Neuropsychological deficits, structural brain changes and excessive daytime somnolence in myotonic dystrophy type 1

Mark James Hamilton

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Abstract

Myotonic dystrophy type 1 (DM1) is a dominantly inherited, multisystem condition, arising from pathological expansion of a CTG trinucleotide repeat. DM1 is characterised by myotonia, weakness and wasting of skeletal muscle, with additional features including ocular cataract, cardiac conduction abnormalities, hypogonadism, and cognitive deficits. The phenotype is highly variable, spanning a clinical continuum from symptom onset in late adulthood, to presence of severe symptoms from birth. The CTG repeat expansion responsible for DM1 is unstable in the germline, with a bias towards further expansion on transmission to subsequent generations. Larger repeats are broadly associated with earlier onset and more severe symptoms, accounting for the clinical anticipation of symptoms seen in affected families. The CTG repeat also shows expansion-biased instability in somatic cells, that is individual- and tissue-specific.

Symptoms arising from central nervous system (CNS) involvement in DM1, such as cognitive deficits, fatigue, apathy, impaired social functioning and excessive daytime somnolence, are often those with the greatest impact on quality of life. With early clinical trials of potential disease-modifying therapies for DM1 already underway, there is a pressing need to identify valid outcome measures for CNS involvement in order to measure response to treatment. Moreover, improved understanding of the relationship between structural brain changes and clinical symptoms is required to identify new targets for therapeutic intervention, as well as highlight potential imaging biomarkers for disease progression.

In this context, we aimed to evaluate the self-reported symptom questionnaires and cognitive tests currently recommended as outcome measures by expert consensus, with regard to their specificity for CNS involvement in DM1. We also aimed to describe the relationship between CTG repeat length and severity of CNS symptoms, in order to consider the validity of genetic measures as a basis for prognostication and patient stratification. By combining our own imaging data with that of a comparable cohort recruited in Iowa, USA, we sought to describe the landscape of volumetric brain changes in a large, case-control cohort with DM1, and to identify the regions in which structural change was most

closely driven by CTG repeat length. Finally, we explored the prevalence and clinical correlates of sleep disorders in our well-characterised cohort.

Forty-seven individuals with a diagnosis of adult-onset DM1 were recruited from the West of Scotland myotonic dystrophy service. Two were subsequently withdrawn from analysis: one due to an incidental finding of a possible glial neoplasm on MRI, and a second because she was found to carry a CTG repeat expansion within the premutation size range. Twenty age-matched control participants were recruited from patients' families and the Scottish Health Research Register. For volumetric analysis of MRI brain, our sample was combined with a second cohort recruited in Iowa City, USA, to give a total sample of 79 adults with DM1 and 58 controls.

Cognitive evaluation revealed the DM1-affected group performed, on average, less well in the Stroop test, Trail Making Tests, Block Design subtest, FAS oral word association, and in the Edinburgh Cognitive and Behavioural ALS Screen compared with controls. Adjustment of performance in the Stroop colour-word and number-letter switching Trail Making Test for basic reading and motor speed respectively, however, considerably attenuated this difference. This observation suggests primary muscle weakness in DM1 may influence performance in these complex cognitive tests, thus undermining their specificity as measures of CNS involvement.

Symptom questionnaires confirmed that DM1-affected participants frequently experienced symptoms of fatigue, excessive daytime somnolence, cognitive difficulties and impaired social performance. Self-reported symptom scores did not correlate with objective measures of brain involvement, such as cognitive performance, questionnaires completed by a proxy or global MRI measures of structural brain change. Instead, self-reported CNS symptoms correlated most closely with symptoms of low mood. Increased self-reporting of low mood and cognitive deficits was more common in participants with milder white matter change on MRI. Together, these findings suggest responses in self-reported symptom questionnaires may be significantly influenced by mood, and confounded by impaired symptom awareness in those with more severe disease.

Self-reported CNS symptoms and cognitive performance did not closely correlate with CTG repeat length, measured as estimated progenitor allele length (ePAL). Three individuals were identified in the Scottish cohort as carrying CCG interruptions within their CTG repeat array. Each reported mild muscle symptoms of DM1, but they were not clear outliers with respect to other clinical or imaging measures.

Regional MRI analysis was undertaken on a combined Glasgow-Iowa cohort, consisting of 79 adults with DM1 and 58 controls. ICV was reduced in DM1-affected subjects. After correction for ICV, age and sex, significantly reduced volume was observed in whole cerebrum, frontal lobe, parietal grey matter, cerebellar white matter, corpus callosum, putamen, accumbens and thalamus. Hippocampus and amygdala volumes appeared larger relative to ICV in the DM1-affected group. CTG repeat length was inversely correlated with volume of occipital grey matter, putamen and thalamus, and positively associated with volume of cerebellar white matter and amygdala. Several affected structures have plausible associations with deficits observed within the DM1 clinical phenotype. For example, the thalamus plays a role in maintenance of wakefulness, and loss of function has been linked to slowing of cognitive processing. A relative increase in volume of structures implicated in the generation of negative emotions (amygdala), alongside reduced volume of structures involved in emotional modulation and motivation (frontal lobe and accumbens) could be hypothesised to underlie socially avoidant traits seen in DM1.

Finally, sleep studies demonstrated altered sleep architecture in DM1, characterised by an increase in slow wave and REM sleep at the expense of stage 2 sleep. Clinically significant sleep-disordered breathing was present in over half of the DM1-affected participants who underwent sleep studies, and was associated with volume loss in frontal and parietal white matter. Risk factors for sleep-disordered breathing included increasing age, male sex, and increasing self-reported muscle weakness and fatigue, although no single clinical measure had strong predictive value.

In summary, our findings confirm major involvement of the CNS in adult-onset DM1. Selection of outcome measures for CNS involvement presents a challenge

for clinical trial design, since self-reporting of symptoms may be confounded by impaired disease awareness or concomitant depression, and performance in traditional tests of executive cognition may be influenced by primary muscle weakness. As such, identification of objective measures, such as brain imaging biomarkers, is an area of considerable unmet need. To this end, we undertook regional volumetric MRI analysis in the largest case-control cohort reported to date, identifying changes in several regions that were driven by CTG repeat length, and may account for key features of the DM1 phenotype. Further analysis of functional correlations, and longitudinal studies using a variety of imaging modalities is warranted to explore these insights further. With respect to current management of patients with DM1, our data also highlight proactive detection and treatment of sleep-disordered breathing, as well as provision of support to overcome low mood and promote social participation, as readily deliverable strategies to improve the wellbeing of people living with DM1.

Table of Contents

List of Tables

List of Figures

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Author's Declaration

This work was undertaken during my employment as a Clinical Research Fellow at the Institute of Molecular, Cell and Systems Biology, University of Glasgow, with an honorary affiliation to the West of Scotland Clinical Genetics Service, NHS Greater Glasgow and Clyde. I am the sole author of this thesis, and all work presented is entirely my own unless stated otherwise.

Experiments to characterise the CTG repeat length and variant repeat sequences in the study cohort were undertaken by Dr Sarah Cumming. I later completed a small number of additional small pool-PCR reactions on participants' DNA for a related project, under Dr Cumming's guidance. Dr Antonio Atalaia produced clinical reports from the raw polysomnography and modified maintenance of wakefulness test traces, which provided numeric data for me to undertake subsequent statistical analyses. The MRI processing that was completed in Glasgow was done with the close guidance and supervision of Dr John McLean. The volumetric MRI analysis presented in Chapter 6 was led by our collaborators at the University of Iowa (Ellen van der Plas, Timothy Koscik, Vincent Magnotta, Joel Bruss, Eric Axelson, Jeffrey D. Long and Peggy Nopoulos). I was fortunate to be able to participate in some of this analysis through a collaborative visit to Iowa City, USA in May 2018.

No part of this work has been submitted for consideration as part of any other degree.

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Abbreviations and definitions

AASM	American Academy of Sleep Medicine
AHI	apnoea-hypopnoea index
ALS	amyotrophic lateral sclerosis
ANCOVA	analysis of covariance
ASO	antisense oligonucleotide
BADS	Behavioural Assessment of the Dysexecutive Syndrome
BDI II	Beck Depression Inventory II
BMI	body mass index
BOLD	blood oxygen level dependent
CBT	cognitive behavioural therapy
CNS	central nervous system
CSF	cerebrospinal fluid
CTG	cytosine thymine guanine
CUG	cytosine uracil guanine
D-KEFS™	Delis-Kaplan Executive Function System
DEX	Dysexecutive questionnaire (a component of the BADS battery)
DM1	myotonic dystrophy type 1
DM2	myotonic dystrophy type 2
DMGV	the Genetic Variation in Myotonic Dystrophy study
DMPK	the *dystrophia myotonica protein kinase* gene
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTI	diffusion tensor imaging
ECAS	Edinburgh Cognitive and Behavioural ALS screen
ECG	Electrocardiogram
EDS	Excessive daytime sleepiness
EDTA	ethylenediaminetetraacetic acid
EEG	electroencephalogram
EMG	electromyogram
ePAL	estimated progenitor allele length
FA	fractional anisotropy
FAS	controlled oral word association test using letters F, A and S
FDSS	Fatigue and Daytime Sleepiness Scale

FEV_1	forced expiratory volume in 1 second
FLAIR	fluid-attenuated inversion recovery
fMRI	functional magnetic resonance imaging
FVC	forced vital capacity
GLA	Glasgow
GM	grey matter
GMV	grey matter volume as % of intracranial volume
Hb	haemoglobin
HFI	hyperostosis frontalis interna
ICV	intracranial volume
IH	idiopathic hypersomnia
IOA	Iowa
IQ	intelligence quotient
LGA	Lesion Growth Algorithm
logAHI	AHI transformed by logarithm with base 10
logPAL	ePAL transformed by logarithm with base 10
MAL	modal allele length
MCRF	magnocerebellar reticular formation
MD	mean diffusivity
MDHI	Myotonic Dystrophy Health Index
meanMWT	mean latency in all trials of MWT attempted
miRNA	micro ribonucleic acid
MIRS	Muscle Impairment Rating Scale
MJH	Mark James Hamilton (the author)
MMSE	mini mental state examination
mMWT	modified maintenance of wakefulness test
MNI	Montreal Neurological Institute
MPRAGE	magnetisation prepared rapid acquisition gradient echo
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MSLT	multiple sleep latency test
MWT	maintenance of wakefulness test
MWT1	sleep latency in the first MWT trial
NHS	National Health Service
NIV	non-invasive ventilation

OMERACT	Outcome Measures in Rheumatology working group
OMMYD	Outcome Measures in Myotonic Dystrophy working group
OSA	obstructive sleep apnoea
OSAS	obstructive sleep apnoea syndrome
PAP	positive airway pressure
PCR	polymerase chain reaction
PPM	permanent pacemaker
PSG	polysomnography
REM	rapid eye movement
RNA	ribonucleic acid
ROC	receiver operating curve
ROI	region of interest
SD	standard deviation
SDB	sleep-disordered breathing
SHARE	Scottish Health Research Register
SMN	Scottish Muscle Network
SOREMP	sleep-onset rapid eye movement
SP-PCR	small pool polymerase chain reaction
SPACE	Sampling perfection with application optimised contrasts using different flip angle evolution
SPSS	Statistical Package for the Social Sciences
SWS	slow-wave sleep
TST	total sleep time
VBM	voxel-based morphometry
VOI	volume of interest
VWML	volume of white matter lesions
WAIS-R	Weschler Adult Intelligence Scale Revised
WASI II	Weschler Abbreviated Scale of Intelligence – Second Edition
WM	white matter
WMV	white matter volume as % of intracranial volume

1 Introduction

1.1 A brief history of myotonic dystrophy

The first description of myotonic dystrophy in the medical literature is credited to Hans Steinert, a German neurologist working from the medical hospital of Leipzig University in the early 1900s [1]. Concurrently and independently, Frederick Batten in England was also preparing the report of a similar series of patients, to be published shortly after [2]. Both described an apparently hereditary neuromuscular condition, with a characteristic slow relaxation of skeletal muscles following voluntary contraction (myotonia). While myotonia was already known to be associated with a distinct condition Thomsen's disease (dominantly inherited myotonia congenita), they additionally observed a phenotype of progressive muscle weakness with atrophy, particularly affecting facial muscles, sternocleidomastoid, proximal muscles of the thigh and the dorsiflexors of the feet. Since muscle wasting is not seen in classical Thomsen's disease, these cases were presented as evidence of a novel neuromuscular disorder.

The perception of myotonic dystrophy as a disease primarily of muscle persists to the modern day, with myotonic dystrophy type 1 (DM1) frequently described as "the most common form of muscular dystrophy affecting adults" [3]. In the decades following Steinert and Batten's first descriptions, however, the publication of additional affected families led to a gradual change in perspective. Summarising varied reports of associations with ocular cataract, testicular atrophy, premature balding, cognitive deficits and other symptoms "too numerous to mention", one author in 1923 concluded:

"Time … has led to a complete change in our conception of the nature of the disease, which far from being one of muscles alone, must now be regarded as a general disease with widespread manifestations."[4]

Around the same time, another key insight into the nature of myotonic dystrophy was made. Through careful observation of nine families (five with common ancestors), German ophthalmologist Fleisher noted that symptoms varied widely, but appeared to generally worsen in successive generations of an affected

family. Asymptomatic generations separated affected families from their common ancestor. The first generation to be affected had mild symptoms, some with only early-onset cataracts. Their offspring in turn tended to have cataracts, muscle wasting and other symptoms we now recognise as classical myotonic dystrophy. In this and the subsequent generation, there was an increased incidence of intellectual disability and childhood mortality (reviewed in [5]).

In 1948, Penrose undertook an analysis of Fleisher and others' reports of this apparent "anticipation" of symptoms. He criticised contemporary theories that anticipation was a mechanism by which the human race acted to purge itself of hereditary disease, and argued that the phenomenon was not explicable by any accepted mechanisms of hereditary. Instead, he proposed that myotonic dystrophy was simply a highly variable disorder, in which ascertainment bias gave the erroneous impression of anticipation of symptoms [6]. The question of anticipation remained contentious for several decades, with Penrose's conclusions frequently seeming at odds with the observations of many clinicians and affected families [7]. In 1989 a systematic assessment of 14 unselected families with myotonic dystrophy by Christian Höweler provided compelling evidence in support of anticipation, demonstrating a decreasing age at onset with transmission in 98% of parent-child pairs [5].

The years that followed would bring an exponential increase in understanding of the molecular mechanisms underlying myotonic dystrophy. This would provide insights into the multisystem nature of the disorder, as well as confirming the genetic basis of anticipation.

1.2 Genetic and molecular mechanisms

1.2.1 Myotonic dystrophy is a repeat expansion disorder

By the 1980's, genetic linkage studies based on polymorphic DNA markers had enabled many genetic traits and disorders to be mapped to a physical location on the human genome. This included myotonic dystrophy, which was found to segregate with markers on the long arm of chromosome 19 [8]. A subsequent process of positional cloning and linkage disequilibrium analysis narrowed the critical region responsible for myotonic dystrophy to position 19q13.3 [9-12]. An

EcoRI restriction fragment was identified within this region that was highly polymorphic in length, but was consistently larger in DM1 affected patients compared with unaffected family members [13]. The fragment also appeared to generally increase in size in successive generations, in association with an earlier onset and greater severity of symptoms [14]. Screening of phage clones spanning the critical region identified a polymorphic CTG repeat as the cause for the variation in restriction fragment length. The CTG repeat lay in the 3' untranslated region (3'-UTR) of a gene coding for a protein kinase that would become known as *dystrophia myotonica* protein kinase (*DMPK*) (Figure 1). Individuals with symptoms of DM1 were found consistently to have at least 50 copies of the CTG repeat, with 1,000 or more present in the most severe cases [15]. Subsequent work demonstrated that alleles greater than around 30 repeats are rare in the general population. Those in the range ~ 38 to 49 repeats, while not expected to be associated with clinical symptoms of myotonic dystrophy, show increased expansion-biased instability on germline transmission, hence carriers of a 'premutation' such as this are at risk of transmitting an allele in the pathogenic range to offspring [16].

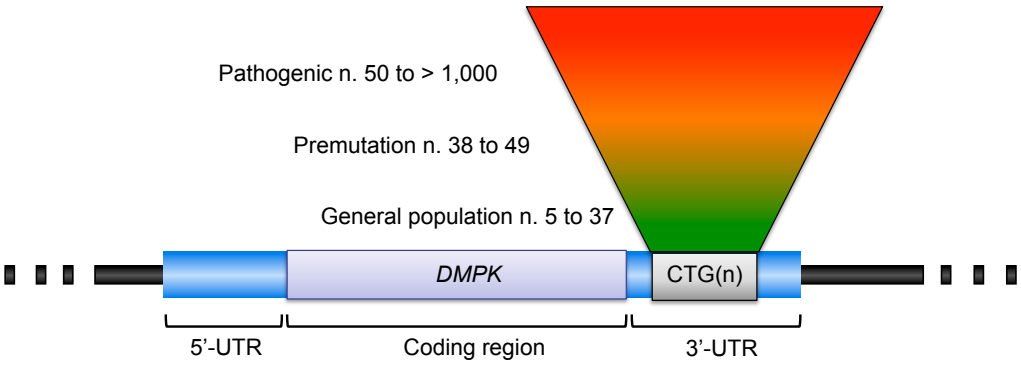

Figure 1: Schematic of the *DMPK* gene, demonstrating the location of the polymorphic CTG repeat in the 3' untranslated region (3'-UTR)

The finding of a polymorphic, unstable repeat expansion as the basis of myotonic dystrophy offered a plausible basis for genetic anticipation and, for the first time allowed, targeted genetic testing for the condition.

1.2.2 Myotonic dystrophy type 2

A proportion of families with clinical features of myotonic dystrophy were identified that did not have CTG repeat expansions on targeted testing. These families also had an absence of severe, congenital-onset cases, and an atypical pattern of muscle weakness, affecting mainly proximal muscles, leading to the name proximal myotonic myopathy (PROMM) [17,18]. The cause of PROMM was subsequently demonstrated to be the expansion of a CCTG repeat in intron 1 of the *CNBP* (cellular retroviral nucleic acid-binding protein 1) gene, previously referred to as zinc finger 9 (*ZNF9*) gene [19]. Myotonic dystrophy due to the *DMPK* repeat expansion would come to be known as myotonic dystrophy type 1 (DM1), and that due to a *CNBP* tetranucleotide expansion myotonic dystrophy type 2 (DM2).

While the core clinical features and underlying molecular mechanisms in DM2 overlap those of DM1 considerably, there are also sufficient differences that distinguish them as separate clinical entities [20]. Given that relative sparing of the central nervous system is a distinguishing feature of DM2 compared with DM1, and acknowledging that very few cases of DM2 are ascertained in the West of Scotland population, the present study of neuropsychological deficits and sleep disorders in myotonic dystrophy will focus primarily on DM1.

1.2.3 Genotype-phenotype correlations in DM1

People affected by DM1 can be stratified clinically according to their age at onset of symptoms (Table 1). Those who develop DM1 symptoms late in adult life (late-onset DM1) may have only premature cataracts, and perhaps mild muscle symptoms but with overall normal life expectancy. Classical DM1 typically involves onset of muscle symptoms in the third or fourth decade of life, and includes core features of cataract, muscle weakness and myotonia and frontal balding in males. Onset of DM1-specific symptoms in childhood defines juvenile-onset DM1, and may be associated with learning or behavioural difficulties. Congenital myotonic dystrophy is the severest form, in which muscle weakness and hypotonia are apparent from birth, and indeed may cause reduced foetal movements and polyhydramnios in the antenatal period. Affected babies may have severe contractures at birth, and require support with artificial ventilation

and parenteral feeding in early life. Those who survive the neonatal period typically have complex needs, including moderate to severe learning difficulties [3]. The divisions between categories are however somewhat arbitrary, and age at onset of symptoms is a highly subjective measure. Therefore, in reality, the phenotype of DM1 should be considered as an overlapping spectrum of clinical severity.

Table 1: Clinical classification of DM1 phenotypes

Subtype of DM1	Typical age at onset of symptoms (years)	Likely clinical features	Approximate number of CTG repeats
Late onset / mild	20 to 70	Cataracts, mild myotonia and distal weakness	50 to 150
Classical	10 to 30	Muscle weakness with myotonia, cataracts, cardiac arrhythmia, fatigue and excessive sleepiness, frontal balding in males	50 to 1000
Juvenile	1 to 10	Learning or behavioural difficulties, continence issues	> 800
Congenital	Birth	Hypotonia, respiratory failure, feeding difficulty, learning disability	> 1000

Adapted from Turner *et al.* [3] with permission from Wolters Kluwer Health, Inc.

A highly polymorphic trinucleotide repeat as the cause of DM1 offers hope that a clinically useful correlation might exist between CTG repeat length and disease severity. Early attempts to explore this relationship, using Southern blotting of restriction digested genomic DNA from blood to size the CTG repeat, confirm a broad trend towards earlier onset of symptoms with larger repeats [21], with a stronger correlation among those with modest-sized expansions [22]. Residual variation in age at onset is large however, equivalent to a difference of 30 years or more in individuals with apparently equivalent repeat sizes [21]. This relationship, therefore, falls far short of a threshold that would allow clinically useful predictions to be made from genetic data: for example, to accurately prognosticate on the likely phenotype based on DNA from chorionic villus sampling in early pregnancy.

A major confounder of genotype-phenotype studies is somatic instability of the CTG repeat. When the repeat size is measured by Southern blotting of restriction digested genomic DNA from blood, the expanded allele appears as a broad smear. This is consistent with individual cells each containing an expanded allele of a different length, implying instability of the repeat leading to mosaicism among somatic cells. This somatic instability is biased towards expansion of the repeat length, and the overall range of repeat sizes present in the cells of a DM1-affected individual increases with age [23]. In early genotype-phenotype studies using traditional Southern blotting methods, CTG repeat length would be inferred from the region of greatest intensity of the smear, or else patients were stratified into groups according to their approximate band sizes [13,21,24]. These methods are somewhat subjective, taking no account of the age-dependent nature of somatic mosaicism, and inevitably would have reduced power to detect correlations.

Small pool PCR (SP-PCR) can resolve somatic mosaicism to reveal the discrete allele sizes present in individual cells [25]. The lower boundary of the allele distribution is relatively preserved (Figure 2), and represents an estimation of the allele size inherited at conception: the estimated progenitor allele length (ePAL). The region of greatest band intensity represents the modal allele length at the time of sampling (modal allele length; MAL). The ePAL has been shown to greatly improve correlations with age at onset of symptoms, a relationship that is further modified by the individual-specific level of somatic instability [26].

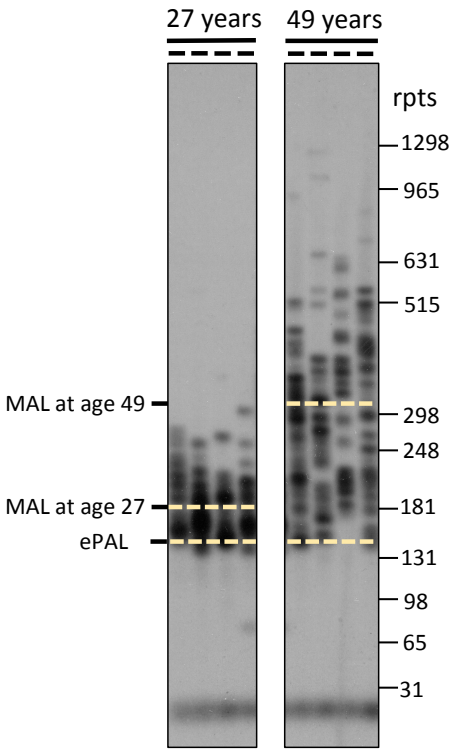

Figure 2: Southern blots of small pool PCR products from the same individual with myotonic dystrophy type 1, sampled at age 27 and 49 years.
The molecular weight marker (right) has been converted to number of repeats (rpts). Note the lower boundary of expanded alleles is relatively conserved, constituting the estimated progenitor allele length (ePAL). The modal allele length (MAL) is substantially greater in the sample taken at age 49 years. Southern blots by Dr Sarah Cumming.

Furthermore, the level of somatic instability varies between different tissues of an affected individual, being particularly high in muscle (Figure 3) and cerebral cortex [27,28]. Since these tissues are severely affected in DM1, this observation suggests somatic instability could account for the tissue-specific nature of symptoms, as well as being a significant driver of disease progression.

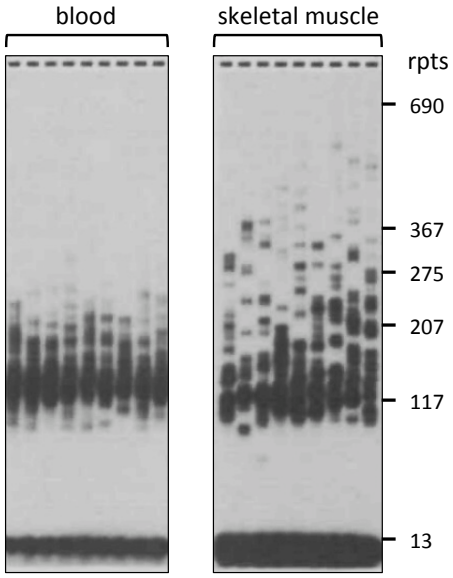

Figure 3: SP-PCR comparison of variation in CTG repeat alleles in blood and muscle from an adult male with DM1
Again, note the lower boundary of repeat sizes is relatively conserved, while the modal repeat length is larger in muscle compared with blood. Adapted from Monckton *et al.* [25] with permission from Oxford University Press.

