02.2006	1.5	0.5	30.03.2010	7
15G	No	D409H/ G202R	18.07.1970	10.02.2006	5.5	8.5	30.03.2010	16.5

Patient ID	Baseline FSIQ	Baseline Chito	Baseline ERT iu/kg	Follow Up FSIQ	Follow Up Chito	Follow Up ERT iu/kg	Seizures at Baseline	Seizures at Follow Up
GERMANY								
1G	N/A	455	116	N/A	3384	63		
2G	N/A	326	66.6	N/A	245	54		
3G	N/A	4022	91.5	88	4294	70		
5G	N/A	737	211	72	271	59	no	yes
7G	N/A	2441	107.7	60	1187	68	yes	yes
8G	N/A	826	120	70	1104	88		
10G	N/A	1544	70.4	79	2278	46		
11G	N/A	237	54.3	85	503	37		
14G	N/A	3562	83	69	3562	48		
15G	N/A	3613	50.9	N/A	118	37	yes	yes

POLISH PATIENTS

Patient ID	Splenectomy	Genotype	Date of Birth	Date of Follow Up Assessment	SST score	Baseline mSST score	Date of Follow Up Assessment	Follow Up mSST score
POLAND								
1P	yes at 23 years	L444P/ D409H	10.01.1971	28.12.2005	6	3	01.12.2009	3
2P	No	L444P/ D409H	17.11.1958	28.12.2005	4	2	01.12.2009	4.5
3P	No	R433S/ R433S	25.10.1996	28.12.2005	3	1.5	01.12.2009	1.5
4P	No	L444P/ L444P	17.06.1978	28.12.2005	6.5	5.5	01.12.2009	5
5P	No	L444P/ L444P	21.01.1998	28.12.2005	6.5	3.5	01.12.2009	4
6P	No	L444P/ L444P	09.09.1996	28.12.2005	3	1.5	01.12.2009	2
7P	No	L444P/ E326K	11.03.1990	28.12.2005	2.5	1.5	01.12.2009	2
11P	No	L444P/ L444P	23.10.1993	28.12.2005	4.5	4	01.12.2009	1.5
12P	yes at 6 years	L444P/ L444P	07.02.1976	28.12.2005	7	6.5	01.12.2009	6
13P	yes at 18 months	L444P/ L444P	21.03.1977	28.12.2005	9	8	01.12.2009	6.5
14P	No	L444P/ L444P	03.03.1997	28.12.2005	7	6	01.12.2009	6.5
15P	No	L444P/ L444P	06.12.1998	28.12.2005	6.5	3.5	01.12.2009	6.5
16P	No	L444P/ L444P	31.05.1990	28.12.2005	6	4	01.12.2009	5
17P	yes at 2 years	L444P/ L444P	11.08.1980	28.12.2005	16	16	01.12.2009	17
18P	yes at 3 years	L444P/ L444P	21.05.1988	28.12.2005	4.5	3	01.12.2009	5
19P	No	L444P/ L444P	22.01.1996	28.12.2005	5	3	01.12.2009	9
20P	No	L444P/ L444P	28.04.1991	28.12.2005	3	1.5	01.12.2009	0.5
21P	yes at 7 years	L444P/ L444P	09.10.1980	28.12.2005	5.5	5.5	01.12.2009	6

POLISH PATIENTS

Patient ID	Baseline FSIQ	Baseline Chito	Baseline ERT iu/kg	Follow Up FSIQ	Follow Up Chito	Follow Up ERT iu/kg	Seizures at Baseline	Seizures at Follow Up
POLAND								
1P	N/A	360	24	N/A	360	13		
2P	96	878	25	96	340	12		
3P	97	4222	55	108	340	12		
4P	71	5922	26	70	830	23		
5P	99	6335	78	99	5540	60		
6P	104	2050	30	115	740	30		
7P	N/A	N/A	26	62	470	19		
11P	88	542	20	90	210	15		
12P	72	6500	30	71	120	30		
13P	N/A	1464	30	89	2060	16		
14P	51	2260	72	50	3230	56		
15P	66	2568	97	50	2560	60	no	yes
16P	72	976	30	95	625	16		
17P	77	15680	30	73	355	20	yes	yes
18P	108	400	30	N/A	7490	72		
19P	92	419	120	87	285	40	no	yes
20P	92	2600	39	90	650	37		
21P	76	12700	50	103	300	30		