1.2.4 Factors influencing somatic instability

The factors that influence somatic instability of expanded trinucleotide repeats are not fully understood. There is some evidence to suggest that sequence variants in genes involved in DNA mismatch repair may be *trans*-acting modifiers [29,30]. In other trinucleotide repeat disorders, interruptions of the trinucleotide repeat array itself by different or "variant" sequences (for example, AGG interruptions within the (CGG)n tract of *FMR1* in fragile X syndrome) are described acting as anchors, reducing the likelihood of misalignment events during DNA processing thus stabilising the repeat [31,32].

Variant repeat sequences have also been described in DM1, and provide further evidence of somatic instability as a major modifier of symptoms. Present in 3 to 4% of a typical DM1-affected population, interruptions in the CTG array may include CCG, CTC or GGC motifs. These most commonly occur near the 3' end [33-36], although interruptions near the 5'-end have also been described [37,38]. The presence of variant repeats appears to affect the mutational dynamics of the expanded DM1 allele, both in somatic cells and in the germline.

In families transmitting interrupted repeats, variant sequences were seen to stabilise both the variant-containing array itself, as well as neighbouring pure CTG sequence. As a result, several families have shown a conspicuous lack of genetic and clinical anticipation [33,34]. Variant repeats also increased stability in somatic cells, offering a plausible explanation for the observation that individuals with interrupted repeats frequently have delayed onset, unusually mild, or atypical patterns of DM1 symptoms [33-36].

1.2.5 Pathogenic mechanisms in DM1

The abnormal expansion of tandem repeats can impact gene function and cause human disease by a variety of mechanisms, including a reduction in gene expression (the primary mechanism in Friedreich's ataxia), or expression of an abnormal protein product (as in Huntington's disease) [39]. Since the CTG repeat in DM1 lies in the 3'-UTR of *DMPK*, an abnormal protein product would not be predicted. Haploinsufficiency of *DMPK* has been explored in mouse models, in which heterozygous disruption of endogenous *DMPK* (-/+) is associated with no perceptible phenotypic difference from wildtype, with the possible exception of mild prolongation of PR interval on electrocardiography. In nullizygous (-/-) mice, a late-onset muscle weakness with non-specific histological muscle changes, and higher grades of heart block have been described [40-42]. However, myotonia, impaired fertility and neonatal morbidity all appear absent from knockout mouse models, arguing against *DMPK* loss-of-function as the major contributor to symptoms in humans with DM1. In contrast, expression of transgenic, untranslated CUG repeat messenger RNA (mRNA) independent of *DMPK* is associated with myotonia, histological evidence of myopathy and early mortality in mice [43]. This more closely recapitulates the phenotype of the human disease, and so supports a toxic mRNA gain of function as a key mechanism.

Expanded CUG-repeat mRNAs are seen to form hairpin-shaped, metastable secondary structures, in which stability increases with greater repeat length [44,45]. Furthermore, there is inefficient transport of DM1 mutant transcripts out from the nucleus to the cytoplasm, and instead they form stable clusters that are retained within the nucleus [46]. These clusters, termed ribonuclear foci, can be visualised by hybridisation with fluorescent probes, and are readily

demonstrable in the nuclei of DM1 patients' fibroblast, cardiac muscle, skeletal muscle, brain and other tissues, including during foetal development in humans with very large CTG repeats [47-49].

A range of potential mechanisms by which expression of expanded CUG repeats, and their sequestration in ribonuclear foci, could cause the widespread phenotypic effects of DM1 have been explored. These include altered regulation of transcription, non-ATG translational initiation to produce homopolymeric expansion peptides, CUG-hairpin induced stress pathways, dysregulation of miRNA processing and function, and usage of alternative polyadenylation sites of several mRNAs (reviewed in [50]).

A key hallmark of cells affected by myotonic dystrophy, however, is the widespread dysregulation of alternative splicing of mRNAs. Major players in this pathway of dysregulated splicing are the mRNA binding proteins muscleblind-like protein (MBNL) and CUG-binding protein 1 (CUG-BP). Under normal conditions, both proteins have a role in the regulation of alternative splicing, frequently acting in opposition [51,52]. MBNL proteins are shown to accumulate both in CUG repeat-containing ribonuclear foci of DM1 [53], and in the CCUG repeat-containing foci of DM2 [54]. This sequestration of MBNL reduces its availability and hence activity within the nucleus. Conversely, the presence of ribonuclear foci results in increased activity of CUG-BP [55]. In this way, the presence of expanded CUG or CCUG repeat mRNA impacts splicing of a whole host of genes that rely on MBNL and/or CUG-BP for post-transcriptional processing. The resulting dysregulated splicing affects a wide range of gene products across different body tissues (Figure 4), providing a plausible basis for myotonic dystrophy as a complex and multi-system disorder. The clinical phenotype of DM1 and current understanding of the mechanisms underlying key features are discussed in greater detail in the following section.

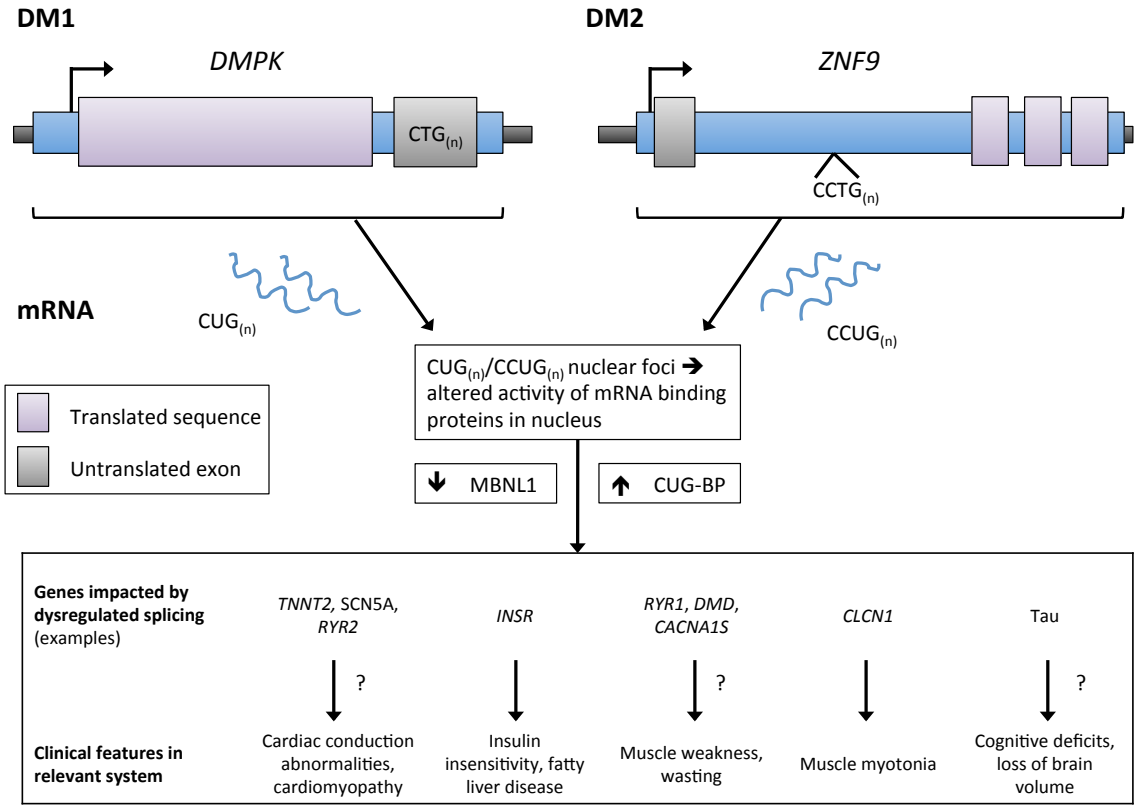

Figure 4: Summary of the key pathological mechanisms in myotonic dystrophy
CUG repeat mRNAs in DM1, or CCUG repeat mRNAs in DM2, are deposited in cell nuclei, where their presence alters the activity of mRNA binding proteins. This includes increased activity of CUG-BP, and reduced activity of MBNL. In turn, there is widespread dysregulation of alternative splicing, affecting products of many genes. Some selected examples are listed, along with their associated clinical correlations. Speculative clinical correlations are marked with '?'. *CACNA1S*, voltage gated calcium channel alpha-1S subunit; *CLCN1*; muscle chloride channel; *DMD*, dystrophin; *INSR*, insulin receptor; *RYR1* and *2*, muscle and cardiac ryanodine receptor respectively; *SCN5A,* voltage gated sodium channel type V, alpha subunit; *TNNT2*, cardiac troponin 2. Adapted from Gatchel *et al*. [56] with permission from Springer Nature.

1.3 Clinical features and current management of myotonic dystrophy

Several decades of cohort studies and molecular research has improved understanding of the mechanisms underlying key clinical features of myotonic dystrophy, and in turn informed a rational approach to medical management of affected individuals. With no disease-modifying therapy yet identified, however, management centres on early recognition of disease sequelae, and prompt implementation of strategies to reduce their impact. Review of patients at least annually is generally recommended [3], and requires a systems-based approach.

1.3.1 Skeletal muscle

Weakness and wasting of skeletal muscle, that is slowly progressive over many years, is a core feature of DM1. Early signs include neck flexion weakness, facial weakness with ptosis, bulbar involvement leading to a characteristic nasal voice, and distal muscle weakness in the upper and lower limbs, affecting grip and causing foot drop respectively. In most individuals, proximal muscle involvement will evolve over time, with implications for walking and balance, although progression to complete wheelchair dependence is unusual [57]. Muscle histology reveals dystrophic change including variation in fibre diameter with a preponderance of type 1 fibre atrophy, a significant increase in internal nuclei, fibrosis and fatty infiltration. The processes driving dystrophic change in muscle are not well described, although dysregulated splicing of genes involved in calcium regulation and excitation-contraction coupling, including *RYR1, SERCA* and *CACNA1S*, are thought to play a role [58].

Muscle myotonia is not universally present, but in those who do experience this symptom its emergence may precede the onset of muscle weakness [3]. Myotonia is thought to occur secondary to dysregulated alternative splicing of *CLCN1*, a transmembrane chloride ion channel widely expressed in the sarcolemma of skeletal muscle [59]. Impaired function of CLCN1 increases muscle membrane excitability, predisposing to self-sustained action potentials with resulting prolonged muscle contraction after stimulus [60]. Myotonia characteristically affects grip, which can be detected easily on clinical examination, and tongue. Percussion over affected muscles helps detect mild myotonia. Electromyographic studies (which nowadays are rarely performed in DM1 patients) detect myotonic discharges, which have a highly characteristic 'dive-bomber' sound. Myotonia symptoms can be improved in DM1 by oral administration of mexiletine, a sodium channel-blocking agent [61]. Theoretical concerns regarding the cardiac safety of mexiletine for this indication have been raised however [62], and hence prescribing practice shows considerable regional variation.

Management of muscle symptoms in DM1 can be supported by appropriate referral to allied health professionals. Falls are common, and may cause significant morbidity including fractures [63], hence input from physiotherapists

and orthotic teams with a view to falls prevention may be useful. Speech may become increasingly slurred with a nasal quality, making communication challenging, and a neuromuscular dysphagia may develop that is compounded mechanically by neck weakness. Choking and aspiration pneumonia are thus significant contributors to morbidity and mortality. As such, communication difficulties or concerns regarding safety of swallow should prompt assessment by a speech and language therapist [64].

1.3.2 Cardiovascular system

Cardiac conduction disease with associated risk of sudden death has been long recognised as a consequence of myotonic dystrophy [65]. Histologically, heart muscle shows interstitial fibrosis, fatty infiltration, myocyte hypertrophy and lymphocyte infiltration. Changes are widespread, but have a predilection for the His-Purkinje system [66]. Clinically, conducting system abnormalities occur in excess of cardiomyopathy, and typically manifest as electrocardiographic (ECG) changes that may evolve slowly over many years. Characteristically, there is gradual lengthening of the PR interval and broadening of the QRS complex that, without intervention, eventually progress to higher grade atrioventricular block and life-threatening bradyarrhythmia [67]. Prophylactic permanent pacemaker (PPM) implantation is recommended for patients with progressive conducting system disease, and cohort studies have identified high-risk ECG features predictive of cardiac morbidity that can help guide clinical decision-making [68,69]. Cardiac management is complicated by observations that conduction abnormalities may be intermittent [70,71], and ventricular dysrhythmias - that would not be mitigated by a permanent pacemaker - are also seen in some patients [72]. Risk factors for ventricular arrhythmias are poorly defined, although focal fatty infiltration [73] and left ventricular impairment [72] may render the myocardium more susceptible. Furthermore, a minority of individuals with DM1 show cardiac muscle repolarisation abnormalities, clinically comparable to Brugada syndrome, due to abnormal splicing of the voltage-gated sodium channel *SCN5A* for its neonatal isoform [74]. International consensus for an approach to cardiac screening is lacking, but clinicians broadly agree that regular assessment of the heart in some form should be offered to all patients, including asymptomatic mutation carriers [67]. High risk ECG changes,

palpitations, dizziness or syncope should prompt urgent further investigation, with consideration of device placement [75].

1.3.3 Ophthalmic system

Ocular cataract is a highly penetrant feature of DM1, and so affected individuals are advised to keep regular check-ups with an optometrist to detect any impact on vision. In the general population, onset of cataracts at a young age (< 55 years) or family history of premature cataracts are highly suggestive of myotonic dystrophy, even in an otherwise asymptomatic individual [76]. As such, some authors have proposed that screening for myotonic dystrophy should routinely be discussed with every patient presenting with premature cataracts [76,77].

Intriguingly, the high penetrance of cataract in DM1 may provide evidence of a pathogenic mechanism independent of mRNA toxicity. This is because pathological CTG repeat expansions causes epigenetic changes at the *DMPK* locus, which reduces expression of the adjacent gene *SIX5* [78]. *SIX5* deficient mice develop cataracts, with a severity proportional to *SIX5* dosage, implicating a possible role for altered expression of neighbouring genes in DM1 pathogenesis [79]. The presence of cataract in DM2, however, argues against reduced expression of *SIX5* as the sole cause of this feature.

1.3.4 Endocrine system

It is intuitive that a widespread dysregulation of alternative splicing might impact endocrine signalling pathways in DM1, and indeed susceptibility to several abnormalities of the hormonal system is described. Features of hypogonadism, such as testicular atrophy, gynaecomastia and impaired fertility, were noted in early descriptions of myotonic dystrophy [80], and are present in around a third of adult males [81]. The sex hormone axis in females has not been widely studied, partly due to difficulties in interpretation due to the normal fluctuations in relation to the menstrual cycle, but available data suggest a trend towards reduced fertility, increased spontaneous abortion rate and a blunting of libido [82,83].

Relative insulin resistance, and consequent hyperinsulinaemia, is also more common in DM1 cohorts [84]. The pre-mRNA of insulin receptor (IR) undergoes dysregulated alternative splicing in DM1 skeletal muscle, resulting in a switch from production of the wildtype protein to an exon 11-skipped form (IR-A), that is associated with lower signalling and hence a decreased metabolic response to insulin [85]. In most cases, insulin resistance does not meet the diagnostic threshold for diabetes mellitus, though the incidence of diabetes is somewhat elevated compared to the background population [83]. Insulin insensitivity may also contribute to the incidence of non-alcoholic fatty liver disease observed in DM1 [86], although the relative contribution of genetic factors compared with obesity and sedentary lifestyle [87,88] have not been elucidated.

Finally, longitudinal data also suggest a marginally increased susceptibility to abnormalities of thyroid function, and to parathyroid adenoma in DM1. Taken together, these findings suggest patients with myotonic dystrophy should be screened regularly for evidence of treatable endocrine dysfunction, including diabetes mellitus [83].

1.3.5 Malignancy

DM1 is not traditionally regarded as a cancer predisposition syndrome. However, meta analysis of large patient cohorts has revealed a marginally increased incidence of several malignancies, including endometrial, thyroid, testicular and colorectal cancers as well as cutaneous melanoma [89]. Neoplasms of brain may also be over-represented in DM1 [90]. No specific protocol for increased cancer surveillance has been recommended, though patients should be encouraged to participate in population screening programmes. Further, it can be speculated that a high background incidence of non-specific symptoms, impaired symptom awareness and possibly reduced assertiveness in seeking further investigation may lead to delayed diagnosis of cancer in patients with DM1, compromising survival from potentially curable malignancies. Clinicians should therefore be mindful of the risk of malignancy, and keep a low threshold for investigation of new symptoms.

1.3.6 Respiratory system

Progressive respiratory impairment and associated infective complications are an important cause of mortality in myotonic dystrophy [91,92]. Weakness of muscles of respiration causes an insidious onset of restrictive respiratory defect, including reduced forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Ability to clear respiratory secretions may also be impaired secondary to weak cough, increasing susceptibility to lower respiratory tract infection and hampering recovery from them [93]. While resting hypoxia or hypercapnoea may be observed, progression of respiratory muscle weakness is very slow [94], and an overt respiratory failure requiring daytime ventilation is not commonly seen. Conversely, patients seem exquisitely vulnerable to hypoventilation during sleep, particularly during the hypotonia of deep sleep. Sleep disordered breathing (SDB) is, therefore, a frequent finding, and may manifest in the absence of apparent daytime respiratory impairment [95]. Practice varies both regionally and internationally with regard to screening for SDB [93], but a low threshold for investigation is generally agreed and so respiratory symptoms, such as recurrent infections, daytime sleepiness or morning headaches (suggestive of hypercapnoea) should prompt further investigation.

Treatment may be offered to patients with significant SDB in the form of positive airway pressure (PAP) therapy or, for patients with more significant respiratory compromise, formal non-invasive ventilation (NIV). The masks and devices used for these therapies can be perceived as uncomfortable and intrusive, and as such adherence to therapy is notoriously low in DM1-affected cohorts [96]. Furthermore, sleepiness symptoms may not improve even with consistent use of ventilation in DM1 [97], supporting a contribution of central nervous system (CNS) factors to daytime sleepiness symptoms. In light of this, wakefulness-promoting agent modafinil has also been widely used to treat somnolence symptoms. Several small studies have examined its use in DM1, generally finding the drug to be safe, but with variable evidence of efficacy [98-103]. The great majority of respondents in a large UK survey of DM1-affected families reported a marked or dramatic symptomatic benefit from modafinil use [104]. However due to a lack of robust randomised control trial data, modafinil

is not formally licenced in Europe for use in myotonic dystrophy [105], and hence access to the drug varies with local prescribing practices.

The pathogenesis of SDB and excessive daytime sleepiness are therefore complex, with both central and peripheral factors playing a role. There is a major unmet need to better understand the basis of these important aspects of the disease, both to develop clinically meaningful methods to quantify their severity, and to identify new targets for treatment. The CNS features of DM1, and their relevance to sleep disorder are therefore discussed in greater detail as follows.

1.4 The neuropsychological phenotype

1.4.1 Congenital and juvenile onset DM1

An apparent effect of DM1 on the CNS, with consequent neuropsychological features, has been recognised since its early descriptions [106]. Among those affected by the most severe, congenital-onset form of myotonic dystrophy, overt intellectual disability is a consistent finding, and is frequently accompanied by additional neurodevelopmental diagnoses, most notably autism spectrum disorders [107-109]. Measures of IQ among those with the juvenile-onset form of DM1 are also typically lower than predicted for age, although do not invariably lie within the range associated with learning disability [108]. Additional neurodevelopmental diagnoses are likewise more prevalent in juvenile-onset DM1, including attention deficit-hyperactivity disorder, autism spectrum disorder, anxiety disorders and alexithymia (an impairment of emotional awareness), with consequent high rates of school delay [110,111].

1.4.2 Adult onset DM1

The cognitive phenotype observed in adult-onset DM1 broadly consists of more subtle deficits. Affected individuals do not exhibit a global intellectual disability when IQ is measured by standard tools, such as the Weschler Adult Intelligence Scale Revised (WAIS-R) or Mini Mental State Examination (MMSE) [112-114], although distribution may be shifted towards the lower end of the general population range. Case-control studies using more targeted tools highlight impairment particularly in aspects of executive functioning, including planning,

attentional control, conceptual reasoning and set shifting [112,115]. Deficits are also observed in performance of tasks requiring visuospatial processing, such as the Rey-Osterreith figure copy [107,116,117] and block design tests [112]. Deficits in attention are evidenced by poorer performance in the Trail Making Test A [107] and Serial 7 subtest of the MMSE [118]. A recent evaluation of 101 individuals with DM1 across five cognitive domains (visuospatial, executive, attention, memory and language) found those with adult-onset DM1 typically exhibit impairment in at least two to three domains, although memory and language were comparatively spared except among older patients with a longer disease duration [119]. Longitudinal studies confirm the progressive nature of cognitive symptoms [120-122]. Follow up of one cohort over 9 years, including ninety adults with adult-onset DM1, noted that decline was most marked in domains of verbal memory, visual attention and processing speed. The authors highlight similarities to normal cognitive aging, proposing that decline in DM1 could be seen as a "progeroid" process [123].

In addition to deficits of cognition, changes in personality and social functioning are also described in DM1, which may evolve with progression of other symptoms. A consistent finding is that people with DM1 are more likely to exhibit avoidant personality traits, that might include reluctance to seek new experiences, make new friends or form intimate relationships [115,124,125]. In the general population, such behaviours are thought to arise from feelings of social inadequacy, or fear of rejection [115], and indeed people with DM1 appear prone to low self-esteem, sensitivity to criticism and introversion [124,125]. A tendency to obsessive-compulsive traits, such as rigidity in routine or in matters of morality, and passive-aggressive traits, such as being inclined to become sulky or argumentative in workplace situations, is also reported [125]. Impaired social cognition may offer some insight into the reasons for withdrawal in DM1. An ability to interpret others' facial expressions, and being able to imagine a scenario from another person's perspective ('theory of mind') are fundamental to satisfying and successful social interactions. Several reports suggest an impairment of such social cognition in DM1 [126-128], which in concert with executive difficulties and poverty of facial expression could hamper ability to gain rapport during everyday social interactions. A preference for

isolation may therefore, in some part, reflect previous negative experiences of social encounters.

A distinct, but related trait frequently encountered in DM1 is that of apathy. Apathy describes a lack of goal-directed behaviours, frequently accompanied by a lack of emotion, interest or concern. The resulting physical inactivity, deferral of duties and apparent indifference of individuals with DM1 may be noted by partners, parents or carers, and can be a source of considerable frustration [129]. Signs of apathy inevitably overlap other central features of DM1, although attempts to quantify apathy in a targeted way suggest this symptom is distinct from depression [130], and fatigue or sleepiness [131]. An inverse correlation of apathy symptoms with performance in tests of basic attention and global cognition suggest that this symptom is also proportional to overall CNS disease burden [130].

1.5 Excessive daytime sleepiness and fatigue

1.5.1 Clinical description

Complaints of excessive daytime sleepiness (EDS) and fatigue are extremely common in DM1, present in up to 90% of symptomatic individuals [132]. EDS in DM1 is characterised by an increased sleep requirement, with a tendency to sleep for long periods overnight, as well as being liable to doze off or nap in the daytime, particularly if attention is not held [133]. Overnight sleep may be restless, however, and patients often find that naps are unrefreshing, and fail to erase the urge to sleep more. This feature in particular draws parallels with a distinct condition, idiopathic hypersomnia (IH) [134].

In clinical practice, it can be challenging to distinguish symptoms of EDS from more general symptoms of fatigue, which might encompass muscle aches due to physical exertion, or mental exhaustion without sleepiness. In DM1, fatigue and EDS show a strong tendency to occur in parallel, with EDS in particular rarely encountered without comparable complaints of fatigue. This has led some authors to suggest they likely represent effects of a common underlying process [135]. Evidence to support this includes the observation that fatigue in DM1 occurs considerably in excess of that reported in neuromuscular conditions with

comparable physical impairment, suggesting a central contribution to fatigue symptoms [136].

Together, EDS and fatigue have the potential to profoundly impact quality of life. Patients burdened by these symptoms report higher depression symptoms and lower health-related quality of life scores in general. There is also evidence to suggest EDS may confound some of the negative personality changes of DM1, with sleepier patients more likely to have higher neuroticism, and lower openness, agreeableness and conscientiousness traits [135]. Patients, and perhaps more frequently their families, readily volunteer anecdotes to demonstrate impacts of EDS on ability to function in areas such as employment, caring for children, driving, and more general ability to enjoy life through social integration and participation [104]. As with apathy symptoms, a tendency to doze can be misconstrued as indifference or laziness, and can lead to increased stress and conflict with family or carers [137].

1.5.2 Sleep research in DM1

Polysomnography (PSG) and multiple sleep latency tests (MSLT) have been widely employed in case-control studies in an attempt to understand the basis of EDS. Compared with controls, marked differences in overnight sleep architecture are described in DM1, including a greater total sleep time, but with greater fragmentation of sleep by frequent awakenings [138,139]. Furthermore, individuals with DM1 spend comparatively longer in the deeper, slow wave and rapid eye movement (REM) stages of sleep [95,140]. An increase in periodic limb movements (“restless legs”) is also a frequent finding [95,138].

The MSLT objectively confirms the presence of excessive somnolence, with subjects falling asleep on average more quickly than controls, although very short sleep latencies (< 8 minutes) are relatively rare [95,138]. Quantitative assessment of sleepiness in DM1 is complicated by the fact that mean sleep latency itself generally does not correlate with self-reported sleepiness scores in this group [95,139]. The MSLT also reveals the presence of sleep onset REM periods (SOREMP), defined as the abnormal presence of REM sleep within 15 minutes of sleep onset, in some individuals [95,140-142]. SOREMP are traditionally associated with the condition narcolepsy, in which affected

individuals may experience cataplexy, sleep paralysis, lucid dreams, hypnagogic hallucinations as well as disturbed night time sleep [143]. Cataplexy-like or sleep paralysis symptoms are uncommon in DM1, though have been reported and appear more common in patients reporting severe sleepiness symptoms [140].

In many neuromuscular disorders, weakness and fatigue of respiratory muscles, combined with reduced chest wall compliance, can provide a substrate for nocturnal hypoventilation, particularly during the hypotonia of REM sleep. Furthermore, bulbar muscle weakness may predispose to upper airways obstruction, resulting in fragmented sleep and daytime somnolence and hence obstructive sleep apnoea syndrome (OSAS) [144]. SDB is a frequent finding in DM1, and may include a diverse range of respiratory events including obstructive apnoeas, centrally-mediated apnoeas, hypoventilation, and periodic breathing [145,146]. The prevalence of significant SDB (defined as five or more respiratory depression events per hour of sleep) is variable between studies, ranging from around 13% to 86% [134,140]. SDB may be present in the absence of any abnormality in daytime respiratory assessments [145-147], and occurs more commonly in DM1 than in other disorders with a similar pattern of muscle weakness, suggesting that a central dysregulation of breathing during sleep may contribute to its prevalence [148].

Fragmentation of sleep due to desaturation events may at first appear to offer a plausible explanation for EDS in DM1. Evidence does not support this hypothesis however, since numerous studies describe no clear difference between respiratory parameters of patients with high subjective sleepiness and decreased sleep latency, from those who report no sleepiness. Reports describe both cases of EDS occurring in the absence of SDB [134,140,142], and clinically significant SDB in patients who deny EDS symptoms [146]. There is some evidence to suggest an increased percentage of stage 4 sleep, as well as a greater number of SOREMPS are predictive of EDS [140], but otherwise no clear correlations have emerged among the measures of sleep architecture, SDB, self-reported EDS and other clinical parameters (reviewed in [149]).

Despite considerable circumstantial evidence pointing to a major contribution of CNS involvement to excessive sleepiness and sleep disorder in DM1, few studies

have combined CNS measures with detailed phenotyping of sleep in a single cohort.

In normal physiology, wakefulness is driven by an activating arousal system that originates in the upper brainstem. Two major pathways are described (Figure 5). In the first (yellow), fibres project from the upper pons to activate the reticular and relay nuclei of the thalamus. The second pathway (red) activates neurons in the lateral hypothalamic area, and basal forebrain, and may be influenced by neurohumeral factors such as orexin and hypocretin. Both pathways regulate widespread projections to the cerebral cortex that, during wakefulness, stimulate the cortex to be active and ready to receive information. Lesions affecting these pathways at the junction of midbrain and forebrain cause profound sleepiness, as occurs in encephalitis lethargica [150].

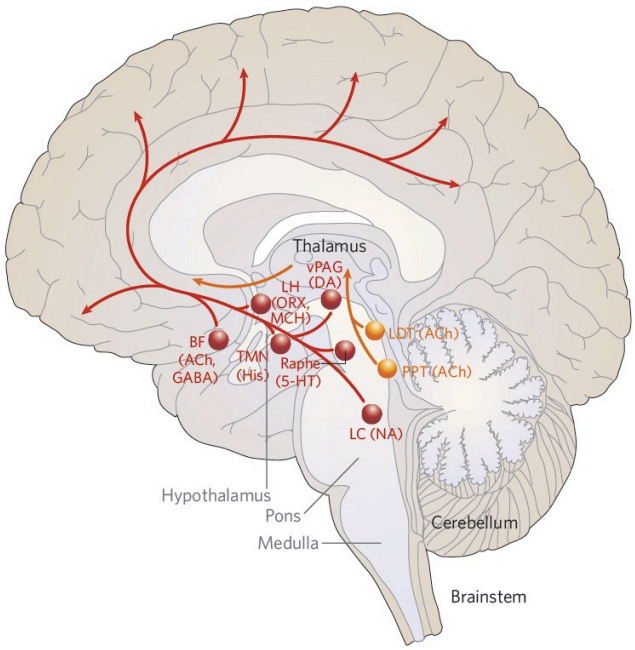

Figure 5: Schematic demonstrating the key components of the ascending arousal system
The system consists of two branches. In the first (yellow), the reticular and relay nuclei of the thalamus receive input from the upper pons, which in turn regulate transmission of information to the cerebral coretex. The second branch (red) does not pass through the thalamus directly, but instead activates neurons in the lateral hypothalamic area before projecting to the cortex. Ach: acetylcholine; PPT: pedunculopontine nucleus; LDT: laterodorsal tegmental nucleus; TMN: tuberomammillary nucleus; His: histamine; DA: dopamine; 5-HT: serotonin; LC: locus coeruleus; NA: noradrenaline; LHA: lateral hypothalamus; ORX: orexin; MCH: melanin-concentrating hormone; BF: basal forebrain; GABA: gamma aminobutyric acid. Reproduced with permission from Saper *et al.* [150].

A group based at the Ichihara Hospital in Tokyo has undertaken several clinicopathological studies, using necropsy data from presumably the same series of eight DM1 patients, compared with controls. A single case report describes a female with DM1 affected by daytime hypersomnia, SDB and tendency to hypoventilation. Following her death from pneumonia at age 53, histological examination of brain revealed severe gliosis and neuronal loss, particularly affecting the tegmentum of the brainstem that could account for a central impairment of respiratory drive [151]. In group comparisons, they found more severe neuronal loss in the medullary reticular formation [152] and medullary arcuate nucleus [153] of the brainstem of three DM1 patients with hypoventilation, when compared with DM1 individuals without respiratory abnormalities and controls. Greater loss of serotonin (5-HT)-containing neurons in the dorsal raphe nucleus and superior central nucleus was also found in those with a history of excessive somnolence symptoms [154]. A single case control study has examined correlations between PSG measures and brain imaging in 10 subjects with DM1, finding a possible association between atrophy of the rostrum and genu of corpus callosum and shorter sleep latency [139]. There is therefore a clear need to build on these preliminary observations, to further define the relationship between the CNS effects of DM1 and EDS symptoms.

1.6 Brain imaging

1.6.1 Structural MRI

Magnetic resonance imaging (MRI) is a widely used method of medical imaging. It is based on the principal that hydrogen atoms will align their spin when exposed to an external magnetic field. If the field is removed, radio frequency energy is emitted. By varying the parameters of a sequence of magnetic pulses applied, and detecting the energy emitted, it is possible to distinguish between different tissues based on the relaxation properties of its constituent hydrogen atoms. Pulse sequences and detection times may be varied to achieve different levels of contrast between specific tissues. Broad classes of imaging include structural MRI sequences, which seeks to provide static anatomical information, and functional MRI (fMRI) that is used to explore dynamic physiological changes over time. Core sequences of standard structural imaging measure the T1 (longitudinal) and T2 (transverse) relaxation time of tissues [155]. In brain

imaging, T1-weighted sequences provide particularly good anatomical definition of cerebral cortex and fat-containing structures, while in T2-weighing the highest signal intensity is for fluid, which can be particularly useful to identify inflammatory processes such as oedema, tumour or infarction [156].

Structural MRI sequences have been widely applied in case-control studies of DM1, readily demonstrating the presence of brain abnormalities that are highly variable. Gross structural abnormalities include a progressive, diffuse atrophy of white and grey matter, and the presence of white matter lesions that are hyperintense on T2 weighted imaging (Figure 6) [157-159]. A subtype of structural imaging, diffusion tensor imaging (DTI), also reveals the presence of diffuse microstructural disruption, even in apparently normal-looking white matter [160]. Correlations of structural abnormalities with clinical and genetic measures have been explored using a range of different modalities.

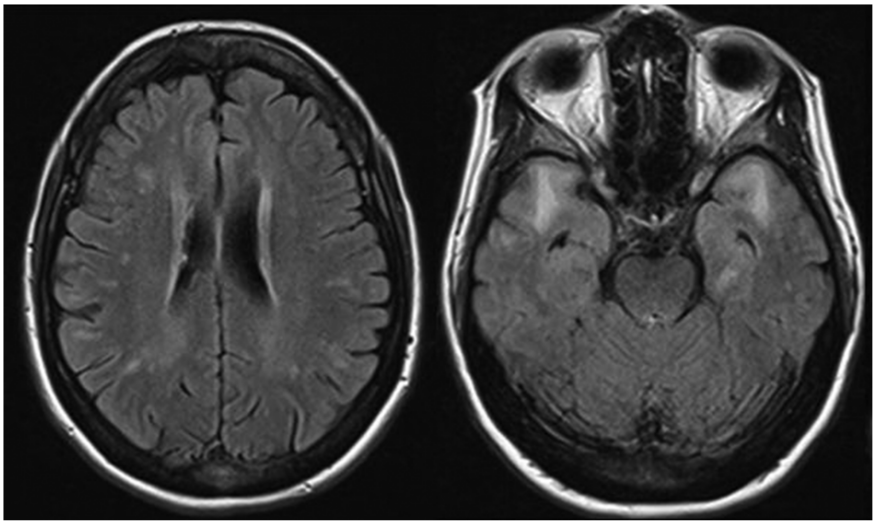

Figure 6: Axial T2 FLAIR images of a 49 year old woman with DM1, demonstrating hyperintense lesions in white matter, particularly affecting the anterior temporal lobes. Reproduced from Cabada *et al.* [161] with permission of Oxford University Press.

1.6.1.1 Voxel based morphometry

A large number of studies have compared structural MRI of brain in DM1 to control subjects (reviewed in [162]). The majority have used a voxel-based morphometry (VBM) approach to identify regional differences in both grey and white matter, as well as exploring linear correlations of volumetric changes with clinical measures.

VBM studies reveal that grey matter atrophy is widespread in DM1 when compared with controls, affecting frontal, temporal, parietal and occipital cortices as well as subcortical grey matter structures, including thalamus, putamen and caudate [163-171]. A handful of specific structure-phenotype correlations have been described; for example, total supratentorial volume correlating with flexibility of thinking and inversely with muscle impairment rating scale (MIRS) [167], or between several cortical regions and performance in a delayed recall memory task [170]. Sample sizes were small however, with each study including 34 or fewer individuals with adult-onset DM1, and so it is perhaps not surprising that no structure-phenotype correlations were consistently described across studies. Similarly, some authors also reported correlations of regional grey matter atrophy with CTG repeat length [163,168], while others found no relation [164,169].

Reduced volume of white matter measured by VBM is likewise described in DM1 [164,166], though in some cases the difference did not reach statistical significance [167]. Automated segmentation of white matter volume may be confounded by the presence of the T2 hyperintensities that are common in DM1, and furthermore volume alone gives no information about the structural and functional integrity of the white matter tracts. In most cases, therefore, additional methods were employed to quantify the severity of white matter change.

1.6.1.2 Quantitative measurements of white matter hyperintensities

White matter hyperintensities are commonly seen on structural MRI imaging in DM1, occurring in the subcortical grey matter of all major lobes as well as the periventricular regions [162]. The distribution of lesions may be similar to appearances seen in small vessel ischaemia, and appearance may be severe enough to be mistaken for other vascular CNS disorders such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) [172].

Methods to quantify the severity of white matter hyperintensities include visual grading scales. These typically involve a trained operator scoring specific lobar regions usually according to defined a four-point scale, running from absent

lesions, to extensive confluent abnormalities. Specific protocols have been proposed by Fazekas [173] and Wahlund [174]. These methods are however comparatively labour intensive, requiring significant training to achieve inter-rater consistency. Furthermore, the categorisation of patients into relatively broad groups limits the power to detect more discrete linear relationships. A more accurate measure of white matter hyperintensity can be achieved by software-driven methods, which identify lesions in an automated or semi-automated manner, and output a total lesion volume for a given 3-dimensional MR image. In DM1, studies measuring total lesion load generally concur that the volume of hyperintensities increases with age. Some also report correlations with neuropsychological impairment [161,169], including mini-mental state examination (MMSE) score [168,171], and CTG repeat length [168]. Other studies found no clinical or genetic correlations with white matter lesions [167,170].

1.6.1.3 Diffusion tensor imaging

An additional MRI method that has proven powerful in characterising white matter change, including in myotonic dystrophy cohorts, is diffusion tensor imaging (DTI). DTI sequences map and characterise the diffusion of water in three dimensions. In biological systems, the mean diffusion of water can be influenced by microstructure of a given tissue, and so DTI has a practical application in detecting microstructural changes that may not be apparent on gross structural imaging. Within free fluid, such as cerebrospinal fluid, diffusion of water is random in all directions, described as ‘isotropic’ diffusion (Figure 7A). In intact white matter, there is a mean tendency for water to diffuse longitudinally in relation to axons. Non-random diffusion is described as ‘anisotropic’ (Figure 7B). For a given voxel, the key scalar measures in DTI are fractional anisotropy (FA), a measure of whether diffusion is constrained in any direction, and mean diffusivity (MD), a measure of the total amount of diffusion occurring within that voxel. In the context of white matter imaging, microstructural disruption of parallel white matter tracts would be predicted to cause a decrease in FA, and an increase in MD (Figure 7C) [175].

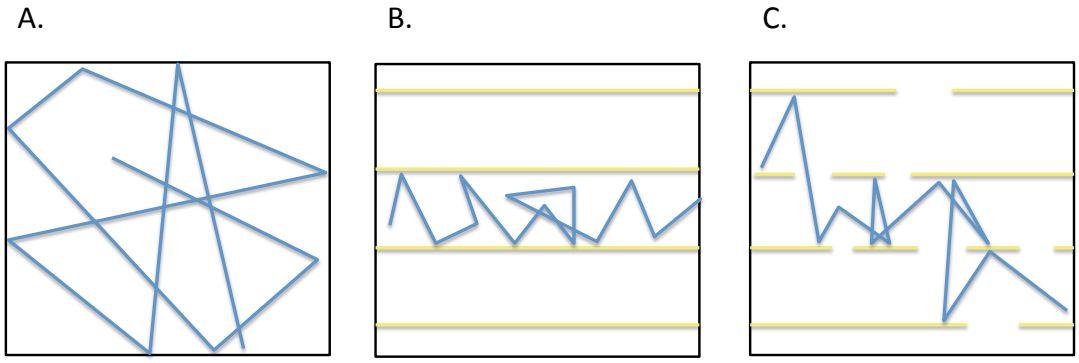

Figure 7: Schematic diagram showing the hypothetical path of diffusion of a single water molecule in (A) cerebrospinal fluid, (B) intact white matter, and (C) disrupted white matter

The direction of greatest diffusivity (the eigenvector) can be used to estimate the trajectory of white matter fascicles using 'tractography' algorithms, and can be thus applied to map the path of specific white matter tracts (*e.g.* Figure 8).

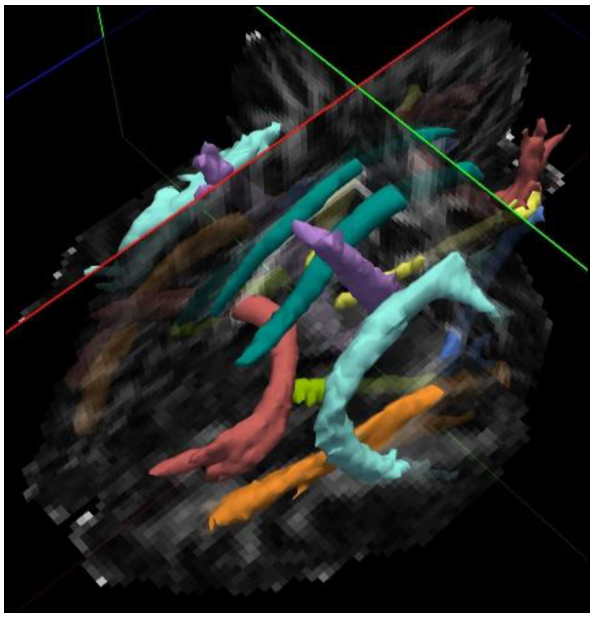

Figure 8: An example of white matter tracts derived from diffusion tensor imaging
This image was produced using the TRACULA (Tracts Constrained by Underlying Anatomy) processing stream. Purple = corticospinal tract; red = forceps major & forceps minor; teal= cingulum bundle (cingulum gyrus component); light green = cingulum bundle (angular gyrus component); light blue = superior longitudinal fasciculus (temporal component); orange = inferior longitudinal fasciculus; medium blue = uncinated fasciculus; yellow = thalamic radiations. Reproduced from Wozniak *et al.* with permission from Elsevier.

DTI measures have been applied in several DM1 case-control studies, measuring white matter integrity either in nominated regions of interest [160,161,163,176],

or within whole tracts by a tract-based spatial statistics approach [166,168-171,177]. These studies broadly describe DTI changes of decreased FA and increased MD occurring ubiquitously in white matter of individuals with DM1, and increasing with age. DTI changes are even present in children with juvenile or congenital onset DM1, in whom they correlate strongly with global intelligence [178]. In mixed DM1 cohorts, correlations are reported between DTI measures and neuropsychology scores including motor performance [166], working memory and processing speed [177], Addenbrookes Cognitive Examination Revised (ACE-R) orientation and attention subscores [169], MMSE scores [168,171], verbal memory [170] and visuospatial impairment [161,170]. Again however, no specific structure-phenotype associations have been consistently described across studies. In some cases, the strength of correlation was reduced if severely affected, congenital-onset cases were excluded [171]. Minnerop *et al*. also noted an apparently counter-intuitive observation that participants with lower FA values, thus more severe white matter disruption, tended to report less fatigue and depressed mood [166].

Regarding correlations with genetic data, relationships between regional FA values and CTG repeat lengths are described in some studies [168,171,177], but were found to be absent in others [163,169,178].

1.6.2 Functional imaging

In contrast to the static nature of structural MRI, fMRI seeks to capture regional changes in brain metabolism that vary with time. Functional MRI (fMRI) relies on the fact that biological processes of cognition (the propagation of action potentials, release and subsequent scavenging of neurotransmitters, and so on) are energy-dependent. When a region of the brain is activated by a cognitive task, there is a localised increase in energy requirement, causing a depletion of oxygen, and production of waste metabolites including carbon dioxide and hydrogen ions. The normal homeostatic response of localised vasodilation is initiated by these processes, and transiently increases delivery of oxygen, indeed typically delivering slightly more oxygen than required to overcome the deficit. In blood, the entire process is therefore characterised by an initial build-up of deoxygenated haemoglobin (Hb) and decrease in oxygenated Hb, followed by a compensatory increase in oxygenated Hb and decrease in deoxygenated Hb

compared with the resting state. This change is detectable by MRI, since there is a change in the magnetic field surrounding red blood cells depending on the oxygen state of haemoglobin, which affects blood's relaxation times. At high field strength, this blood oxygen-level dependent (BOLD) signal is particularly strong in capillaries, giving good spatial specificity.

Experiments utilising fMRI commonly involve a form of stimulus, such as a visual stimulus or manual task, to induce an alternation between cognitive states in the subject ("control" and "experimental" conditions). Imaging is undertaken over a period of time, in which the stimulus is applied intermittently, with longer periods of control condition between stimuli to allow a return to resting state. A statistical test can then be applied to map areas of activation in response to the experimental condition [179]. Imaging by fMRI may also be undertaken in the resting state alone, to deduce functional connections between spatially separated brain regions. This is based on the principal that a variation in BOLD signal that is synchronous over time between different brain regions implies a functional connection between them; in this way, maps of functional connectivity can be derived.

Few studies have yet applied fMRI in DM1. The data available suggest widespread abnormalities on resting-state fMRI compared with controls, with a broad pattern of decreased connectivity in anterior regions (Figure 9), and increased connectivity in posterior regions. The anterior cingulate and orbitofrontal cortices are particularly affected by reduced connectivity. Since these nodes act as 'hubs' with projections to other cortical nodes, this could offer a plausible link to cognitive deficits.

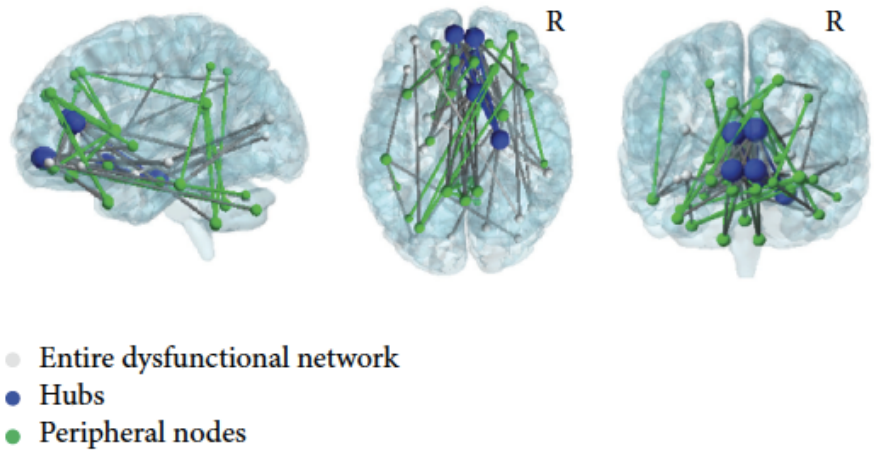

Figure 9: Networks of reduced functional connectivity identified in DM1 subjects compared with controls
The authors applied a Bonferroni correction to identify the most critical nodes of the dysfunctional network (blue and green). 'Hubs', characterised by a large number of connections, were identified in the anterior cingulum, oribitofrontal cortices and right parahippocampal gyrus. Peripheral nodes were located in prefrontal, temporal and parietal cortices, and the cerebellum. Reproduced from Serra *et al*. [180] under Creative Commons Attribution License.

Conversely, there is an increased connectivity between the supplementary motor area and cerebellum that may represent a compensatory remodelling of neural networks in response to peripheral motor impairments [180]. Similar findings were made on fMRI undertaken during a motor task (a repeated a finger to thumb opposition movement), during which there was increased activity compared with controls in premotor cortex, again postulated to represent a compensatory reorganisation of neural networks involved with movement [181]. Toth and colleagues demonstrated an increased BOLD signal in the supplementary motor area and dorsal anterior cingulate complex during grip myotonia in eight patients who experience this symptom, which was absent in a DM1 positive control group without myotonia. This observation was interpreted as representing a cortical attempt to terminate the abnormally sustained muscle contraction, rather than to suggest a central origin for myotonia itself [182]. A specific association of functional connectivity in the left parietal cortex with schizotipic personality traits, that might include apathy, disorganised behaviour and fixed ideas, has also been proposed [183].

1.7 Current research context

1.7.1 Clinical trials in DM1

Growing insight into the molecular mechanisms underlying DM1 has led to several areas of encouraging progress towards targeted therapies. Perhaps the most promising of these is development of the IONIS-DMPK-2.5Rx antisense oligonucleotide (ASO) molecule by Ionis Pharmaceuticals™. ASOs are short strands of synthetic nucleic acids, designed to bind to a complementary sequence of mRNA. Traditionally, they have been used in knock-down experiments, since binding of the ASO to a mRNA prevents translation, either by physically blocking the translational machinery, or by flagging the mRNA for destruction by RNase H [184]. In mouse models, systemic administration of an ASO complementary to exon 15 of the *DMPK* mRNA, just downstream of the (CUG)n repeat, reduces abundance of expanded CUG repeat RNA in muscle by 70%, and is associated with correction of dysregulated splicing, and improvements in muscle strength and muscle histology [185]. A phase 1/2a trial of an equivalent ASO in humans, IONIS-DMPK-2.5Rx, was undertaken in 48 patients with DM1, and concluded in 2017. The drug was well tolerated, and showed some encouraging trends in biomarker responses. However, the amount of drug present in affected muscle, measured on biopsy, did not meet the target for therapeutic efficacy. Ionis therefore elected not to advance IONIS-DMPK-2.5Rx, but rather aim to redevelop the core ASO to improve delivery to muscle [186].

Theorising that social withdrawal and physical inactivity in DM1 can produce a cycle of thoughts and behaviours that perpetuate low mood, fatigue symptoms and muscle atrophy, a large randomised control trial was recently undertaken of cognitive behavioural therapy (CBT) in DM1; the OPTIMISTIC study [187]. CBT has a strong evidence base in psychiatric medicine, where it may be used in treatment of depression, obsessive-compulsive disorders and chronic fatigue states among others, and there is some evidence to suggest it is beneficial over exercise therapy alone for treating fatigue in muscle disorders [188]. Participants in OPTIMISTIC completed CBT modules that were tailored to individual need, with common themes including improving sleep patterns, starting new activities, increasing physical activity and improving relationships

with others. Results demonstrated a significant increase in capacity for activity and social participation, reflected by increased DM1-ActivC© score [189].