UK PATIENTS

Patient ID	Splenectomy	Genotype	Date of Birth	Date of Follow Up Assessment	SST score	Baseline mSST score	Date of Follow Up Assessment	Follow Up mSST score
UK								
1UK	No	L444P/ L444P	22.09.1994	01.03.2006	3	1	03.11.2009	1.5
2UK	No	L444P/ L444P	11.11.1994	01.03.2006	3	2	16.02.2010	1.5
3UK	No	L444P/ L444P	08.05.1992	01.03.2006		6.5	19.03.2008	12.5
4UK	No	L444P/ L444P	13.11.1993	01.03.2006	4	2	01.04.2010	1.5
5UK	No	L444P/ L444P	17.08.1987	01.03.2006	9	12	24.03.2010	12
6UK	No	L444P/ L444P	19.11.1996	01.03.2006	7	6.5	17.03.2010	6
7UK	No	L444P/ L444P	21.11.2000	01.10.2008	4	3	21.01.2010	4
8UK	No	L444P/ E233D	11.12.1999	01.03.2006	15.5	13	11.03.2010	8
9UK	No	L279P/ G243V	05.07.2001	01.03.2006	14.5	12	16.09.2009	26
14UK	No	L444P/ L444P	03.05.1998	01.03.2006	4	2	25.02.2010	2.5
16UK	No	K198T/ L444P	16.07.2004	14.02.2008	7.5	6	20.11.2010	20.5

UK PATIENTS

Patient ID	Baseline FSIQ	Baseline Chito	Baseline ERT iu/kg	Follow Up FSIQ	Follow Up Chito	Follow Up ERT iu/kg	Seizures at Baseline	Seizures at Follow Up
UK								
1UK	80	807	172.5	N/A	374	120		
2UK	90	2485	489	N/A	1700	240		
3UK	40	1664	104	N/A	N/A	N/A		
4UK	60	767	101	N/A	478	115		
5UK	50	592	87.8	N/A	N/A	98.5	yes	yes
6UK	81	497	170.7	N/A	557	117		
7UK	N/A	N/A	N/A	N/A	N/A	N/A		
8UK	N/A	401	117	N/A	388	112		
9UK	N/A	N/A	N/A	N/A	739	91		
14UK	70	3015	N/A	N/A	2331	113		
16UK	N/A	N/A	N/A	N/A	N/A	N/A	no	yes

UK PATIENTS RECRUITED FOR GAIT AND MRI ANALYSIS

Patient ID	Splenectomy	Genotype	Date of Birth	Presneted for MCID evaluation	GAITRIte	Diffusion Imaging
UK cNGD						
1UK	No	L444P/ L444P	22.09.1994		x2	N/D
2UK	No	L444P/ L444P	11.11.1994			9.10.2009
3UK	No	L444P/ L444P	08.05.1992			N/D
4UK	No	L444P/ L444P	13.11.1993			3.11.2009
5UK	No	L444P/ L444P	17.08.1987			N/D
6UK	No	L444P/ L444P	19.11.1996	YES	x3	24.9.2009
7UK	No	L444P/ L444P	21.11.2000			N/D
8UK	No	L444P/ E233D	11.12.1999	YES	x3	N/D
9UK	No	L279P/ G243V	05.07.2001		x2	2.10.2008
14UK	No	L444P/ L444P	03.05.1998			N/D
16UK	No	K198T/ L444P	16.07.2004	YES		N/D
UK TYPE I						
UK-A	No	L444P/ R463C	8.2.1996		28.10.09	28.10.09
UK-B	No	N37OS/ D409H	17.2.2003		12.11.09	12.11.09
UK-C	No	N370S/ 55bpdel	21.9.2001		31/03/2010	16.12.09
UK-D	No	N370S/ ?	29.8.1995		27.1.10	27.1.10
UK-E	No	NOT KNOWN	06.03.2001		16.4.10	N/D