Finally, a phase 2 trial of tideglusib, an inhibitor of glycogen synthase kinase 3β (GSK3β), has recently been completed in the UK for DM1 [190]. It has been demonstrated that activity of GSK3β is increased in the presence of expanded CUG repeat RNA. This in turn suppresses cyclin D3-dependent kinase 4, whose normal function involves modulating activity of CUGBP1 through phosphorylation. In this way, overactivity of the GSK3β pathway might contribute to CUGBP1-mediated dysregulation of alternative splicing. In the DM1 mouse model, inhibition of GSK3β caused a reduction in muscle weakness and myotonia [191]. The GSK3β pathway has also been implicated in other disorders, and so safety (though not efficacy) of tideglusib has been established through previous trials in Alzheimer's dementia and progressive supranuclear palsy [192,193]. Unlike ASO, tideglusib would be predicted to cross the blood-brain barrier, which may account for the decision to target recruitment for the recent trial at participants with juvenile or congenital onset DM1, in whom the CNS phenotype is most pronounced.

1.7.2 Priorities in CNS research

Taken together, central symptoms of fatigue, excessive daytime sleepiness, cognitive impairment and social difficulties account for a major portion of the disease burden of DM1 [132]. It is therefore crucial that these symptoms are regarded as a priority for therapeutics research, at least equal in importance to physical impairment due to muscle weakness. Indeed, it is conceivable that central symptoms of apathy and fatigue could be a barrier to patients benefitting from an effective therapy for peripheral muscle symptoms, since physical activity is likely to be necessary to drive recovery of muscle strength.

International expert working groups, including the outcome measures in myotonic dystrophy group (OMMYD), and the DM-CNS group [194-196], have proposed key areas of need to improve understanding of CNS disease, and thus improve and enhance readiness for clinical trials within the myotonic dystrophy community. Major issues raised include the need for a standardised, consensus approach to neuropsychological assessment in DM1. This would both improve the

value of natural history studies, since data collection from diverse populations could be harmonised, and provide a core set of cognitive outcome measures for use in clinical trials. Furthermore, while brain changes seen on imaging are relatively well described in DM1, the clinical correlates of specific structural changes are less well understood. Larger studies, using a range of imaging markers, robust methods of phenotyping and ideally longitudinal design are required to identify imaging biomarkers for use in clinical trials. Another major priority is greater understanding of the molecular mechanisms by which the CTG repeat expansion leads to structural brain changes and relevant CNS symptoms. Development of patient registries, further work on animal models of CNS disease, identification of cerebrospinal fluid biomarkers and greater understanding of the neurodevelopmental phenotype encountered in juvenile and congenital-onset DM1 have also been highlighted as important [194-197].

1.7.3 Myotonic dystrophy research in the West of Scotland

In the West of Scotland, all adults with a diagnosis of myotonic dystrophy are invited to attend annual review appointments co-ordinated by the West of Scotland Regional Genetics Service. Around 250 individuals with DM1 attend each year, with the full clinical spectrum of myotonic dystrophy represented. For around six years preceding the present study, individuals within this cohort had been invited to provide blood DNA samples for a longitudinal genetic study, the Genetic Variation in Myotonic Dystrophy study (DMGV), led by Prof Darren Monckton at the University of Glasgow. A large proportion of this cohort have therefore undergone genotyping by SP-PCR for CTG repeat length, and those with variant repeats further investigated by PacBio sequencing. As such, this DM1 cohort is perhaps the most genetically well-characterised in the world. In addition, clinical services in the West of Scotland have a long history of supporting both research and service improvement work in neuromuscular disorders, forming a strong foundation of clinical expertise. In particular, the formation of the Scottish Muscle Network (SMN), a national managed clinical network, has enabled clinicians working with neuromuscular disorders to drive changes to deliver high and equitable standards to care to patients. Notable services associated with the SMN include the dedicated myotonic dystrophy review clinic, and the specialist respiratory service for neuromuscular disorders currently based at the Queen Elizabeth University Hospital in Glasgow.

In light of the above factors, the West of Scotland myotonic dystrophy population represents a highly favorable cohort in which to conduct DM1 clinical research. Since all patients attend a single service irrespective of their social circumstances, there is the potential to obtain a large sample with minimal selection bias. In addition, the availability of relevant expertise in multiple medical specialties offers an opportunity to investigate various aspects of this multisystem condition in a single sample.

1.8 Aims of the present study

The work presented here aimed to address key questions relevant to the wider landscape of DM1 research, by exploring CNS involvement in a moderate-sized, case-controlled sample from the West of Scotland DM1 population.

The specific aims of the study were as follows:

1. To assess the validity of clinical outcome measures currently recommended by expert consensus for assessment of CNS symptoms in DM1.

2. To explore the relationship between CTG repeat length and the severity of DM1 symptoms, with particular reference to those arising from CNS involvement.

3. To describe volumetric brain changes that occur in DM1, and to identify regional changes that correlate with CTG repeat length, hence hold promise as candidate imaging biomarkers.

4. To describe the prevalence, and both clinical and radiological correlates of sleep disorders in the West of Scotland DM1 population.

1.9 Hypotheses

In relation to these aims, the following hypothesis were formulated:

1. Questionnaire-based tools for symptom quantification in DM1 have been well-validated against other self-reported measures [198-201], or motor

measures [199]. Their validity with respect to objective measures of CNS involvement, however, has not been well explored. We hypothesised that self-reported CNS symptoms may correlate poorly with objective measures of brain involvement (such as severity of MRI change, or performance in cognitive tests), since impaired disease awareness is reported as a prominent feature of the DM1 CNS phenotype [202].

2. We speculated that performance in some of the complex neuropsychology assessments recommended by OMMYD [194,195] might be influenced by peripheral muscle weakness in patients with DM1. This would have implications for outcome measure selection in clinical trials, since interference by peripheral muscle weakness would undermine the specificity of these tests as measures of CNS involvement.

3. To date, no clear relationship has been described between CTG repeat length and the severity of CNS symptoms. We suspected that use of traditional methods in previous genotype-phenotype studies, which take no account of age-dependent somatic mosaicism, have hampered the power to detect these correlations. Since SP-PCR has been shown to improve correlations with age at onset of symptoms compared with traditional methods [26], we anticipated this technique would also increase power to detect genetic correlations with key CNS symptoms, and reveal CTG repeat size to be a major modifier of these.

4. The relationship between CTG repeat length and structural brain change is likewise poorly defined [162]. We hypothesised that, as is the case in Huntington disease [203], brain regions in which structural change is most strongly associated with repeat length are likely to be the same structures that underlie major features of DM1 disease. Hence exploration of structure-genotype relationships may give greater insight into potential therapeutic targets for central symptoms of DM1, as well as highlighting candidate regions for use as imaging biomarkers for clinical trials.

5. Our literature review demonstrates strong evidence to support a central basis for excessive daytime somnolence symptoms in DM1, but the nature of this has not been elucidated. We speculated that loss of neurons of the

ascending arousal system, involving the upper brainstem, hypothalamus or thalamus, or reduced integrity of its cortical projections, could be a major contributor to excessive sleepiness symptoms.

6. Finally, we noted that, in the general population, obstructive sleep apnoea is associated with structural brain changes, predominantly affecting white matter [204], that are held to represent hypoxic damage secondary to desaturation during sleep. However, the very high prevalence of sleep-disordered breathing in DM1, even in comparison to neuromuscular disorders with similar patterns of weakness [148], has prompted some authors to suggest that central control of breathing may be dysregulated in this group. We therefore hypothesised that structural changes associated with SDB in DM1 may be twofold; with neuronal loss affecting both regions of brain vulnerable to hypoxic damage, and regions implicated in control of breathing, such as the central pattern generator of the medulla [205].

2 Methods

2.1 Ethical approval

The study underwent ethical review and was approved by the West of Scotland Research Ethics Committee 4 (Reference: WOS 15/WS/0189). Genetic analysis was undertaken as part of a pre-existing study, the Genetic Variation in Myotonic Dystrophy study (DMGV), which also approved by the West of Scotland Research Ethics Committee (08/S0703/121). All study participants provided written, informed consent.

2.2 Inclusion and exclusion criteria

Criteria for inclusion of study participants are summarised in Table 2.

Table 2: Study inclusion criteria

DM1-affected subjects	Control Subjects
Age 18 years of age or over Genetic confirmation of myotonic dystrophy type 1 Able to consent and willing to participate throughout the duration of the study Previously enrolled, or willing to enrol in the DMGV study	Age 18 to 75 years No known major chronic medical or neurological condition

Subjects were excluded if any of the following were present: Inability to give informed consent; Formal diagnosis of congenital or juvenile onset DM1, or clinical evidence to suggest this (e.g. history of DM1-specific symptoms, learning or behavioral difficulties with onset under the age of 16); Severe concurrent medical condition that would prevent participation in study procedures (e.g. cardiac failure with pulmonary oedema); History of major head trauma with loss of consciousness greater than a few minutes or with significant medical sequelae.

Patients with contraindications to MRI could not participate in the imaging portion of the study. Contra-indications included presence of a permanent cardiac pacemaker, and claustrophobia.

No upper age limit was imposed for DM1-affected subjects, in order to maximise the pool of potential recruits. It was known that few elderly DM1-affected patients existed in the target population; hence interference from background age-related cognitive decline was expected to be minimal. The upper age limit for controls was initially set at 65 years. However, after recruitment of a DM1-affected individual who was aged 71 years, the limit for controls was raised to 75 years in order to provide matched data for this subject (substantial amendment approved by West of Scotland Research Ethics Committee 4).

2.3 Recruitment

2.3.1 Determination of sample size

A power calculation demonstrated that a sample size of 40 affected individuals would give 95% power to detect an effect size (r^2) of 0.36 at the p = 0.01 level in regression analysis. Previously, it has been demonstrated that ePAL accounts for 71% of the variation in age at onset (r^2 = 0.71) in DM1 [26]. Given the subjective nature of determining age at onset of symptoms, and the quantitative nature of the measures we planned to undertake, we believed that a sample size of around 40 affected individuals would give very high power to detect meaningful structure-phenotype and genotype-phenotype correlations.

2.3.2 Cohort

DM1-affected individuals were identified for recruitment by review of the DM1 disease database curated by the West of Scotland Clinical Genetics Service. Records of living patients with DM1, who had ever attended annual review appointments with the myotonic dystrophy service, were screened for suitability to participate in the study. Patients whose records suggested they would not meet inclusion criteria outlined above were not contacted. Patients known to have a permanent pacemaker were not actively targeted for recruitment, since they would not be able to complete the MRI portion of the study, although ethical approval permitted up to ten individuals with MRI contra-indications to be included and complete the protocol excluding scanning.

DM1-affected participants were approached by letter and invited to participate in the study. The letter included an “expression of interest form”, which could

be returned to the principal investigator using a freepost envelope. In the majority of cases, the letter was sent approximately two weeks before the patient's scheduled annual review, enabling a member of the clinical team to discuss the study and offer recruitment during that appointment. Awareness of the study was also raised through a news story on the website of Muscular Dystrophy UK, and their associated social media accounts.

First choice for recruitment of control participants was from the families of DM1-affected participants, in order to maximise the concordance of variables such as socio-economic background, educational background and diet. During home visits to DM1-affected participants, the investigator (MJH) discussed the study and need for control participants with any relatives present. If they expressed willingness to volunteer, the investigator left an information sheet, expression of interest form and freepost envelope in their possession. To avoid coercion, no further attempt was made to contact these relatives if no expression of interest form was received.

To supplement the control cohort, recruitment of the remaining subjects was undertaken through the Scottish Health Research Register (SHARE) [206], a voluntary register by which National Health Service (NHS) patients in Scotland can make their electronic records available for research, and express willingness to be contacted for relevant studies. Once recruitment from patient families was complete, an application was made to SHARE to provide contact details for study participants meeting the entry criteria, targeted to the age range necessary to complete the age-matched control cohort.

2.4 Measures of cognition and symptom severity

2.4.1 Neuropsychology assessments

The rationale for selection of the specific neuropsychology outcome measures used, and the nature of the tests themselves are described in greater detail in Chapter 4.

Participants all completed a commercial form of the Stroop test (Golden and Freshwater, Stoelting Co; 2002), including word, colour and colour-word tasks;

the five Trail Making Tests from the Delis-Kaplan Executive Function System; and the Block Design test from Weschler Abbreviated Scale of Intelligence – Second Edition (WASI II).

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was also included in the test battery. The ECAS was devised as a screen for patients with amyotrophic lateral sclerosis, in whom domains affected by neuropsychological impairment can overlap those affected in DM1 (executive, verbal fluency, language, social cognition). The screen also assesses some non-ALS specific domains (memory and visuospatial functions) [207].

The ECAS includes a controlled word association test for the letter 'S'. Two additional conditions were applied for each subject, using the letter 'F', then 'A', to complete an FAS oral word association test. The instruction given to subjects was "I am going to give you a letter of the alphabet, and I would like you to say as many different words as you can beginning with that letter, but not names of people, places or numbers". The number of correct responses for each letter in one minute was recorded.

2.4.2 Self-reported outcome measures

All participants were asked to complete the Fatigue and Daytime Sleepiness scale [201], Beck Depression Inventory II (BDI II) [208], the visual analogue scale from the McGill Pain Questionnaire [209], bodily pain items from Short Form-36 [210] and the self-rated dysexecutive questionnaire (self-DEX) from the Behavioural Assessment of the Dysexecutive Syndrome battery [211].

DM1-affected subjects were further asked to complete the Myotonic Dystrophy Health Index (MDHI) [200] and DM1-ActivC© questionnaires [198]. They were also asked to nominate a close friend, relative or carer to complete a second DEX questionnaire (The Informant-DEX). Specific tools are described in greater detail as follows.

The MDHI is a large questionnaire, which asks subjects to rate the impact of 114 separate symptoms on their quality of life, using a six-point Likert scale. The MDHI was designed to evaluate DM1 patients' overall perception of their health,

as well as providing scores in 17 subdomains identified directly by patients as having greatest potential impact on quality of life [200]. The subdomains assessed are: mobility, upper extremity function, ability to do activities, communication, social satisfaction, social performance, fatigue, pain, myotonia, gastrointestinal issues, swallowing, vision, emotional issues, sleep, cognitive impairment, hearing and breathing. The subscores show good concurrent validity with traditional tools to measure similar concepts [199]. Raw scores were returned to the MDHI authors at the University of Rochester, where they were converted to centile scores.

The DM1-ActivC© is a measure of activities of daily living in DM1. Subjects are asked to rate 25 items with a motor theme (e.g. 'Stand on one leg') on a 3-point Likert scale ('without difficulty, with some difficulty, unable to do'). The questionnaire was produced by Rasch analysis from a large sample of DM1-affected patients, and shows strong test re-test reliability [198]. Raw DM1-ActivC© scores were returned to the tool's authors at Maastricht University Medical Centre, where they were converted to standardised centile scores.

Similarly, the Fatigue and Daytime Sleepiness Scale (FDSS) is a 12 item questionnaire, using a 3-point Likert scale, developed specifically for DM1 patients by Rasch analysis of existing tools including the Epworth Sleepiness Scale (ESS) [201]. This is thought to be superior in DM1 to the traditionally used ESS, since in its standard form the ESS is shown to have weak internal consistency in DM1 patients [212]. Raw FDSS scores were also returned to the tool's authors at Maastricht University Medical Centre, where they were converted to standardised centile scores.

The Behavioural Assessment of the Dysexecutive Syndrome (BADS) is an assessment battery developed to address concerns that established neuropsychology tools may not accurately measure the impact of executive dysfunction in everyday 'real life' situations [211]. We elected to include the Dysexecutive (DEX) questionnaire from the BADS battery, to determine if this tool is sensitive for the social difficulties experienced in DM1. Both the self-reported Dex questionnaire (self-DEX) and a second completed by a close relative or carer (Informant-DEX) were included.

Well-established tools were used to assess mood and pain. These were the Beck Depression Inventory II (BDI II) [208], the visual analogue scale from The short-form McGill Pain Questionnaire [209], and bodily pain items from Short Form-36 [210].

The full battery of neuropsychology assessments, self-reported measures and proxy-reported measures is summarised in Table 3.

Table 3: Summary of neuropsychology and self-reported outcome measures completed by participants

Neuropsychology assessments	Self-reported measures	Proxy-reported measures
Stroop test D-KEFS™ Trail Making Tests WASI-II Block Design subtest FAS oral word association ECAS	MDHI DM1-ActivC© FDSS Self-DEX BDI-II McGill pain scale SF-36 pain items	Informant-DEX

The measures underlined were applied to DM1-affected subjects only, and the remainder were applied to all participants. BDI-II, Beck Depression Inventory II; D-KEFS™, Delis-Kaplan Executive Frontal System, ECAS, Edinburgh Cognitive and Behavioural ALS Screen; FDSS, Fatigue Daytime Sleepiness Scale; MDHI, Myotonic Dystrophy Health Index; SF-36, Short Form 36. WASI-II, Weschler Abbreviated Scale of Intelligence – Second Edition.

2.4.3 Application of outcome measures

DM1 affected participants were offered the option to undertake neuropsychology assessment in their home environment, or in a hospital clinic room. Control participants were assessed in a hospital clinic room.

A single operator (MJH) applied all self-reported outcome measures and neuropsychology assessments, after training by Jonathan J Evans, Professor of Applied Neuropsychology, University of Glasgow. Tools were each applied according to the test manual or author's instructions.

2.5 MRI acquisition and processing (Glasgow site)

2.5.1 Image acquisition

Prior to commencement of scanning, efforts were made to harmonise the imaging protocol with that of a separate DM1 imaging study, led by Prof Peggy Nopoulos at the University of Iowa. The Monckton group had existing collaborative links with Prof Nopoulos' team, and so the potential for images from both studies to be analysed as a pooled cohort was anticipated.

Key imaging sequences for the analysis presented here were T2-w turbo spin echo (TSE); T2-w BLADE™ dark fluid; T2-w Sampling perfection with application optimised contrasts using different flip angle evolution (SPACE) dark fluid; T1-w magnetisation prepared rapid acquisition gradient echo (MPRAGE).

For DM1-affected participants, a slot for MRI scanning was arranged within a short number of weeks from recruitment and neuropsychology assessment. Control participants underwent MRI scanning on the same day as recruitment and neuropsychology assessment. Imaging was carried out at the Glasgow Clinical Research Facility at the Queen Elizabeth University Hospital, Glasgow. Remuneration of reasonable travel expenses, or provision of transportation by taxi free of charge was offered to all participants to attend. Height and weight were recorded prior to scanning.

Scans were acquired on 3T Siemens Prisma MRI scanner (Software version: VE11B, Erlangen, Germany) with a 20 channel head and neck receiver coil.

Diffusion tensor imaging and resting state functional MRI sequences were also acquired during the scanning session. Analysis of these sequences is planned, but is not included in the work presented here.

2.5.2 Lesion filling and quantitation of white matter abnormalities

To quantify the total volume of white matter lesions, T1-weighted 3D MPRAGE and the T2-weighted SPACE dark fluid sequences were analysed using a Lesion Growth Algorithm (LGA) (http://www.applied-statistics.de/lst.html) [213], from the Lesion Segmentation Toolbox. The LGA creates a lesion probability map,

from which the number of and total volume of white matter hyperintensities can be derived.

Prior to determining major brain tissue class volumes, the T1-w 3D MPRAGE images were lesion-filled using the LST toolbox. This sought to minimise error in the tissue segmentation process due to the white matter lesions [214].

2.5.3 Segmentation of major tissue classes

To quantify grey matter, white matter and cerebrospinal fluid (CSF) volumes, the 3D T1-weighted sequences were loaded to Statistical Parametric Mapping software (SPM12; http://www.fil.ion.ucl.ac.uk/spm/). The built-in automated segmentation tool was used with default settings to segment the three tissue classes for each image, from which individual tissue volumes and total intracranial volume were derived.

2.5.4 Pre-processing for voxel-wise statistics

To enable voxel-wise statistics to be applied to the imaging data, the brain images first had to be aligned and warped to an average template. The DARTEL tool within SPM was applied to all DM1-affected and control segmented grey and white matter images, in order to simultaneously align grey and white matter to produce the average template. Images were then smoothed and normalised to a standardised brain template (the Montreal Neurological Institute; MNI; T1-w 152 template), following the 'Normalise to MNI Space' module built into SPM using default settings [215].

2.5.5 Voxel-wise statistics

Voxel-wise statistical modelling (both group comparisons and linear models) was undertaken following methods in tutorial material produced by Prof John Ashburner, University College London [215]. Smoothed grey matter and white matter images, normalised to MNI space, were used for analysis. Total intracranial volume data were loaded to the 'Global Normalisation' menu for all analyses. Additional covariates and the stringency of correction for multiple comparisons will be specified for each model as results are presented. Where it was necessary to closely define the anatomical location of significant clusters,

MNI co-ordinates were extracted from SPM, converted to Talairach co-ordinates using BrainMap GingerALE 2.3.6 (brainmap.org) and uploaded to Talairach Client (www.talairach.org).

2.6 MRI acquisition and processing (Iowa site)

Data presented in Chapter 6 arose from collaboration between the University of Glasgow group and the team led by Prof Peggy Nopoulos, Department of Psychiatry, University of Iowa. The MRI pre-processing and segmentation work outlined as follows were undertaken by imaging scientists of the Iowa team. Key contributors were Timothy Koscik, Vincent Magnotta, Joel Bruss and Eric Axelson. Analysis was led by Dr Ellen van der Plas and Prof Peggy Nopoulos. During a one-week collaboration visit to Iowa City in May 2018, MJH was given detailed instruction regarding the image processing pipelines from the Iowa imaging team, and was able to participate in the latter stages of analysis with guidance from Dr van der Plas and Prof Nopoulos. Dr van der Plas provided MJH with a file containing the output data from volumetric analysis (after adjustment for effects of site, discussed below), and the necessary codes for R statistical software to perform analysis. Final statistical analysis of this dataset was performed by Prof Jeffrey D. Long, Department of Psychiatry and Department of Biostatistics, University of Iowa.

2.6.1 Recruitment of the Iowa Cohort

Participants imaged at the University of Iowa Hospital were recruited from across the United States of America through advertisements distributed by the Myotonic Dystrophy Foundation and word-of-mouth. Healthy control participants were either spouses of affected participants, or recruited from the local community through advertisements. Travelling expenses were provided.

2.6.2 Image acquisition

The research scanner available at the University of Iowa Hospital changed during the course of the study. Initial scans were acquired on a Siemens Trio 3T scanner (n.52), while latter scans were acquired on a GE Discovery 3T scanner (n.27).

2.6.3 Production of group template

A group template for volumetric analysis and was produced from the Advanced Normalisation Tools (ANTs; http://stnava.github.io/ANTs/) pipeline. The final group template was warped to Montreal Neurological Institute (MNI) space.

2.6.4 Volumetrics

Lobar and subcortical regions of interest (ROI) were segmented by a multi-atlas, joint label fusion method using BRAINSTools (https://github.com/BRAINSia/BRAINSTools), BRAINSAutoWorkup pipeline. This method seeks to reduce segmentation errors by applying multiple atlases to each image, with weighted voting for each voxel [216].

The BRAINSTools pipeline provided volumetric data for the following structures: cerebellum, cerebrum, cerebrum grey matter, cerebrum white matter, cerebellum vermis, cerebellum grey matter, cerebellum white matter, corpus callosum, frontal lobe, occipital lobe, parietal lobe, basal ganglia, temporal lobe, thalamus, frontal grey matter, frontal white matter, occipital grey matter, occipital white matter, parietal grey matter, parietal white matter, temporal grey matter, temporal white matter, caudate, putamen, pallidum, hippocampus, amygdala and accumbens.

2.6.5 Intracranial volume correction

Comparison of brain regional volumes between groups of individuals requires adjustment for variation in head size. This has traditionally been achieved by expressing the ROI as a proportion of ICV; *i.e.* ROI/ICV. There is considerable evidence, however, to suggest that many brain structures do not demonstrate a linear proportional relationship to ICV in humans [217]. Instead, structures are commonly related to ICV by a power law principle; *i.e.* $ROI = \alpha * ICV^{\beta}$, where α is a constant scaling component, and β a power component. The value of the power component (β) is often less than 1, particularly for subcortical structures, hence assuming a linear relationship would over-estimate the predicted ROI from a given ICV [218]. A power-proportion method, deriving values for α and β from the sample data, has been shown to be highly effective in removing the effect of ICV for volumetric comparisons [219]. A power-proportion method was therefore

used to adjust our volumetric data for ICV, by estimating β for each ROI from the study data, and dividing the ROI volume by ICV^{β}.

2.6.6 Multi-site harmonisation

Analysis presented in Chapter 6 involves harmonisation of MRI data acquired at two sites; the Institute of Neurological Sciences in Glasgow and the University of Iowa Hospital in Iowa City. Three distinct MRI platforms were used; a Siemens Prisma scanner in Glasgow, and one GE plus one Siemens scanner in Iowa. Several steps were taken to address potential variation in bias field between sites, that might in turn lead to inter-site variation in the automated segmentation of tissue volumes.

Raw T1 weighted and T2 weighted images acquired at the Glasgow site were transferred to the Iowa imaging team for processing. Prior to automated segmentation, the N4 bias correction algorithm for Advanced Normalisation Tools (ANTS; http://stnava.github.io/ANTs/) [220] was applied. This algorithm was developed as an improvement to the preceding nonparametric nonuniform normalisation (N3) algorithm [221]. The N3 algorithm uses an iterative method, which incrementally derives a non-parametric model of tissue intensity from the data itself, in order to reduce tissue intensity variation. N3 has been widely applied with in multi-site imaging research with good results [222]. The N4 algorithm builds on N3 by adding an improved B-spline smoothing strategy, as well as modifying the iterative optimisation scheme, to improve convergence performance [220].

The BRAINSTools AutoWorkup pipeline for automated segmentation of regions of interest (ROI) itself runs an iterative process for bias correction, alongside tissue segmentation and registration [223].

Finally, the raw values for ROI volumes output by BRAINSTools were further adjusted using ComBat (www.bu.edu/jlab/wp-assets/ComBat/Abstract.html), a statistical framework available for R Statistical software. ComBat was first designed to eliminate batch effect in analysis of microarray expression data [224], where total number of batches is small and hence alternative statistical methods to eliminate batch effect are not appropriate. ComBat applies an

empirical Bayes framework to estimate the model parameters that represent the batch effect by pooling information across different measurements. This is based on the assumption that the batch effect will affect many measures in similar ways. In other words, ComBat harmonisation estimates the data distribution for each site or batch, and adjusts individual values to remove this distribution, while retaining variation that is not due to the effect of site or batch. Since its development, ComBat has been shown to be effective in preserving biological variation whilst addressing the effect of site for multi-centre imaging studies [225,226].

2.6.7 Statistical analysis of Glasgow-Iowa cohort data

Analysis of imaging data from the combined Glasgow-Iowa cohort was undertaken using R statistical software. Preliminary analysis was performed using the 'DM1 Analysis' programme written by Dr Ellen van der Plas and Dr Timothy Koscik (May 2018, Iowa City, USA). Final analysis was undertaken by Prof Jeffrey Long, Department of Psychiatry and Department of Biostatistics, University of Iowa, also using R statistical software.

2.7 Sleep studies

2.7.1 Polysomnography

(PSG) was undertaken using an Embletta® MPR PG with ST+ proxy (Natus Incorporated, Pleasanton, CA), a portable recording device. The device was configured to measure:

- Abdominal effort
- Thoracic effort
- Nasal flow (oronasal thermistor and nasal airflow cannula)
- Oxygen saturation (SpO_2)
- Electrocardiogram (two sites)
- Subject position
- Electroencephalogram (EEG) at ten sites
    - frontal (L+R), middle (L+R), occipital (L+R), mastoid (L+R), reference, ground

- Electro-oculogram (EOG) at one site bilaterally
- Chin electromyogram (EMG) at three sites
- Leg EMG at two sites bilaterally

This setup is consistent with SCOPER categorisation S_1 C_3 O_1 P_2 E_1 R_1, based on the Collop criteria for out-of-centre sleep study devices [227]. The operator (MJH) attended around 20:00 on the evening of PSG to attach the equipment. The patient was asked to state their intended "lights off" time, in keeping with their usual bedtime, and the device was set to start recording at this time. The device was programmed, and study data downloaded using RemLogic™ software (Natus Medical Incorporated, USA).

2.7.2 Modified maintenance of wakefulness test (mMWT)

The morning after PSG, the operator returned at approximately 09:00, having asked the subject to be awake in time for their arrival. The overnight PSG data was downloaded, before the device was reprogrammed to complete the mMWT.

At 09:30, the participant was asked to return to their bedroom, sit on top of the bed outside of the covers, and try not to fall asleep. They were instructed not to clap, sing, read, use electronic devices or otherwise use physical distraction. The light was turned off, and EEG recorded for 40 minutes. Tracings were not monitored in real-time; hence the test could not be terminated early if the subject fell asleep. The participant was asked to repeat a further two 40-minute mMWT sessions at 11:30 and 13:30 if they were willing to do so. Reasonable efforts were made to minimise disturbance during mMWTs, although complete elimination of ambient noise (e.g. traffic outside) was not always possible given the nature of the domiciliary setting.

2.7.3 Scoring of sleep studies

Sleep studies were scored and reported by Dr Antonio Atalaia (Newcastle University), in accordance with the American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated events (Version 2.4; www.aasm.org). Classification of respiratory events is summarised as follows:

2.7.3.1 Apnoea

A respiratory event was scored as an apnoea if there was both a drop in peak signal excursion by ≥ 90% of baseline using an oronasal thermal sensor or alternative apnoea sensor, and the duration of the ≥ 90% drop in signal was ≥ 10 seconds. An apnoea was scored as obstructive if the event was associated with continued or increased respiratory effort throughout its duration, and central if respiratory effort was absent. Absent respiratory effort in the initial portion of the event, that resumed in the second portion was classed as "mixed".

2.7.3.2 Hypopnoea

Events were scored as hypopnoeas if a drop in signal excursion was detected and the following three criteria were fulfilled: drop of ≥ 30% of baseline detected by nasal pressure or other hypopnoea sensor, the duration of the ≥ 30% drop in signal was ≥ 10 seconds, and there was either a desaturation of ≥ 3% baseline or the event was associated with arousal. Hypopnoeas were considered obstructive if snoring was present, there was increased inspiratory flattening of nasal pressure compared to baseline breathing, or there was associated thoracoabdominal paradox that was not present during pre-event breathing. If none of these features was present, the hypopnea was considered a central event.

Dr Atalaia provided PSG reports for each subject using the RemLogic™ standard template. The reports provide extensive data in relation to sleep, but pertinent results for the analysis presented here are: sleep efficiency, latency to specific sleep stages (stage 1, stage 2, slow wave and REM sleep), respiratory events classified as above, total apnoea-hypopnoea index (AHI), and details of sleep architecture (% total sleep time in stage 1, stage 2, slow wave and REM sleep). For mMWTs, a hypnogram report was produced. Latency to first 30 seconds of sleep was used for analysis.

2.7.4 Capillary blood gas measurement

Training in capillary blood gas measurement was provided to MJH by members the nursing team from the Long Term Ventilation Service, Queen Elizabeth University Hospital, Glasgow. The sample was taken on same day as MRI, and

was performed prior to scanning. For the majority of subjects, this was around 10:00, though time of day was variable depending on available scanning slots. Patients were placed in a seated position and asked to relax. Vasodilator cream was applied to the earlobe, and left for approximately 10 minutes. The earlobe was then cleaned with an alcohol swab, before a sterile needle was applied to pierce its lower edge. Free-flowing blood was collected in a glass capillary tube, before being transferred to a portable device positioned next to the patient for analysis. One of two devices was used for all analyses; an ABL 77, or ABL 80 Flex (both by Radiometer Medical®). Both devices were used by the Long Term Ventilation Service for general clinical use, hence were subject to regular calibration and maintenance.

2.8 Venous blood sampling

A single venous blood sample was collected from each DM1-affected participant for genetic analysis using standard aseptic technique. Approximately 8 ml of whole blood was extracted, into a BD Vacutainer® containing ethylenediaminetetraacetic acid (EDTA). The West of Scotland Genetics Service diagnostic laboratory carried out DNA extraction from whole blood. DNA was then forwarded to the Monckton group at the University of Glasgow.

DM1-affected participants were additionally offered the option to provide two further blood samples, to be stored in the Newcastle MRC Centre Biobank for Rare and Neuromuscular Diseases, University of Newcastle. The Biobank aims to facilitate research progress in neuromuscular disorders by making anonymised patient biomaterial available to researchers [228]. Consenting subjects provided two extra samples of around 8 ml; one collected in EDTA, and the other into a Vacutainer® containing clot activator. The clotted sample was then centrifuged at 1500 *g* for 10 minutes. After centrifugation, 200 µl aliquots of serum were transferred to cryotubes. Samples were stored at -80°C within three hours of collection. Batched samples, anonymised by study number, were shipped to the Biobank on dry ice by 24-hour courier.

2.9 Genetic analysis

Genotyping of the CTG trinucleotide repeat in DM1 participants was undertaken by small-pool PCR (SP-PCR) as previously described [25], using the flanking primers DM-C and DM-DR. Four reactions, each using 300 pg blood genomic DNA template, were performed for each patient. Where necessary, PCRs were supplemented with 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich UK) and the annealing temperature was reduced to 63.5°C. When DMSO had been added to the PCRs, the amplicons were first purified using the QIAquick PCR purification kit (Qiagen UK).

PCR products were resolved on a 1% agarose gel, and visualised by Southern blotting. The probe used for Southern blotting was a PCR product with 56 CTGs amplified using DM-C and DM-DR.

CTG repeat lengths were estimated by comparison against DNA fragments of known length in the molecular weight marker, using CLIQS 1D gel analysis software (TotalLab UK Ltd.). The lower boundary of the expanded molecules in SP-PCR was used to estimate the inherited, or "progenitor" allele length (ePAL) [25], which is the major determinant of age at onset of symptoms [26], while the region of greatest band intensity constitutes the modal allele length (MAL) at the time of sampling. Samples were also screened for presence of sequence variations within the CTG trinucleotide repeat ("variant repeats") by exposure to AciI enzyme (New England Biolabs UK Ltd; restriction site 5'-CCGC-3') [34].

Dr Sarah Cumming, University of Glasgow, performed genetic analysis for the study participants, many of whom had previously provided DNA samples for the pre-existing DMGV study. MJH performed a small number of further small-pool PCRs on isolated lymphocyte DNA from selected patients as part of a separate study.

2.10 Additional clinical data

Supplementary clinical measures, not collected as part of the core study protocol, were obtained from electronic medical records relating to the subject's annual appointments at the myotonic dystrophy review clinic (West of

Scotland Clinical Genetics Service). Data were extracted from the appointment most closely contemporaneous to recruitment and MRI scanning (for analysis presented in Chapters 2 to 5), or closest to PSG (for analysis presented in Chapter 7). All of these appointments had been with one of two clinicians - MJH or Dr Bob Ballantyne, the majority Dr Ballantyne.

Data extracted from electronic clinical records were Muscle Impairment Rating Score (MIRS) [229], Epworth sleepiness score, electrocardiogram (ECG) measures, liver function tests (LFTs) and haemoglobin A1c (HbA1c).

2.11 Statistical analysis

Block Design standard score and Stroop test subscores were converted to age-adjusted T-scores using normative data provided in the test manual. D-KEFS Trail Making scores were likewise converted to age-adjusted scaled scores using the test manual. Progenitor allele length (ePAL) was converted to a logarithm with base 10 (logPAL) for statistical analyses in which a normal distribution was desirable.

Statistical analysis was predominantly undertaken using Statistical Package for the Social Sciences (SPSS, Version 24.0; IBM 2015). Cohen's d effect size was calculated using G*Power (version 3.1) [230]. Linear regression analyses, and all analyses pertaining to the joint Glasgow-Iowa cohort were carried out using R statistics software (version 3.3.2; www.r-project.org). To address the issue of multiple comparisons, the Benjamini-Hochberg correction was applied to linear regression analyses presented in chapters 3 to 4, using an on-line tool (Macdonald JH, 2014; http://www.biostathandbook.com/multiplecomparisons.html) with a false discovery rate of 0.05.

3 Recruitment, demographics and self-reported symptoms

3.1 Introduction

This section summarises the process by which suitable subjects were identified within the West of Scotland DM1 population, and describes the baseline clinical, genetic and self-reported symptom characteristics of the cohort recruited.

The experience of recruitment to the present study, which aimed to enrol 40 individuals with adult-onset DM1 from the West of Scotland, is highly relevant to the current landscape of DM1 research. While work continues internationally to identify molecular targets, develop therapeutics and validate outcome measures [194,195,197], the availability of sites with suitable infrastructure to host clinical trials in neuromuscular disease has become a major limiting step in the United Kingdom [231]. At the time of writing, the National Hospital for Neurology and Neurosurgery, Queen Square, London and the John Walton Muscular Dystrophy Research Centre, Newcastle are the major UK hubs for DM1 clinical research. A recent workshop, focussing on Duchenne muscular dystrophy, suggested both are nearing capacity to host clinical trials in muscle disease, and emphasised a need for other UK centres to build the means to deliver such studies [232]. Therefore the present study will provide useful insights into whether it is feasible to recruit a cohort of suitable size for phase I to II drug trials from a single UK regional centre.

This chapter also aims to provide an overview of the genetic characteristics and symptoms reported by the cohort recruited. It could be hypothesised that voluntary recruitment to clinical research will be heavily biased towards the ascertainment of mildly affected individuals, because symptoms including apathy, fatigue and social communication difficulties, as well as physical limitation can hamper DM1 patients' engagement with healthcare [233]. This might include those with small CTG repeat sizes, and those with variant repeat sequences, since these have been linked to milder DM1 symptoms overall [234]. A homogeneous, mildly affected cohort may be undesirable for many clinical studies, and so we sought to determine whether a recruitment strategy that included approaching all suitable patients attending annual clinic review, with

provision of home visits and taxi transport where required, could successfully lead to recruitment of a DM1 cohort with diverse phenotypes.

3.2 Results

3.2.1 Preliminary screening

The process of recruitment of DM1-affected participants is summarised in Figure 10. The records of 224 patients appearing on the West of Scotland myotonic dystrophy database were screened for suitability to participate. Forty-eight were deemed unsuitable based on review of electronic records (Table 4), the majority due to a documented diagnosis of congenital or juvenile onset myotonic dystrophy, or learning disability diagnosed in childhood likely due to DM1. These patients were not contacted. One patient had a high burden of white matter change seen on MRI brain, which had previously been discussed at a neuroradiology multidisciplinary meeting and was felt to be in excess of that which would be expected due to DM1. The patient had been investigated for possible comorbid CADASIL, though no mutation was identified. While this patient's phenotype may have simply represented a severe end of the DM1 neuroradiological spectrum, the decision was made not to offer recruitment given the possibility of a second, unrelated diagnosis. The patient's functional status was also understood to be poor.

Screening identified 17 individuals with permanent pacemakers or implantable cardiac defibrillators. These individuals were not actively approached to participate, since a cardiac device would present an MRI contraindication. Ethical approval did allow up to 10 individuals with MRI contraindications to be included, however, and the final cohort included three individuals with pacemakers. One was invited to participate in error, as electronic records did not make clear she had a pacemaker, and two had independently become aware of the study, and informed a treating clinician of their wish to participate. The clinician in turn forwarded their details to the study team.

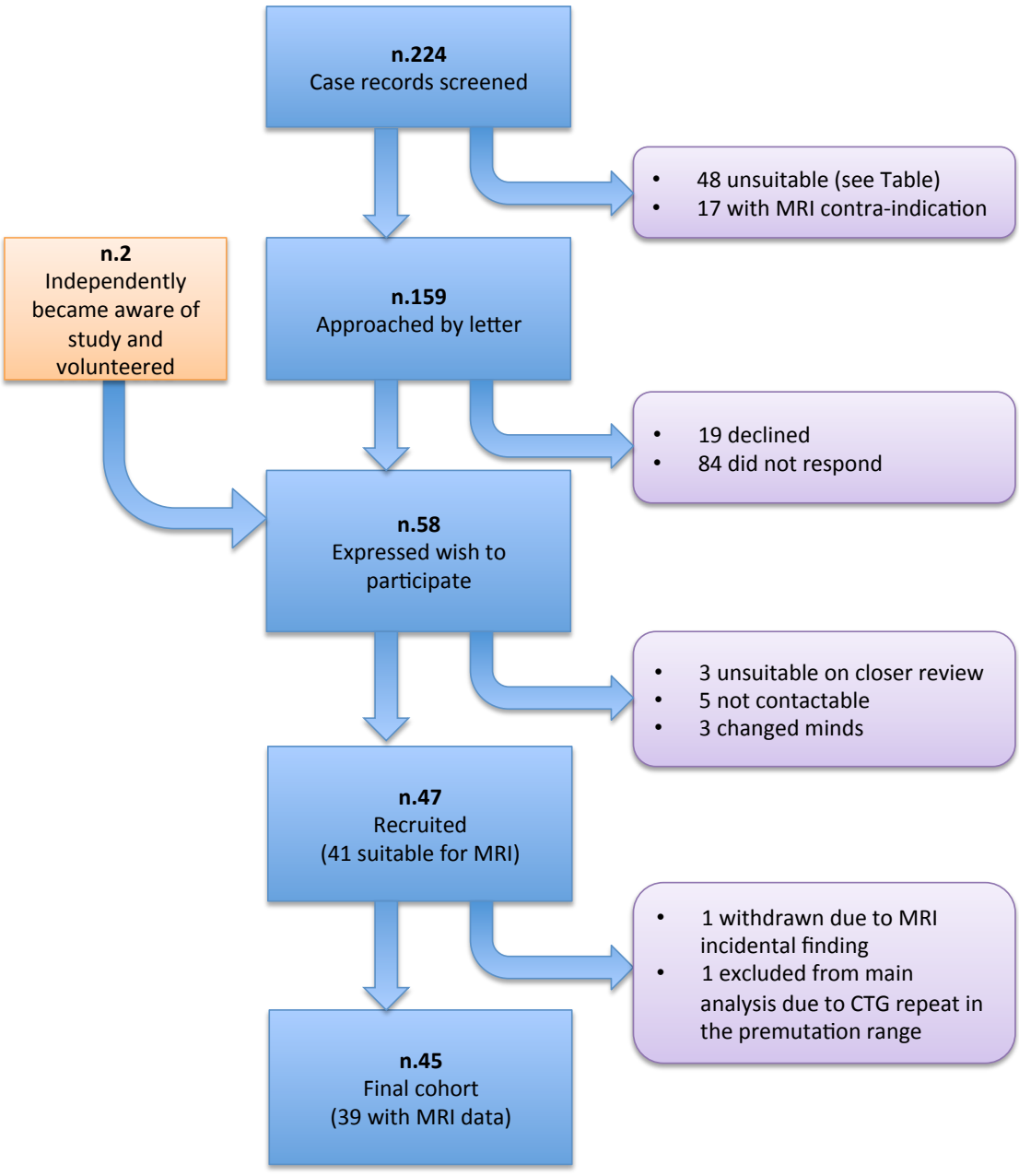

Figure 10: Summary of recruitment process for DM1-affected individuals

Table 4: Reasons for ineligibility of 48 potential participants identified from screening of the DM1 clinical database

Reason for screening fail	n.
Congenital or juvenile onset, or documented learning disability	31
Age > 75 years	1
Age < 18 years	1
History of severe head injury	2
Active cancer	2
Major visual impairment*	2
Deafness*	1
Radiologically confirmed stroke	1
Possible comorbid CADASIL	1
Bedbound	1
Childhood meningitis causing learning disability	1
No engagement with service since diagnosis	1
Active drug dependency	1
Currently pregnant	1
Recently deceased	1
Total	**48**

*Patients with hearing or visual impairment were excluded if these were considered severe enough to impact performance in neuropsychology assessments

Invitation letters were sent to 159 potential participants. Nineteen forms were returned declining participation. Fifty-eight patients expressed willingness to participate, either by returning the expression of interest form, or by verbally stating their wish to take part during a clinic visit. Of these, three were unsuitable on closer screening (one with severely reduced visual acuity, one with frequent use of illicit drugs, and one with childhood onset DM1), five did not respond to subsequent attempts to make contact, and three changed their minds after more detailed discussion of the protocol. This left 47 DM1-affected subjects suitable for recruitment.

3.2.2 The study cohort

3.2.2.1 Baseline demographics

Forty-seven DM1-affected individuals completed the recruitment process. One, a 33 year old female (DMN-057A), was subsequently withdrawn due to an incidental finding of a left frontal lesion on MRI brain, with a differential diagnosis of a glial neoplasm or developmental abnormality. She was referred for neurosurgical opinion, and on most recent review after 10 months without intervention, imaging appearances of the lesion were stable.

A second subject (DMN-005A) was withdrawn from the main portion of analysis, after her diagnosis of DM1 was revised based on genetic data produced by the study. This 52-year-old female had been tested for DM1 seventeen years previously, following the diagnosis of her sister who had given birth to a congenitally affected child. At that time, the subject complained of muscle aches and fatigue. Based on a molecular diagnostic report stating heterozygosity for an expanded allele greater than 30 repeats, she was informed that she had "mild myotonic dystrophy". Analysis of her sample by SP-PCR as part of the present study estimated ePAL to be 47 repeats, with a MAL of 51 repeats. Data from subsequent MiSeq analysis (which would be considered more accurate for smaller alleles) showed a stable expansion of around 43 repeats. Re-evaluation of the patient's sample by the NHS diagnostic service by triplet-primed PCR also estimated the expanded allele to be 43 repeats. On clinical review, the subject was found to have no DM1-specific signs. In light of her molecular and clinical findings, this participant's diagnosis was revised to one of a premutation carrier, not a DM1-affected individual. The events leading to the initial diagnosis were highlighted for further investigation through NHS standard procedure for reporting adverse incidents (Datix® system). This subject had already completed the study protocol including MRI imaging, neuropsychology assessment, PSG and mMWT before the anomalous genetic result was identified.

Six of the remaining recruits completed the protocol excluding MRI due to contraindications (three with permanent pacemaker, two claustrophobia and one high body mass index). Therefore forty-five DM1-affected participants, 39 with MRI data, were included in the final analysis.

Twenty control participants were also recruited. Twelve volunteers were obtained from patients' families, and eight from SHARE. A summary of the participants' baseline characteristics is provided in Table 5. DM1-affected and control cohorts were well matched for age (p = 0.783) and educational history (p = 0.882), while controls were more likely to have ever been smokers (p = 0.016).

Table 5: Characteristics of DM1-affected and control cohorts

	DM1-affected	Control	p
Number	45	20	-
Female: number (%)	26 (58%)	8 (40%)	0.282¶
Age: mean (SD)	46.87 (12.37)	46.06 (13.14)	0.754*
Years of education: mean (SD)	14.38 (2.83)	14.48 (3.19)	0.971*
Smoking status Never : Former : Current (ratio)	27 : 7 : 11	5 : 14 : 1	<0.001¶
Muscle impairment rating scale (MIRS) 1:2:3:4:5 (ratio)	3:7:10:23:2	-	-
ePAL: mean number of CTG repeats (SD)	235 (121)	-	-
MAL: mean number of CTG repeats (SD)	479 (253)	-	-

* Independent samples t-test; ¶Chi-square test

Four DM1-affected participants were currently prescribed modafinil, and none were using mexiletine. Eight (18%) were taking medication for vascular risk factors; five had treated hypertension only, two hypercholesterolaemia and one had both. In the control group, five (25%) had treated vascular risk factors (one hypertension only, two hypercholesterolemia and two both). None of the study participants had diabetes mellitus.

3.2.2.2 Genetic characteristics

The DM1-affected cohort was comparatively heterogeneous with respect to ePAL and MAL. The ePAL ranged from 56 to 572 repeats (mean 235, SD 121), and MAL from 65 to 998 repeats (mean 477, SD 253). A significant bias was observed relating to repeat size and age, with older participants significantly more likely to have a smaller CTG repeat (Figure 11, p = 0.049, Adj R^2 = 0.066).

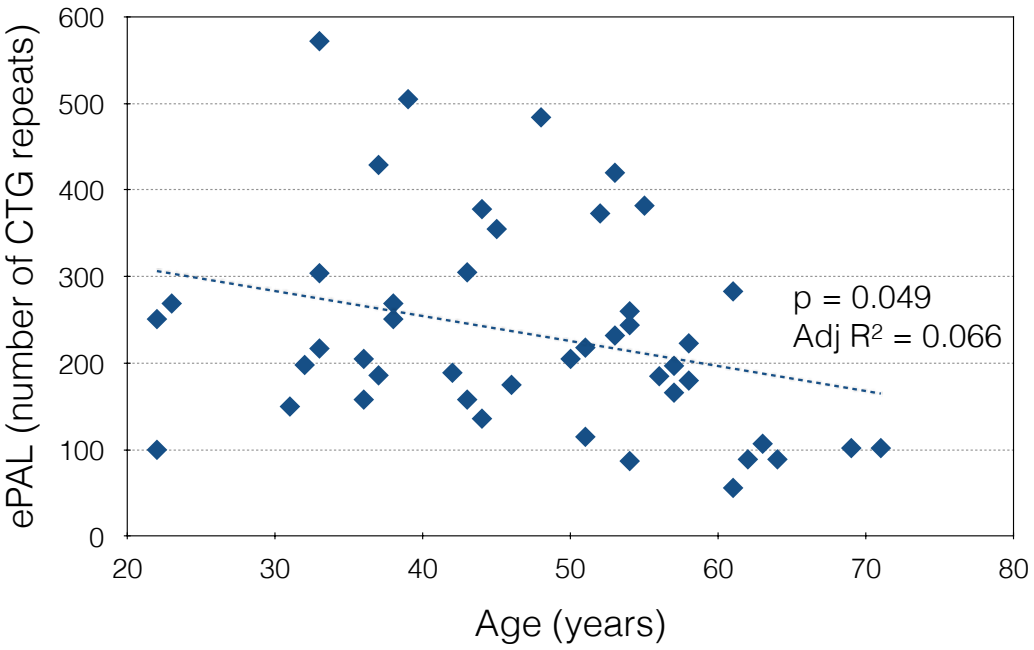

Figure 11: Estimated progenitor allele length (ePAL) plotted against age in the DM1-affected cohort.
Data show a significant trend towards shorter repeats in older study participants.

Three individuals tested positive for the presence of variant trinucleotide repeats by AciI digest (Figure 12). These were DMN-006A, a 36-year-old male; DMN-052A, a 22-year-old female; and DMN-059A, a 33-year-old male. All three individuals were from the same extended family, in which the structure of the variant repeat had previously been characterised by PacBio sequencing through work undertaken by Dr Sarah Cumming (Figure 13).

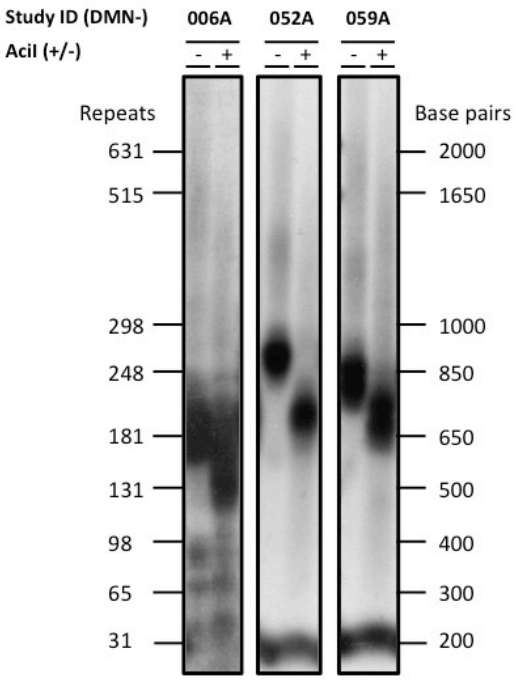

Figure 12: Southern blots demonstrating digestion of PCR products from subjects DMN-006A, 052A and 059A by Acil enzyme.
Southern blots produced by Dr Khalidah Nasser (DMN-006A) and Dr Sarah Cumming (DMN-052A and 059A).

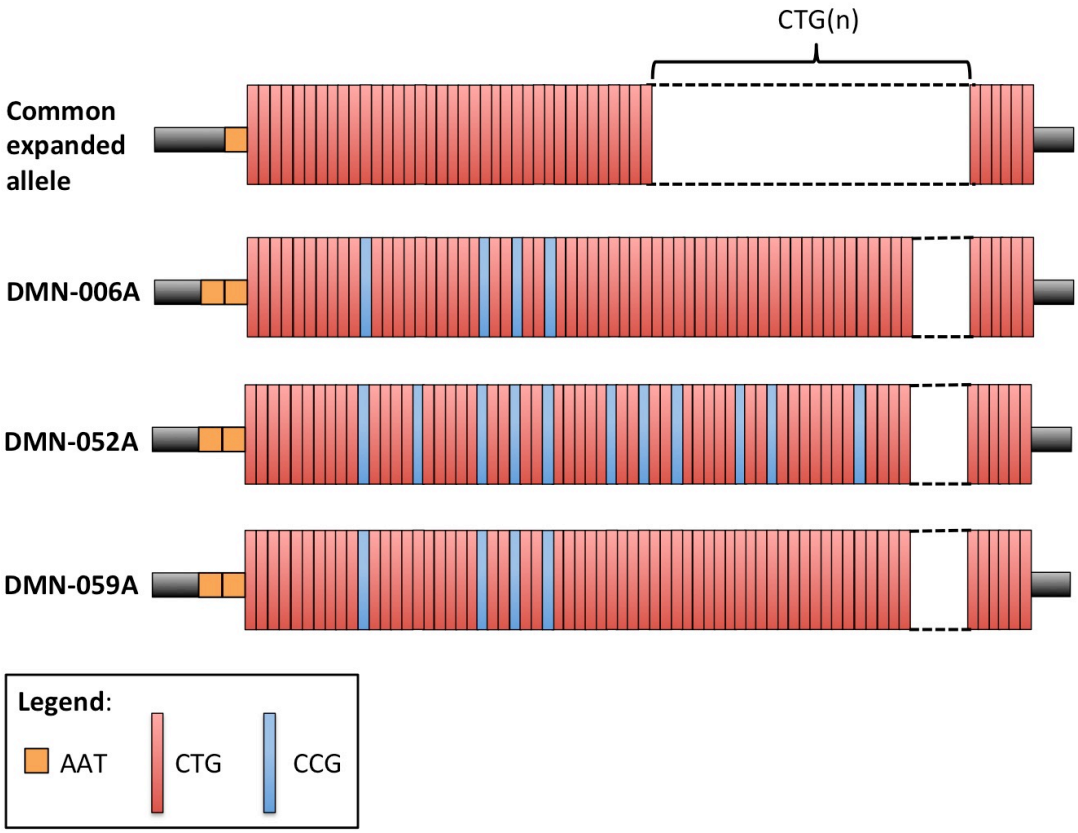

Figure 13: Consensus expanded repeat allele structures of three individuals who tested positive for the presence of variant repeats by Acil digest, determined by PacBio sequencing.
Based on analysis by Dr Sarah Cumming.

3.2.3 Summary of self-reported symptoms

Compared with controls, DM1-affected participants reported greater fatigue, low mood and pain on FDSS, BDI II and McGill visual analogue scales respectively (Table 6). There was a trend towards greater everyday executive dysfunction as measured by the self-DEX questionnaire, and greater pain reported by SF-36, though these differences did not reach statistical significance (p = 0.102, 0.061 respectively).

Table 6: Self-reported symptom scores in DM1-affected patients versus controls

	Affected N	Control N	DM1-affected participants: Mean (SD)	Control participants: Mean (SD)	Effect size (Cohen's D)	p	
Self-DEX	45	20	17.89 (11.60)	12.70 (8.05)	0.520	0.102	↑
BDI II	45	20	11.22 (8.76)	5.05 (5.09)	0.858	0.001	↑
FDSS centile Score	45	20	37.47 (15.59)	17.00 (9.03)	1.607	<0.001	↑
SF-36 Pain Items	45	20	4.76 (2.48)	3.45 (1.61)	0.627	0.061	↑
McGill Pain Scale	45	20	22.18 (24.03)	9.20 (13.73)	0.663	0.046	↑
DM1-ActivC©	46	-	69.78 (20.00)	-	-	-	↓
MDHI total	46	-	27.78 (21.66)	-	-	-	↑

Data were not normally distributed for any of the outcome measures included (defined as $p < 0.05$ in Shapiro Wilk test of normality), hence a non-parametric Mann Whitney U test was applied for comparison of means. BDI II = Beck Depression Inventory II; FDSS = Fatigue and Daytime Sleepiness Scale; MDHI = Myotonic dystrophy health index. Direction of the arrows indicates the trend that is associated with more severe symptoms.

Fatigue, low mood and pain symptoms in DM1-affected participants frequently reached a threshold that could be considered clinically significant. Twenty-seven (60%) had an FDSS score greater than two SDs above the mean score of controls. Thirteen (29%) had a BDI II score greater than 13, the threshold for mild depression suggested for clinical use by the author's manual [235]. Seventeen (38%) rated bodily pain as "moderate" or greater on SF-36. Patients frequently experienced two or more of these symptoms together (Figure 14).

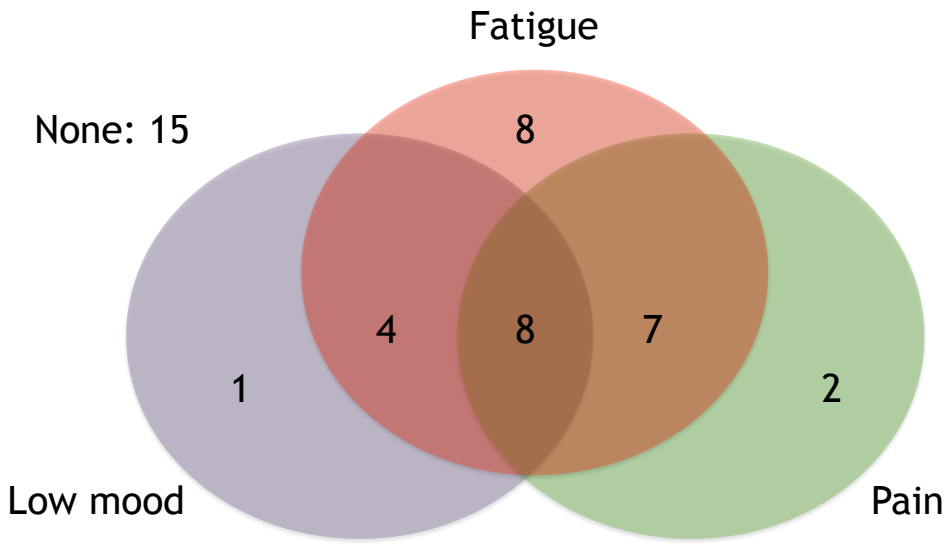

Figure 14: Venn diagram demonstrating the number of DM1-affected individuals reporting clinically significant low mood, fatigue and pain.

3.2.4 Relationships between self-reported outcome measures

Relationships between self-reported measures of specific symptoms were explored by linear regression analysis. A correction for multiple testing was applied using a Benjamini Hochberg calculation via an on-line tool, (Macdonald JH, 2014; http://www.biostathandbook.com/multiplecomparisons.html), with a false discovery rate 0.05.

Following correction for multiple comparisons, there remained significant co-linear relationships among symptoms of fatigue, pain and low mood. For example, BDI II score correlated with FDSS score ($p < 0.001$; Adj $R^2 = 0.394$; Figure 15A) and with McGill pain scale ($p < 0.001$; Adj $R^2 = 0.255$). In turn FDSS also correlated positively with McGill pain scale ($p < 0.001$, Adj $R^2 = 0.321$).

A very strong relationship was observed between mood, measured by BDI II, and self-reported CNS symptoms in general. This included cognitive problems (MHDI cognitive impairment subscale; $p < 0.001$, Adj $R^2 = 0.626$), executive difficulties (self-DEX; $p < 0.001$, Adj$R^2 = 0.480$) and impairment in social functioning (MDHI social performance subscale; $p < 0.001$, Adj $R^2 = 0.441$) (Figure 15B to D).

Subjects' rating of their own everyday executive dysfunction (self-DEX) correlated significantly, but weakly with the same scale completed by a proxy (informant-DEX; p = 0.006, Adj R^2 = 0.147).

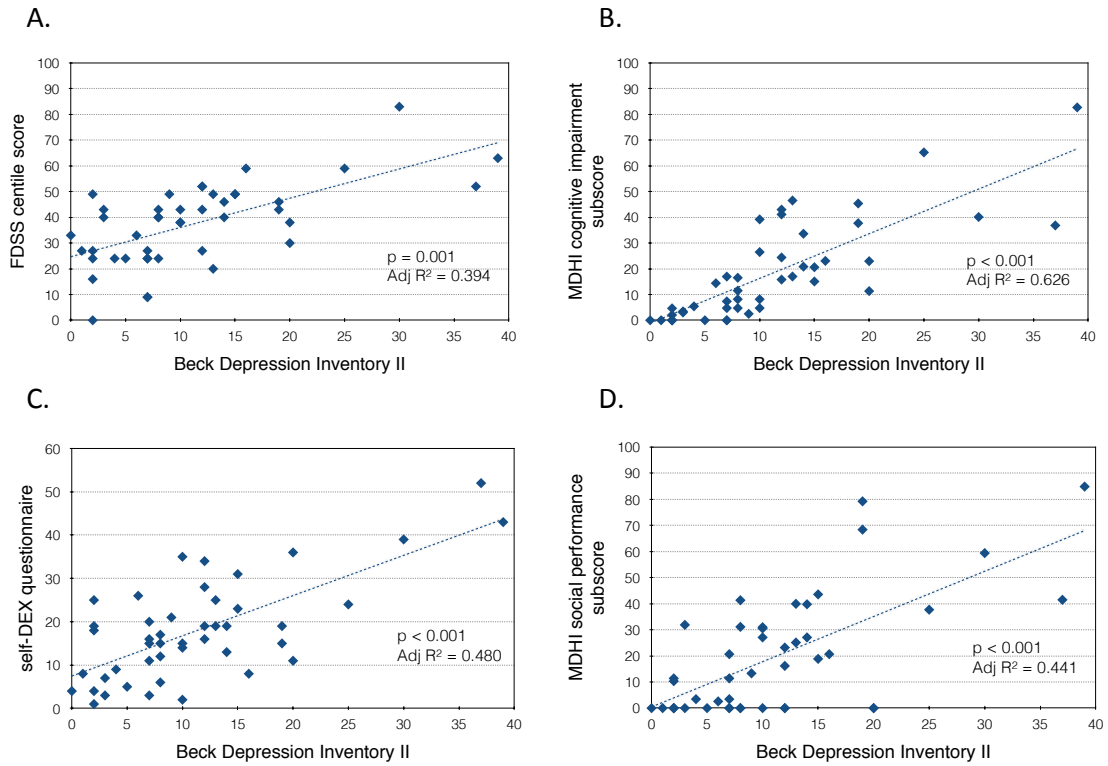

Figure 15: Scatterplots demonstrating highly significant relationship between Beck Depression Inventory II score and self-reported (A) fatigue, (B) cognitive impairment, (C) executive difficulties and (D) impaired social performance

Comparison of MDHI subscores with other self-rating scales for similar themes showed good correlations. Mobility (p < 0.001; Adj R^2 = 0.746), upper extremity function (p < 0.001; Adj R2 = 0.531) and ability to do activities (p < 0.001; Adj R^2 = 0.684) subscales all correlated inversely with DM1ActivC© score as expected. BDI II score correlated with the emotional issues subscale (p < 0.001; Adj R^2 = 0.611), FDSS centile score correlated with the fatigue subscale (p < 0.001; Adj R^2 = 0.504), and both SF-36 pain items and McGill pain scale correlated well with MDHI pain subscale scores (p < 0.001; Adj R^2 = 0.524 and Adj R^2 = 0.582 respectively).

3.2.5 Genotype and self-reported symptoms

In univariate analysis, logPAL did not correlate with performance in any of the neuropsychology assessments, or with any self-reported symptoms. Since logPAL represents an estimation of CTG repeat size at conception, its influence on phenotype would be expected to be age-dependent. Hence we also explored correlations with logPAL in a multivariate model [age + logPAL + (age*logPAL)], demonstrating a significant correlation with the MDHI mobility subscale ($p = 0.008$, Adj $R^2 = 0.194$) only.

Increasing MAL significantly correlated with greater physical impairment measured by MDHI mobility subscale ($p = 0.001$, Adj $R^2 = 0.211$) and DM1-ActivC© score ($p = 0.006$, Adj $R^2 = 0.143$).

The three individuals identified by as carrying variant trinucleotide repeats - a 22-year-old female, a 33-year-old male and a 36-year-old male - all reported minimal physical impairment due to their DM1 symptoms. Their centile scores on DM1ActivC© were 100, 88 and 93, despite an ePAL of 251, 217 and 158 repeats respectively. It is interesting to note that two of the three also reported minimal to absent symptoms on the MDHI (total scores 0.27 and 1.47; mean score of cohort 27.77). The third had a total score on MDHI 0f 52.78, which is considerably greater than the cohort mean. The same individual also scored their depression symptoms as exceptionally high (BDI II = 37). Since the questions on MDHI are somewhat emotive, compared with the more factual DM1-ActivC© ("How much do the following symptoms impact your life?", compared with "Can you run; yes, no, or with difficulty?"), this further supports a relationship between low mood and increased self-reporting of symptoms. Of note, muscle signs in the same individual objectively assessed by a Specialty Doctor in clinical genetics (BB) were rated as MIRS 2, consistent with minimal signs of DM1.

3.3 Discussion

3.3.1 Recruitment

Forty-five individuals with adult-onset DM1 were successfully recruited from the West of Scotland population, who were able and willing to undertake a comparatively demanding study protocol. This represents a yield of just over 20% from the pool of 224 unselected individuals with DM1 who were initially screened. Exclusion of those with childhood onset forms and MRI contraindications narrowed the available pool considerably. It is widely acknowledged that fatigue, apathy and physical impairment in DM1 are commonly associated with both reduced social participation and impaired engagement with medical care [233]. It had been anticipated that the same factors might hamper recruitment to the study, and so meeting our target for recruitment is, in itself, a significant finding.

Several local factors served to support effective recruitment to the present study. The existence of the DM1 specialist clinic meant that most families had an established, positive relationship with clinicians involved prior to being invited to participate. Furthermore, previous recruitment to the DMGV study, and various related public outreach efforts by Professor Monckton and colleagues, meant that many families were also aware of the rationale of the ongoing genetic research at the University of Glasgow. The provision of taxi transport free of charge, and the offer of home visits also supported the inclusion of patients with significant disability or socio-economic disadvantage. Despite these factors, successful recruitment to the present study also likely reflects a broader willingness on the part of the DM1 patient community to participate in clinical research. With a view to future clinical trials, our success in meeting the recruitment target provides evidence that sample sizes suitable for phase I to II trials can potentially be achieved from single regional services in the UK, and suggests clinical researchers should not be unduly pessimistic regarding uptake among DM1-affected cohorts.

3.3.2 Baseline characteristics

The cohort was highly heterogeneous with regard to age, severity of symptoms and CTG mutation profile. Most individuals had established muscle symptoms of myotonic dystrophy, as evidenced by a modal MIRS score of 4 (indicating mild to moderate proximal muscle involvement along with distal weakness), although the full range of MIRS scores were represented. CTG repeat length was also highly variable. The absence of modal allele sizes above 919 repeats is consistent with the exclusion of childhood onset forms of DM1.

A bias was observed with regard to age and repeat length, which was not entirely unexpected. Since the diagnosis of myotonic dystrophy in a family is frequently ascertained by the birth of a severely affected child, the archetypal family structure includes a parent with classical onset, and a grandparent with a late onset phenotype, and hence small repeat expansion. In addition, reduced life expectancy associated with larger repeats may also account for their absence among older participants [92]. Since many features of DM1 overlap those of normal aging, the relative contributions of repeat size and normal aging to CNS phenotypes may therefore be quite disparate at the extremes of age in this cohort. It is therefore important to consider this bias as a potential confounder in statistical analysis of the study data going forward.

3.3.3 Self-reported outcome measures

The patient group was substantially burdened by symptoms of low mood, fatigue and pain. The strong mutual correlations that were observed between somatic symptoms and mood support imply a close interaction between mental wellbeing and physical symptoms in DM1. This model is in keeping with the rationale for the recent OPTIMISTIC trial of a cognitive behavioural therapy-based intervention as a treatment for experienced fatigue [187]. Patients with lower mood reported more symptoms in general, particularly relating to cognition and social performance. From the data presented at this stage, it is not clear whether those patients who perceive their cognition or social functioning to be impacted are also those whose CNS involvement would be considered more severe by objective measures. The lack of a strong correlation between self-DEX

and informant-DEX, however, hints at a possible impairment of insight with respect to central symptoms, which has been previously reported [202].

It is desirable to minimise the burden of participation in clinical research for individuals with DM1, especially because study visits for future clinical trials are likely to include a range of multi-system assessments including effort-intensive measures of muscle strength. It is therefore useful to minimise any redundant or duplicate outcome measures within a study protocol. Consistent with previous data [199], we noted that individual domain subscores within the MDHI correlated well with other measures of similar themes. This supports the MHDI as a good stand-alone measure for the quantitation of self-reported symptoms in DM1 studies. This is with the caveat that, from the data presented to this point, it is not clear whether MDHI scores are consistent with objective measures of disease severity. Further, correlations of subscores relating to central symptoms with BDI II score, however, suggest that, responses to MDHI may be influenced by the subject's mood. This observation urges caution against the use of self-reported scales alone for the measurement of CNS symptoms in DM1; an issue which will be explored in greater detail in subsequent chapters.

3.3.4 Genotype-phenotype correlations

With the exception of the MDHI mobility subscale, we did not detect significant correlations between CTG repeat length and self-reported symptoms after correction for multiple comparisons. A small cohort size, and selection for adult onset DM1 only (excluding severe phenotypes and thus large repeat sizes) may have contributed to the absence of detectable correlations. Furthermore, self-reported symptom questionnaires are somewhat subjective, particularly in relation to central symptoms such as fatigue and cognitive difficulties, and so may not closely reflect the severity of the primary disease process.

It is however noteworthy that the three individuals identified with variant repeats reported particularly mild muscle impairment in DM1-ActivC©. This adds to growing evidence that individuals with DM1 due to variant repeats may be statistical outliers in terms of disease severity [234], and thus reinforces a role for robust genotyping, including screening for variant repeats, in future DM1 clinical studies, particularly drug trials.

3.3.5 Summary

In summary, a cohort of 45 individuals with adult-onset DM1 and 20 suitable control participants were successfully recruited and completed baseline symptom questionnaires. Self-reported problems with fatigue, cognitive difficulties and impaired social performance were frequently reported, and positively correlated with measures of low mood. A priority for further investigation is therefore to further characterise CNS involvement in the same cohort, using neuropsychology assessment and imaging. This will enable exploration of whether symptom severity scores reported by individual subjects are consistent with the severity of CNS disease measured by objective means.

4 Neuropsychology assessment

4.1 Summary

This chapter summarises the selection, administration and results of neuropsychology assessments applied to the study cohort. Cognitive outcome measures recommended by international expert consensus were applied to 45 individuals with adult-onset DM1, and 20 age-matched controls. The test protocol was well tolerated, and demonstrated impairment with moderate to large effect sizes across a range of cognitive domains in the DM1-affected cohort compared with controls. Hypothesising that primary muscle involvement, resulting in dysarthria or upper limb weakness, may disadvantage DM1-affected subjects in some cognitive tests, an adjustment was applied to test scores where possible to eliminate the effect of any basic speed limitation. In a key test of cognitive flexibility, which had initially showed a large effect size in DM1 subjects compared with controls, the difference was no longer significant after adjustment for basic motor speed. Possible interference by primary muscle weakness undermines specificity of some cognitive assessments for brain involvement, and hence their appropriateness for use as CNS outcome measures for clinical trials. Most cognitive measures did not show strong correlations with CTG repeat length, possibly reflecting the high level of background variation in cognitive performance in the general population.

4.2 Introduction

Selection of objective measurements of cognition for use in DM1 research studies is challenging. The cognitive phenotype in adult-onset DM1 is one of relatively mild impairment [236], hence a test battery would need to be highly sensitive to impairment in the relevant cognitive domains in order to detect meaningful changes. Further, traits including apathy, fatigability, and rigidity of personality that can form part of the DM1 clinical phenotype [233] mean that an excessively complex or burdensome protocol is likely to be met with high rates of disengagement and dropout.

The identification of valid measures of cognition has achieved greater urgency with the advent of clinical trials of potential disease modifying therapies [197]. Both the Outcome Measures in Myotonic Dystrophy (OMMYD) working group [194,195], and DM CNS taskforce [196], have highlighted the need for a validated, consensus approach to the measurement of cognition as a major priority for clinical trial readiness. To identify and endorse potential outcome measures as suitable for use in clinical trials, the OMMYD group apply the OMERACT (Outcome Measures in Rheumatology) filter, a process first devised for selection of clinical trial outcome measures for rheumatologic disorders [237]. The OMERACT filter requires that a measure is truthful (*i.e.* measures the aspect of disease that is intended), discriminates between situations of interest (for example, allows classification of disease states, or is sensitive to change over time), and is feasible within the constraints of the intended study.

Regarding outcome measures for cognition in DM1, OMMYD has recommended four neuropsychology assessments, based on evidence from previous case control studies. These are: the Stroop test [112,115,238-240], Trail Making tests A and B [112,115,238,241], the Block Design subtest from Weschler Adult Intelligence Scale-Revised (WAIS-R) [112,238,240,241], and the FAS controlled oral word association [112,238,241]. Stroop and Trail Making tests are commonly used in clinical and research contexts, broadly as measures of executive function [242]. The Block Design subtest is considered an assessment of visuospatial cognition [243], while the FAS controlled oral word association primarily demands verbal fluency [244]. These case control studies demonstrate that individuals with DM1 are consistently able to complete these assessments in a clinical study setting, and that the tests reproducibly detect impairment in the patient group compared with controls.

While existing data support the sensitivity of these tools to impairment in DM1, and their feasibility in the context of clinical research, no study to our knowledge has explored their specificity for brain involvement in DM1 (their 'truthfulness' with respect to the OMERACT filter). We hypothesised that performance in complex neuropsychology assessments rewarding rapid completion of a manual task, such as the Trail Making test and Block Design tests, or rewarding rapid speech, such as the Stroop test, may be compromised

by a more basic speed limitation that could in turn be influenced by peripheral muscle weakness in DM1. Further, additional central symptoms not directly related to cognition, such as fatigue and depression, also represent potential confounders to performance in cognitive tests.

The specificity of the test battery for central nervous system deficits, and in particular whether performance is influenced by peripheral muscle impairment, is an important consideration for future clinical trials. If performance in a particular neuropsychology assessment is limited, for example, by the speed of fine motor movements, then a drug that selectively treats muscle weakness or myotonia could erroneously give the impression of having improved cognition in the domain measured by the test. This is directly relevant to the evaluation of *DMPK*-targeting antisense oligonucleotides, since these drugs are thought to poorly penetrate the blood-brain barrier if administered peripherally [185].

To explore these issues, we applied the neuropsychology tests recommended by OMMYD to the study cohort, along with an additional short cognitive test battery designed to avoid interference by peripheral muscle weakness. A correction step was applied to the Stroop, Trail Making and Block Design tests to quantify the relative contributions of basic speed limitation and higher cognitive deficits to performance in these tests. Cognitive performance was compared against self-reported symptom measures relating to mood, fatigue and motor impairment, and correlations with CTG repeat lengths were explored.

4.3 Methods

4.3.1 Stroop Test

The Stroop test is widely used in clinical and research contexts as a measure of executive function [242]. The colour-word task is the key executive component of the Stroop, requiring the subject to suppress a habitual impulse (to say the written word), while performing an unfamiliar task (saying the colour of ink). It is however recognised that additional cognitive factors, including attention and basic processing speed, also contribute to performance in all elements of the Stroop test [245].

Our protocol used a commercial version of the test (Golden and Freshwater, Stoelting Co; 2002), comprising three A4-size stimulus cards printed on white paper. The word card consisted of 5 x 20 columns of colour names, written in black ink. The colour card was a similar 5 x 20 matrix of semantically meaningless symbols (X) in coloured ink. The colour-word card comprised the words from the first card written in colours from the second, in which the words and colours did not match. An illustrative example, modified from the source stimulus cards to observe copyright, is provided in Figure 16.

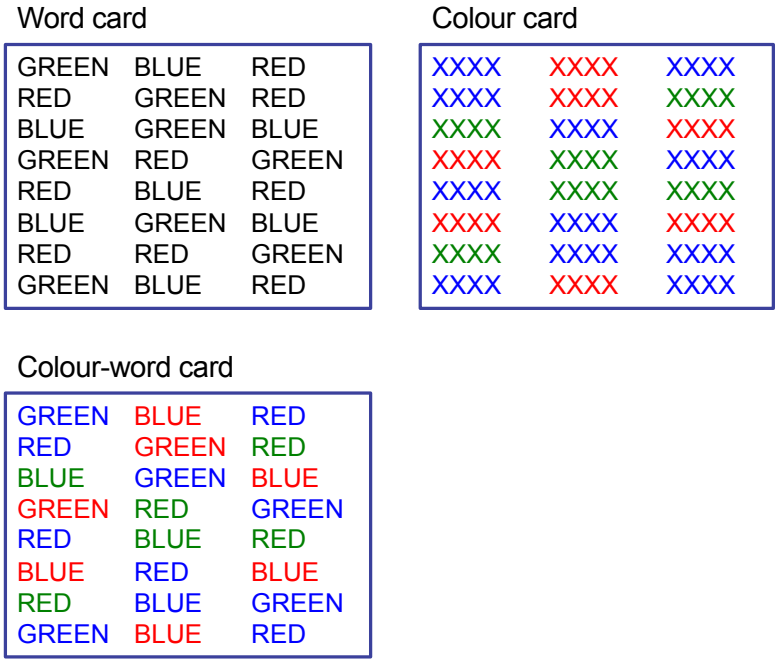

Figure 16: Examples of word, colour and colour-word cards from a Stroop test

In the word and colour tasks, the participant was asked to read aloud as many words or colours respectively as possible, within a 45 second time limit. For the colour-word card, the participant was asked to read aloud the colour of the ink, not the word that was written, and again complete as many as possible within 45 seconds. The subject was prompted to self-correct if any errors were made.

To adjust for basic reading speed, a predicted score for the colour-word task was calculated from the raw word and colour scores, using the nomogram in the test manual [246]. The difference between the predicted and actual performance in colour-word constituted the Interference score.

Individual task scores (word, colour, colour-word) and the interference score were converted to a T-score for analysis, using normative data and tables from the test manual [246]. A T-score allocates 50 points for the age- and education-predicted score, with 10 points equivalent to one standard deviation. For example, a 35 year old male with 12 years' education would be expected to read 100 words in 45 seconds on the word card. If the subject scored 85 words in this test, the difference between actual and predicted scores would be calculated as 85 - 100 = -15. This is equivalent to a T-score of 39 (just greater than one standard deviation below the predicted score).

4.3.2 D-KEFS™ Trail Making Tests

Trail Making tests are likewise commonly used in neuropsychology assessment. The typical version of the test involves two conditions. In Trail Making Test A, the participant is asked to connect circled numbers in numerical sequence (1,2,3, *etc*.) as quickly as possible. In Trail Making Test B, the subject connects circled letters and numbers in an alternating sequence (1, A, 2, B, 3, C, *etc*.). Several cognitive processes are thought to contribute to Trail Making test performance, including processing speed, sequencing, mental flexibility and visual-motor skills. Broadly, Test A is believed to rely more on visual scanning and motor speed, while Test B reflects higher functions such as cognitive flexibility [247], although some data do not support a strong distinction between the two tasks [248].

Our protocol used the D-KEFS™ version of the Trail Making tests [249], which seeks to more accurately quantify the constituent cognitive processes involved in Trail Making tests, by applying five separate conditions. In each, the subject is presented with a sheet of paper, approximately A3 size, printed with letters and numbers within circles. The subject is asked to complete each task, using a pen, as quickly as possible. In the first task, ('visual scanning'), the subject should make a mark through every number '3' they can see. The second, 'number sequencing', is analogous to Trail Making Test A, with subject asked to draw a line connecting numbers only in numerical order. Similarly, the third ('letter sequencing') involves connecting letters only in alphabetical order. The fourth trail, analogous to Trail Making Test B (number-letter sequencing), is the most cognitively demanding, in which the participant is asked to connect numbers and

letters in alternating order, without missing any (1, A, 2, B, 3, C, *etc.*). An example of a number-letter sequencing task is provided in Figure 17, although the D-KEFS™ version is larger, requiring 31 correct connections.

To allow correction for basic motor speed the D-KEFS™ Trail Making test also includes a fifth task, in which the subject completes the same number of connections as in the number-letter sequencing task, but instead follows a pre-determined trail, indicated by a heavy dotted line. This allows a 'motor contrast' score to be calculated, by subtracting the time taken to complete the motor task from the number-letter sequencing score.

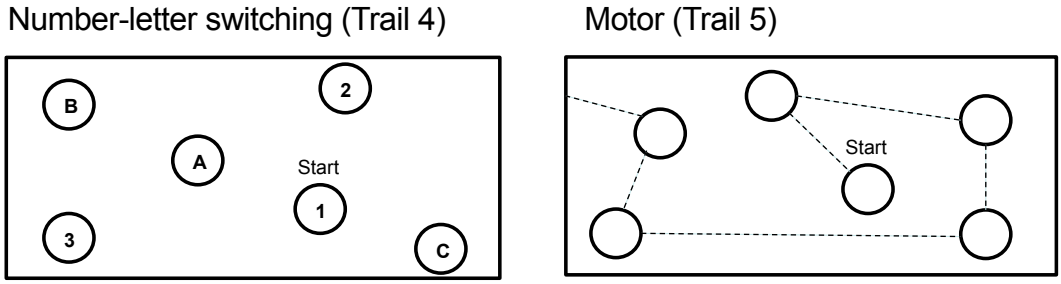

Figure 17: Examples of number-letter switching and motor Trail Making tasks.
These illustrative examples are smaller than the trails included in D-KEFS™, which include 31 separate connections each.

Trail Making test scores were converted to age-adjusted scaled scores for analysis, using normative data and tables provided in the test manual. A scaled score of 10 is consistent with the mean predicted score for the subject's age, with 3 points equivalent to one standard deviation.

4.3.3 Block Design subtest from WASI-II

The Block Design subtest is a measure of visuospatial cognition. Performance has been shown to correlate with measures of everyday spatial ability, such as map-reading, and tendency to participate in activities requiring visuospatial skills, such as building items around the home [243].

The Block Design subtest from the WASI-II test battery was used due to local availability and pricing. The test was applied according to the manufacturer's

manual [250]. Participants were provided with at first four, and then nine patterned blocks (Figure 18) and asked to arrange the blocks to match a series of ‘designs’, provided on stimulus cards. There was a time limit to complete each stimulus (45 to 60 seconds for smaller designs, 120 seconds for larger designs), and the points awarded were stratified, to reward completion in a faster time. If the subject failed to complete a design within the time limit, no points were awarded. If a subject was unable to complete two designs in a row, the test was terminated.

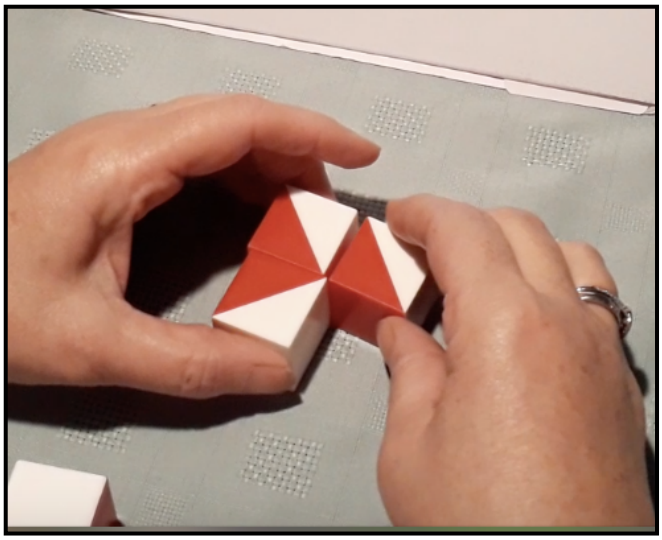

Figure 18: The Block Design subtest involves manual manipulation of small plastic blocks in a time-dependent manner, which we hypothesised may disadvantage DM1 patients with distal weakness

The time-weighted scores were converted to an age-adjusted T-score using normative data from the manual. These scores were recorded as the ‘standard score’. The raw number of designs correctly completed before termination of the test, with no weighting for speed, was also recorded as the ‘non-adjusted score’.

4.3.4 Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

The ECAS is a paper-based cognitive assessment, originally designed for use in people with amyotrophic lateral sclerosis (ALS). The assessment can be applied in around 20 minutes, and includes 16 short tasks, including spelling, a reverse digit span, sentence completion/response inhibition and recall of details from a

short story. This provides subscores in domains of language, verbal fluency, executive, memory and visuospatial functioning [207]. The ECAS has good concurrent validity with conventional cognitive screens such as the Montreal Cognitive Assessment Tool (MoCA) and Frontal Assessment Battery, but crucially, includes no tasks that require manual dexterity [251].

Subjects' answers were recorded on a combined stimulus and scoring sheet. The raw final scores from each subdomain, as well as total ECAS score, were used for analysis.

4.3.5 FAS controlled oral word association

FAS controlled oral word association is a test of verbal fluency. Completion requires cognitive flexibility, and the ability to comprehend and express language, produce internal speech and inhibit inappropriate responses [244]. In this test, the subject is asked to say as many words as they can think of beginning with a specific letter, excluding proper nouns ("the names of people or places") and numbers. The number of appropriate responses within one minute is recorded.

The ECAS test includes a controlled word association test for the letter 'S'. Two additional conditions were therefore applied for each subject, using the letter 'F', then 'A'. The sum total number of appropriate responses from the three conditions was used for analysis.

4.3.6 Statistical analysis

Comparison of means and linear regression analyses were undertaken as outlined in Chapter 2. Correction for multiple testing was addressed by Benjamini-Hochberg correction, which was applied as a single test to data from all linear regression analyses presented in Chapters 3 to 4. A false discovery rate of 0.05 was used.

Cohen's D effect sizes were calculated for each comparison of means test, using G*Power (version 3.1) [230]. Cohen's D represents the difference between the mean score of each group, divided by the average of their standard deviations.

An effect size of D ~ 0.2 is considered small, ~0.5 a moderate effect, and greater than 0.8 a large effect [252].

4.4 Results

4.4.1 Completion of assessments

Complete Stroop test data were not obtained for four DM1-affected participants. One because the tool was not available, one male could not complete the colour tasks due to red-green colour blindness, and a third became frustrated and disengaged during the colour-word task. Data from a fourth was excluded as she was reported to have a diagnosis of visual stress (Meares-Irlen syndrome). This participant had above-average scores in most cognitive domains, but in the Stroop test her basic reading speed was exceptionally slow, such that it was not possible to assign a T-score based on normative data from the Stroop test manual.

For two DM1-affected participants, it was not possible to complete Trail Making and Block Design tests during the home visit due to lack of a suitable flat surface. These tests were completed in a clinic room when the patient attended for MRI a short number of weeks later. Otherwise, all DM1-affected and control participants tolerated the full neuropsychology test battery on the same day as completing the self-reported outcome measures.

4.4.2 Comparison between DM1 affected and control subjects

Comparison of neuropsychology scores from DM1-affected participants with control participants are summarised in Table 7. The DM1-affected group had lower scores on average in all elements of the Stroop, D-KEFS™ Trail Making, Block Design and FAS oral word association tests. The mean total score for ECAS was also lower in the DM1-affected group ($p = 0.004$), though subscores for verbal fluency and memory only approached statistical significance ($p = 0.112$, 0.085). Visuospatial and language subscores of ECAS showed a significant ceiling effect in DM1-affected participants, with 24 (53%) and 15 (33%) respectively gaining the maximum possible score in these subsections.

Table 7: Neuropsychology test scores of DM1-affected and control participants

	Affected n.	Control n.	DM1-affected participants: Mean (SD)	Control participants: Mean (SD)	Effect size (Cohen's d)	P
Stroop test (T-score)						
Word task	43	20	39.42 (11.39)	48.95 (6.67)	1.021	<0.001
Color task	42	20	36.71 (10.87)	49.30 (7.23)	1.364	<0.001
Color-word task	41	20	41.95 (10.56)	53.80 (7.98)	1.266	<0.001*
Interference	41	20	47.34 (6.88)	51.60 (6.88)	0.619	0.022*
D-KEFS™ Trail Making (scaled score)						
1. Number scanning	45	20	9.00 (2.71)	11.50 (2.12)	1.028	0.001
2. Number sequencing	45	20	7.84 (3.77)	12.00 (1.59)	1.438	<0.001*
3. Letter sequencing	45	20	8.27 (3.86)	12.50 (2.04)	1.370	<0.001*
4. Number-letter sequencing	45	20	8.42 (4.25)	11.70 (2.00)	0.988	0.001*
5. Motor	45	20	8.96 (3.32)	12.55 (1.32)	1.421	<0.001*
Motor contrast score	45	20	9.51 (3.74)	9.15 (2.48)	0.113	0.221*
FAS word association						
Number of words	45	20	37.00 (10.92)	47.10 (11.11)	0.917	0.001
WASI-II Block Design						
Standard score (T-score)	45	20	37.49 (9.88)	52.15 (8.49)	1.592	<0.001
Non-adjusted score	45	20	7.58 (2.73)	10.75 (1.41)	1.459	<0.001
ECAS						
Language	45	20	26.58 (1.94)	27.20 (2.07)	0.309	0.012*
Verbal fluency	45	20	17.51 (3.60)	18.70 (3.91)	0.317	0.112*
Executive	45	20	35.80 (6.17)	39.35 (5.80)	0.593	0.013*
Memory	45	20	17.04 (3.81)	18.90 (2.83)	0.554	0.085*
Visuospatial	45	20	11.13 (1.25)	11.85 (0.37)	0.781	0.010*
Total score	45	20	108.07 (11.74)	116.00 (9.50)	0.743	0.004*

Comparison of means was carried out as an independent samples t-test if data were normally distributed in both groups (defined as $p > 0.05$ in Shapiro Wilk test of normality). If data were not normally distributed in one or both groups, a non-parametric Mann Whitney U test was applied. P values that relate to a non-parametric test are marked *. In all assessments, a higher score is consistent with better cognitive performance.

D-KEFS™ = Delis Kaplan Executive Frontal System; ECAS = Edinburgh Cognitive and Behavioural ALS screen; WASI-II = Weschler Abbreviated Scale of Intelligence

T-scores: age predicted score = 50, standard deviation = 10. Scaled score: age predicted score = 10, standard deviation = 3.

4.4.3 Adjustments for basic speed

The effect sizes detected in simple word and colour elements of the Stroop tests were similar in magnitude to that observed in the colour-word task (Cohen's D = 1.021, 1.364 and 1.266 respectively). Elimination of basic reading speed by calculation of the Stroop Interference score attenuated this effect size to a smaller, although still significant 0.619.

In the D-KEFS™ Trail Making tests, DM1-affected participants also took considerably longer on average than controls to complete each trail. A large Cohen's D effect size was demonstrated in each subtest, including the motor trail (Cohen's D = 1.421). The difference between DM1-affected subjects and controls in the number-letter switching trail was no longer significant after correction for basic motor speed by calculation of the motor contrast score.

In the WASI-II Block Design test, the large effect size between affected and control participants was very slightly attenuated when weighting for speed was removed. The effect size remained very large however (Cohen's D = 1.592 versus 1.459 for the standard and non-adjusted score respectively), suggesting motor impairment is not the major driver of impaired performance in this assessment.

4.4.4 Self-reported symptoms and cognitive performance

The relationship between self-reported symptoms and performance in neuropsychology assessments was explored by linear regression analysis. Self-DEX, BDI-II, FDSS and McGill pain scores alone did not correlate with performance in any of the neuropsychology assessments. Similarly, the cognitive impairment subscore of MDHI was not significantly associated with performance in any of the cognitive tests (a weak inverse correlation was detected with Stroop Interference score; $p = 0.008$, Adj $R^2 = 0.142$; although this was no longer significant after correction for multiple comparisons).

Greater physical impairment measured by DM1ActivC© was significantly associated with poorer performance in the visual scanning ($p = 0.001$, Adj $R^2 = 0.198$) and motor task ($p < 0.001$, Adj $R^2 = 0.305$) of the D-KEFS™ Trail Making tests, as well as total standard score ($p < 0.001$, Adj $R^2 = 0.229$) and non-adjusted score ($p < 0.001$, Adj $R^2 = 0.210$) of the Block Design subtest. Executive impairment rated by a proxy (Informant-DEX) showed an inverse correlation with Stroop word score only ($p = 0.003$; Adj $R^2 = 0.181$).

4.4.5 Genotype-phenotype correlations

In univariate analysis, logPAL did not correlate with any of the neuropsychology test scores. Since logPAL represents an estimation of CTG repeat size at conception, its influence on phenotype would be expected to be age-dependent. Hence we also explored correlations with logPAL in a multivariate model [age + logPAL + (age*logPAL)], demonstrating a significant correlation with the Block Design non-adjusted score ($p = 0.004$, Adj $R^2 = 0.212$) only. Increasing MAL significantly correlated with poorer performance in the Block Design standard score ($p = 0.003$; Adj $R^2 = 0.168$).

To investigate the influence of variant repeats on neuropsychology test scores, the three individuals with variant trinucleotide repeats were compared with other DM1-affected participants of a similar age (20 to 40 years) and ePAL size (150 to 250 repeats), which comprised a total of 5 individuals. No significant difference was detected by independent samples t-test in the subscores or total scores from the Trail Making tests, Block Design tests, FAS oral word association

or ECAS (illustrative scatterplots Figure 19A and B), although there was a trend towards better performance among individuals with variant repeats in the Block Design subtest (Figure 19C).

Two of the variant repeat subjects were among the four for whom Stroop data were incomplete; one who had become frustrated and disengaged, and the other's Stroop data was omitted from analysis as is was felt to be compromised by Meares-Irlen syndrome. The remaining participant with variant repeats achieved a Stroop Interference T-score of 60, which was significantly higher than the mean of the five age- and ePAL-matched controls in an independent samples *t*-test (p = 0.005, Figure 19D).

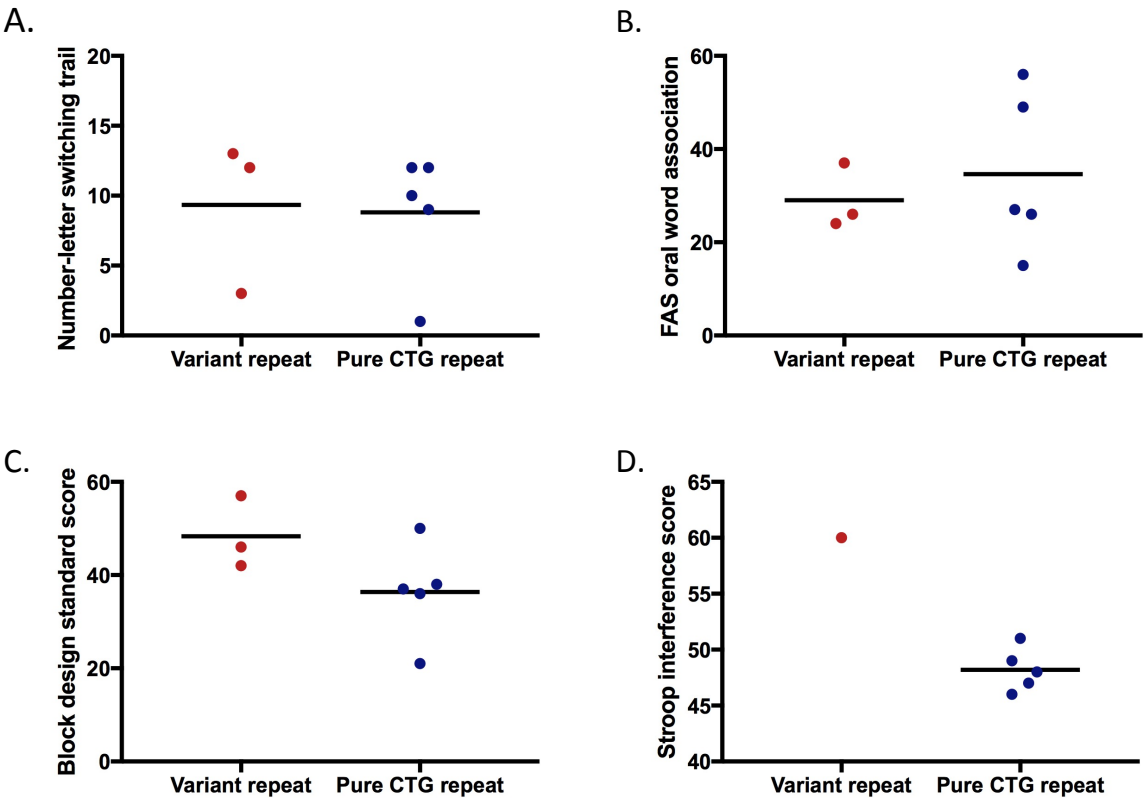

Figure 19: Comparison of neuropsychology assessment scores from individuals with variant repeats with age- and ePAL-matched subjects with pure CTG repeats

4.5 Discussion

4.5.1 Tolerability and sensitivity of the test battery

The neuropsychology test battery was broadly well tolerated by both control and DM1-affected participants. The mean scores of control participants were very close to age-predicted values where normative data were available (12 for scaled scores, 50 for T-scores), suggesting that the tests were appropriately applied. All primary components of the battery detected significant deficits in DM1-affected participants compared with controls, with effect sizes in the region of -0.5 to -1.5 standard deviations, consistent with the findings of previous studies [119,121]. Effect sizes were largest in assessments intended to measure cognitive flexibility (Stroop colour-word test, Trail 4), attention (Stroop word test, Stroop colour test, Trails 2, 3 and 5) and visuospatial processing (the Block Design subtest), which is in keeping with the profile described in other DM1 cohorts [119,123]. These findings therefore support the feasibility and sensitivity of the neuropsychology assessments described for use in clinical studies.

4.5.2 Specificity for central nervous system impairment

Specificity of the neuropsychology battery for central versus peripheral effects of DM1 was less clear, however. The Stroop test and Trail Making tests are broadly considered to be measures of higher, executive cognitive functions [242]. The colour-word task of the Stroop requires the subject to suppress a habitual impulse (to say the written word), and instead perform an unfamiliar task (saying the colour of ink), while the number-letter switching task of the Trail Making test demands cognitive flexibility to switch repeatedly between two unrelated sequences. In reality however, both are complex tasks, and performance depends on additional domains including attention and basic processing speed [245,247]. In both tests, comparison with controls showed a similar, large effect size for a simplified version of the task (the colour and word cards of the Stroop test, and motor task of the Trail Making test) as for the main executive component (the colour-word card and number-letter switching trail). Correction of the Stroop colour-word score for basic reading speed reduced this large effect size to a moderate Cohen’s D value, closer to that of the executive

subscore of ECAS. In the D-KEFS Trail Making tests, correction of the number-letter switching score for basic motor speed eliminated any significant difference compared to controls, suggesting that a more basic speed limitation is the major contributor to poorer performance in the DM1-affected group.

It is unclear whether the basic speed limitation detected in the Stroop and Trail Making tests is predominantly due to the primary muscle effects of DM1, causing dysarthria and upper limb weakness respectively, or due to additional central factors, that might include a slowing of basic processing speed or indeed a lack of drive to complete the task in a competitive way. Of note, we observed a significant direct correlation between both performance in the visual scanning and motor Trail Making tasks and greater self-reported muscle weakness measured by DM1ActivC©, supporting primary muscle weakness as playing a significant role.

In the Block Design test, standard scoring systems are heavily weighted to reward rapid completion of the designs. Hence, we hypothesised that distal muscle weakness in DM1 might account for a major portion of the deficit detected compared with controls. In this test, the large effect size persisted despite elimination of weighting for speed in the non-adjusted score, suggesting the Block Design test is indeed sensitive for impairment of visuospatial cognition in DM1 patients. Although the non-adjusted score improved correlations with ePAL compared with the standard score, this should be interpreted with caution since this value could not be age-adjusted due to a lack of normative data.

A significant contribution of muscle weakness to performance in some of the tests described is a potential confounder for use in drug trials, since a therapy that successfully improves primary weakness or myotonia, and so increases reading speed or manual dexterity, could erroneously give the impression of having impacted cognition. These results, therefore, suggest there would be value in further work to determine the nature of the basic speed limitation detected in DM1 by the Stroop and Trail Making tests and, in particular, to distinguish whether this is related to peripheral muscle impairment or other central factors. This will be explored further in light of imaging findings in the following chapter.

4.5.3 Genotype-phenotype correlations

Correlations of CTG repeat length with performance in neuropsychology assessments were comparatively poor when compared to the relationship between ePAL and muscle measures previously described by our group (Overend *et al*. Manuscript in preparation). As with self-reported outcome measures, small cohort size and selection for adult onset DM1 may have hampered our ability to detect genotype-phenotype correlations in the present study.

In addition, performance in neuropsychology assessments varies considerably in the general population, as a result of diverse multifactorial influences including genetic variation and educational background, as well as overall nutrition, health and wellbeing including sleep habits. Since the neuropsychological phenotype of adult onset DM1 is typically one of mild impairment within the general population range, the effect of CTG repeat length may be too subtle to detect in the present sample size against this background variation. This is in contrast to muscle, in which the disease effect can be associated with a loss of function that is considerably outwith the normal range, hence is more easily detected in comparison to background variation.

Wide background variation may also explain why the three individuals with variant repeats were not obvious outliers in their performance in these tests. Furthermore, the individuals with variant repeat were relatively young (the eldest aged 36 years) and so, since the CNS phenotype in DM1 is progressive with time [123], any modifying effect of variant repeats may be more apparent in older patients.

4.6 Conclusions

In summary, the neuropsychology test battery was well tolerated, and was sensitive to deficits in DM1 reported by previous studies. Impaired performance in two key assessments, the Stroop test and Trail Making tests, was significantly influenced by a basic speed limitation, but it was not absolutely clear whether this limitation reflected primary muscle weakness, or additional cognitive factors. Self-reported cognitive impairment was not predictive of performance in the neuropsychology assessment battery, nor was performance strongly

influenced by CTG repeat length measured in blood. Priorities for further analysis are therefore to compare both self-reported central symptoms and cognitive performance with objective measures of the severity of DM1-related CNS disease, derived from structural imaging of brain.

5 Magnetic resonance imaging: Glasgow site

5.1 Summary

This chapter describes the analysis of MRI data that was undertaken at the Glasgow site. Structural MRI sequences of brain from 39 DM1-affected participants and 20 controls were included in the main analysis. Segmentation of the major tissue class volumes revealed that total intracranial volume tended to be lower in DM1-affected subjects. After correction for intracranial volume, grey matter volume was reduced in the DM1-cohort compared with controls, and greater loss was significantly associated with increasing age, male sex and increasing CTG repeat length. After correction for age and sex, global grey matter volume correlated significantly with performance in several cognitive outcome measures. Using a lesion growth algorithm, the total volume of T2-hyperintense white matter lesions was quantified, demonstrating a marked increase in DM1-affected subjects that was age-dependent. The total volume of white matter lesions positively correlated with executive dysfunction reported by a proxy, while self-reported depression and cognitive problems was more common in those with milder white matter change. A voxel-wise group comparison demonstrates greater volume loss occurring particularly in subcortical regions in DM1 compared with controls, which could provide a plausible substrate for excessive somnolence and some cognitive features.

5.2 Introduction

The preceding chapters have highlighted uncertainty regarding the clinical validity of questionnaires and neuropsychological assessment for evaluation of CNS disease in DM1. Therefore identification of a valid, objective biomarker for brain involvement in DM1 would hold considerable value for future natural history studies and clinical trials. The structural differences that can be observed on brain imaging in DM1 are relatively well described, including diffuse atrophy of whole-brain, and the presence of T2 hyperintense lesions of white matter [162]. However previous studies have yielded inconsistent results in attempts to correlate regional structural changes seen on imaging with CTG

repeat length or aspects of clinical phenotype. A validated imaging biomarker for DM1 therefore remains elusive.

In light of the above challenges, the present chapter aims first to describe the global structural brain changes in the DM1-affected cohort compared with controls, with respect to the major tissue classes (grey matter, white matter and cerebrospinal fluid). The burden of T2-hyperintense lesions in white matter will also be quantified by a semi-automated method. Subsequently, relationships will be explored between the severity of brain changes objectively measured on MR imaging and the level of impairment measured by the clinical assessments described in the preceding chapters (self- and proxy-reported questionnaires and neuropsychology evaluation). Finally, we hypothesised that regional brain changes most specific to DM1, and hence representing the strongest candidates for imaging biomarkers, would be those that demonstrate the greatest volume difference compared to controls, and show the strongest correlations with CTG repeat length. An exploratory analysis of potential candidate regions was therefore undertaken using a voxel-based morphometry approach.

5.3 Results

5.3.1 Completion of MRI scanning

Of the 47 individuals initially recruited to the DM1-affected cohort, 41 underwent MRI of brain. The remaining six were excluded due to MRI contra-indications: three had permanent pacemakers, one had previously been declined an MRI due to high body mass index and abdominal girth, and two had claustrophobia. One claustrophobic patient declined to attempt MRI at all, the other attended the MRI suite but felt she could not proceed after lying down on the table. All DM1-affected and control participants who commenced MRI scanning completed the full imaging protocol.

5.3.2 Exclusions

As outlined in Chapter 3, one individual who underwent MRI scanning was excluded from the main analyses after she was found to carry an expanded CTG repeat allele in the premutation range (43 CTG repeats), hence could not be considered to have a diagnosis of DM1 by standard criteria. This individual's data

was however included in linear regression analyses in the exploring the effect of CTG repeat length on global MRI brain measures.

In another individual, MRI scanning revealed a left frontal lesion that was suspicious of a glial neoplasm. It was considered that the presence of a mass lesion would likely confound the semi-automated methods used for tissue segmentation as part of MRI analysis, and may also affect symptomatology and neuropsychological performance. Data from this individual was excluded from all analyses.

Thirty-nine DM1-affected individuals and 20 controls with MRI data were therefore included in the main analyses.

5.3.3 Incidental findings

Research involving MRI of brain carries the chance of identifying unexpected abnormalities of clinical significance. Occasional white matter lesions that appear hyperintense on T2-weighted imaging occur quite commonly in the general population, their prevalence increasing with age [253]. An increased frequency of such lesions can, however, be indicative of exposure to vascular risk factors such as hypertension or smoking, and so should prompt a review of treatable causes [254]. Frequent T2 hyperintense lesions also constitute one of the key hallmarks of DM1 on structural brain imaging [162]. There are no specific MRI features to distinguish the lesions seen in DM1 from those seen in the general population, except that in DM1 they are often more numerous, and may coalesce to form larger, confluent areas of abnormality [162].

In the present study, a letter was sent to subjects advising them to contact their GP to request assessment of blood pressure and cholesterol if the reporting radiologist described their burden of white matter hyperintensities as greater than expected for age. This was a common finding in DM1-affected subjects (*e.g.* Figure 20), but was considered actionable in only one control: a 67-year-old male with a history of atrial fibrillation. This participant was retained in the study, since the total lesion load measured by the automated pipeline was still within the upper limits of the population distribution for his age range [253], and

it is plausible that untreated vascular risk factors might contribute to lesion volume within the DM1 sample also.

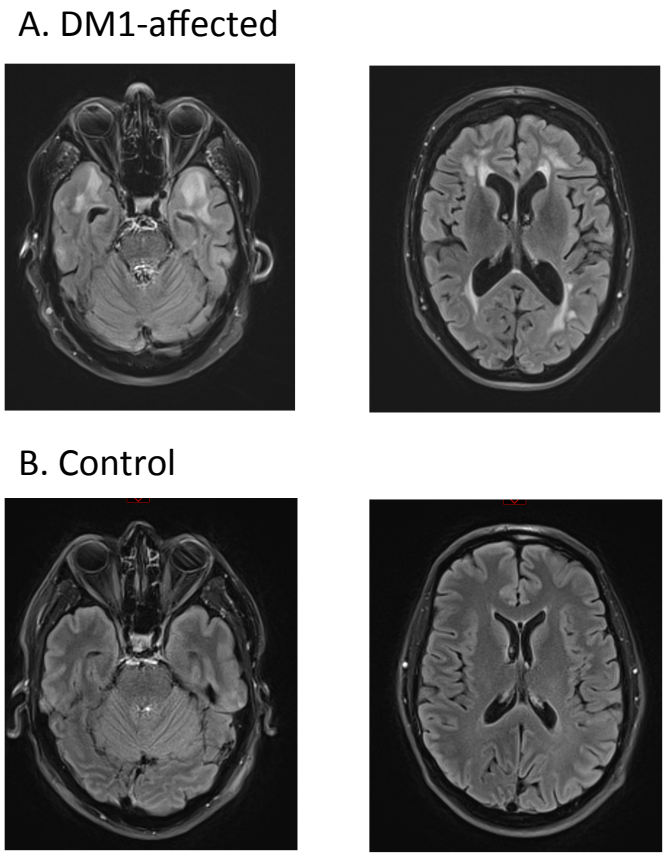

Figure 20: Axial T2 SPACE dark fluid images comparing brain of a 54 year old male with DM1 (A) with an age- and sex-matched control (B)
Note the presence of generalised atrophy, and white matter hyperintensities affecting the parietal lobes as well as periventricular regions in the DM1-affected participant.

Aside from white matter hyperintensities, MRI may also identify other actionable abnormalities such as evidence of previous cerebral infarction, cerebral aneurysms or primary tumours. The expected incidence of such findings in asymptomatic populations is quoted at around 2 to 4% [255]. In the present cohort, seven MRI scans revealed additional actionable incidental findings: four from DM1-affected subjects, and three from controls. Actionable findings are summarised in Table 8.

Table 8: Summary of actionable findings on MRI

Study ID	Finding	Outcome
DMN-012A	Increased T2 signal in the left petrous apex. Differential diagnosis includes possible cholesteatoma.	Subject was recalled for further diffusion-weighted MRI scan. Appearances were consistent with asymmetrically aerated left petrous apex, and therefore benign. Subject reassured.
DMN-014A	T2 signal change observed within the head of the left hippocampus/amygdala. Differential diagnosis included possible neoplasm.	Subject was recalled for further CT and contrast MRI scans. This concluded appearances were benign, likely representing gliosis associated with prominent perivascular space. A further MRI was performed at one year for additional reassurance, with stable appearance.
DMN-018A	A 2 cm, well-defined lesion was observed within left parotid gland.	Patient was referred for specialist opinion of the Ear, Nose and Throat team. A left partial parotidectomy was undertaken. Histology was consistent with a "benign pleomorphic adenoma/benign mixed tumour".
DMN-051C	A 13 x 11 mm slightly lobulated T1 low and T2 hyperintense mass lesion was observed at the deep margin of the right parotid gland.	Patient was referred for specialist opinion of the Ear, Nose and Throat team. A conservative approach was adopted, and imaging appearances were stable on repeat scanning at 1 year without intervention.
DMN-057A	Ill-defined area of altered signal within the left antero-inferior frontal lobe measuring 3 x 2.3 x 1.5 cm. Appearances suspicious of a glial neoplasm.	Subject was referred to the neurosurgical team, who arranged additional imaging and kept the patient under active surveillance. At one year, imaging appearances were stable. The differential diagnosis included a low-grade glioma or cortical dysplasia. This subject was withdrawn from the study.
DMN-063C	High fluid signal present in the right mastoid	Subject had a history of adenoidectomy operation for recurrent otitis media around 6 weeks prior to scan. Findings were communicated to the subject's surgeon, who felt they were consistent with expected post-operative appearances. No further action taken.
DMN-064C	An 8 x 4 mm nodule of apparent grey matter heterotopia was observed adjacent to the trigone of the right lateral ventricle.	Subject was informed of the finding, but reassured that in the absence of a history of seizure, or family history of neurodevelopmental problems, no further action was indicated.

As well as actionable incidental findings, the reporting radiologist commented on benign structural variations in five DM1-affected subjects (Table 9). This included two incidences of calcification of the falx cerebri, which has previously

been reported in patients with DM1 [158], and one of calcification within the superior cerebellar cistern.

Table 9: Summary of non-actionable structural variations identified by the reporting radiologist

Study ID	Finding
DMN-010A	Prominence of the lateral ventricles, likely developmental and longstanding.
DMN-018A	Calcification of the falx cerebri.
DMN-048A	Asymmetrically prominent right frontal sinus, prominent perivascular space within the right basal ganglia.
DMN-055A	Hyperostosis of frontal vault, ossification of anterior falx cerebri. Prominent ventricular system, likely developmental and longstanding.
DMN-057A	Incidental osteolipoma or calcification within the superior cerebellar cistern.

5.3.4 Volumetric analysis

5.3.4.1 Major tissue class volumes

Measures of total white matter volume, grey matter volume and cerebrospinal fluid volume were successfully derived from T1-weighted imaging for all 39 DM1-affected and 20 control participants. Total intracranial volume (ICV) was derived as the sum of these three values.

Group comparison (Table 10) demonstrated that mean ICV was significantly lower in DM1-affected subjects. To adjust for these differences, tissue classes were expressed as a percentage of total ICV for further analysis (grey matter volume, GMV; white matter volume, WMV; cerebrospinal fluid, CSF). After adjustment for ICV, GMV was significantly lower in DM1 subjects compared with controls, while WMV was not significantly different. This suggests that the decrease in whole brain volume (reflected by greater volume of CSF) in DM1 is primarily driven by grey matter loss.

Table 10: Major tissue class volumes in DM1-affected subjects and controls

Measure	DM1-affected (n.39) Mean (SD)	Control (n.20) Mean (SD)	p
Total intracranial volume (ICV) in litres	1.35 (0.10)	1.49 (0.16)	0.001
Grey matter volume as % ICV (GMV)	46.53 (5.79)	51.00 (3.83)	0.003
White matter volume as % ICV (WMV)	29.92 (2.18)	30.76 (1.66)	0.136
Cerebrospinal fluid as % ICV (CSF)	23.56 (6.27)	18.24 (4.56)	0.001

Sex differences were also explored within the DM1 and control groups (Table 11). Total ICV was significantly smaller in females compared with males in both groups. In the DM1-affected group only, GMV was significantly lower in males (44.2% versus 48.4%; p = 0.018), despite the groups being well matched for age (p = 0.280 in independent samples t-test), and female DM1 participants who completed imaging tending to have larger repeat sizes (mean ePAL 256 versus 171 repeats; p = 0.007).

Table 11: Comparison of tissue class volumes by sex in DM1 and control groups

Measure	DM1-affected			Control		
	Female (n.22) Mean (SD)	**Male (n.17)** Mean (SD)	**p**	**Female (n.8)** Mean (SD)	**Male (n.12)** Mean (SD)	**p**
Total intracranial volume (ICV) in litres	1.29 (0.08)	1.43 (0.06)	<0.001	1.38 (0.10)	1.56 (0.14)	0.005
Grey matter volume as % ICV (GMV)	48.35 (4.97)	44.16 (6.05)	0.023	50.78 (4.41)	51.15 (3.58)	ns
White matter volume as % ICV (WMV)	30.04 (1.39)	29.75 (2.44)	ns	31.23 (2.00)	30.44 (1.39)	ns
Cerebrospinal fluid as % ICV (CSF)	21.61 (5.35)	26.08 (6.62)	0.025	17.99 (5.71)	18.41 (3.89)	ns

5.3.4.2 Linear regression analyses of white and grey matter volumes with age, sex and CTG repeat length

In the DM1-affected group, WMV was not significantly associated with age (p = 0.657), MAL (p = 0.437), logPAL (p = 0.237), or age + logPAL + [age*logPAL] (p =

0.289). Inclusion of sex as a cofactor did not bring any of the linear models to statistical significance.

By contrast, a strong inverse correlation was observed between GMV and age in both groups ($p < 0.001$; Adj $R^2 = 0.730$ in the control group, Adj $R^2 = 0.622$ in DM1 subjects). In the DM1-affected group, the inverse correlation between GMV and age improved by inclusion of both age and sex in a multivariate model ($p < 0.001$; Adj $R^2 = 0.665$), (Figure 21). This model improved further with inclusion of age, sex and logPAL (n. = 40 including subject with premutation; $p < 0.001$; Adj $R^2 = 0.$ 0.697. Table 12). Neither BMI nor smoking status improved the fit of the model further.

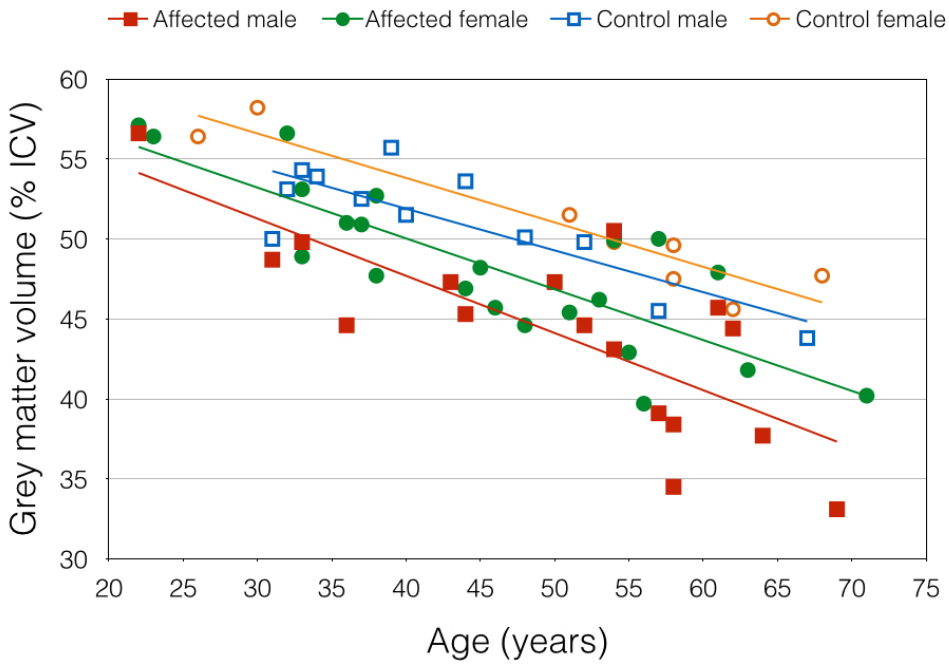

Figure 21: Grey matter volume (GMV) expressed as a percentage of total intracranial volume (ICV) plotted against age in DM1-affected and control subjects
Trend lines suggest a sex effect that was exaggerated in DM1-affected subjects.

Table 12: Significance level of predictor variables in multiple linear regression model GMV ~ age + sex + logPAL, using data from 39 DM1-affected individuals and one premutation carrier

Variable	Standardised beta coefficient	p
Age (years)	-0.815	< 0.001
Sex (male = 0; female = 1)	0.322	0.002
logPAL	-0.245	0.018

5.3.4.3 Quantitation of white matter hyperintensities

With respect to white matter changes, the absence of detectable group differences in WMV between DM1-affected and controls, or correlations with age or genetic measures, suggest white matter volume alone is a poor marker of DM1-related white matter disease. Additional analysis was therefore undertaken using a Lesion Growth Algorithm (LGA) (http://www.applied-statistics.de/lst.html) [213], from the Lesion Segmentation Toolbox, to quantify the total volume of white matter T2 hyperintensities present on each individual's structural imaging.

The mean volume of white matter hyperintensities (VWMH) was considerably greater in DM1-affected participants compared with controls (7.6 ml versus 2.1 ml; $p < 0.001$). No sex difference was observed, either within DM1-affected or control groups ($p = 0.421$, 0.670 respectively). VWMH increased with age in both groups ($p < 0.001$; Adj $R^2 = 0.355$ in DM1 subjects, Adj $R^2 = 0.148$ in controls. Figure 22). This model did not improve with inclusion of sex, CTG repeat length (logPAL or MAL), BMI or smoking status in multivariate models.

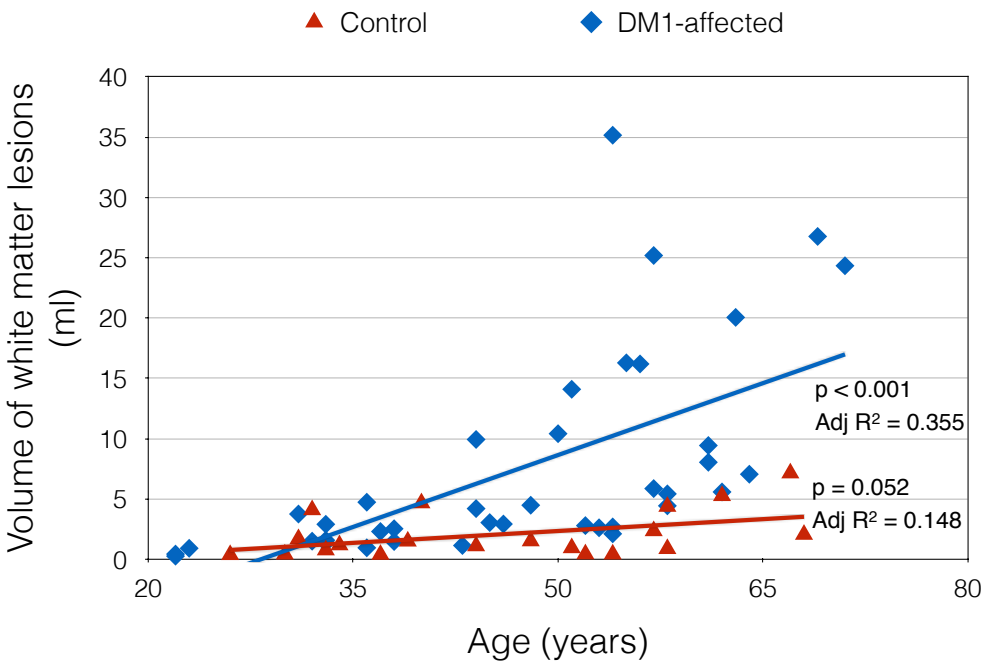

Figure 22: Total volume of white matter hyperintensities plotted against age in DM1-affected and control participants

5.3.4.4 Influence of variant trinucleotide repeats on global MRI measures

Variant repeat status, expressed as a categorical variable, did not significantly improve the best-fit linear models for GMV and VWML (GMV ~ age + sex + logPAL, and VWML ~ age respectively).

Perhaps surprisingly, scatterplots demonstrate that the two male subjects with variant repeats had GMV loss and VWML that was slightly greater than other DM1 subjects of a similar age (Figure 23). Of note, both individuals were current smokers.

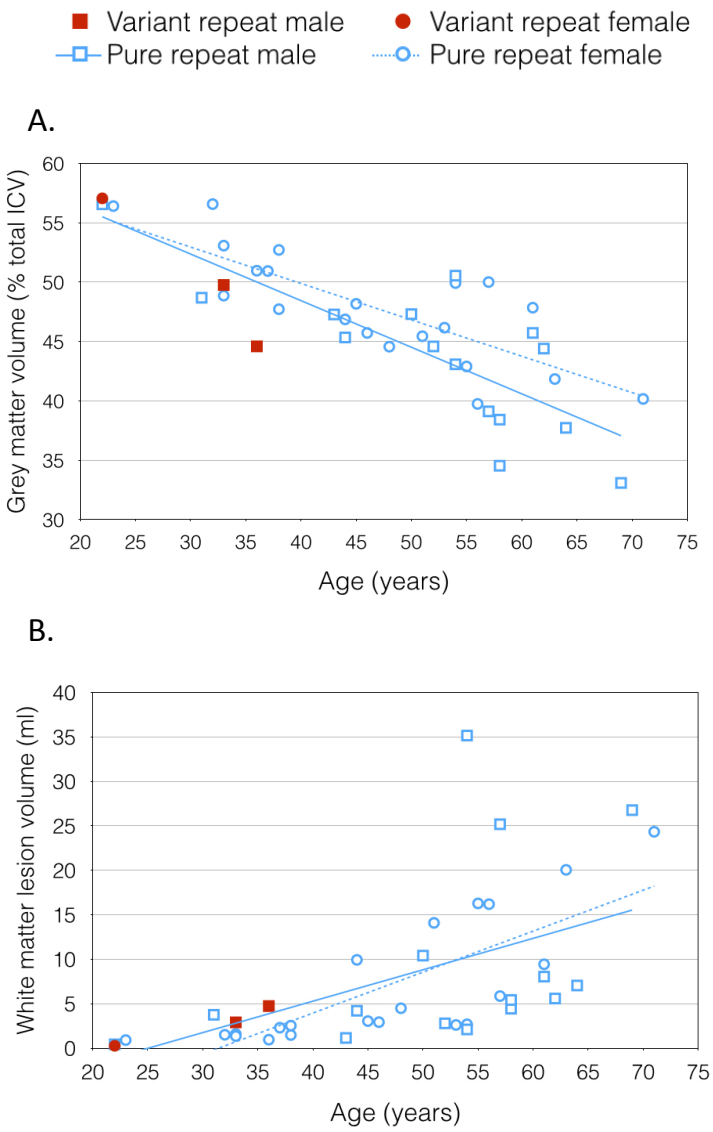

Figure 23: Grey matter volume (A) and volume of white matter lesions (B) plotted against age in the DM1-affected cohort. Individuals with variant repeats are marked in red.

5.3.4.5 Global MRI measures and self- or proxy-reported symptoms

Within the DM1-affected cohort only, relationships between global imaging measures and self-reported central symptoms were explored by linear regression analysis. Neither GMV nor VMWH alone were significantly associated with self-reported fatigue (FDSS), depression (BDI-II), emotional issues (MDHI emotional issues subscale), executive dysfunction (self-DEX), cognitive impairment (MDHI cognitive impairment subscale) or social performance (MDHI social performance subscale). On visual inspection of scatterplots, however, there was a trend towards increased self-reporting of depression, emotional issues, cognitive

impairment and social difficulties in patients with milder VWMH (Figure 24A). The same trend was not evident when compared with GMV (Figure 24B).

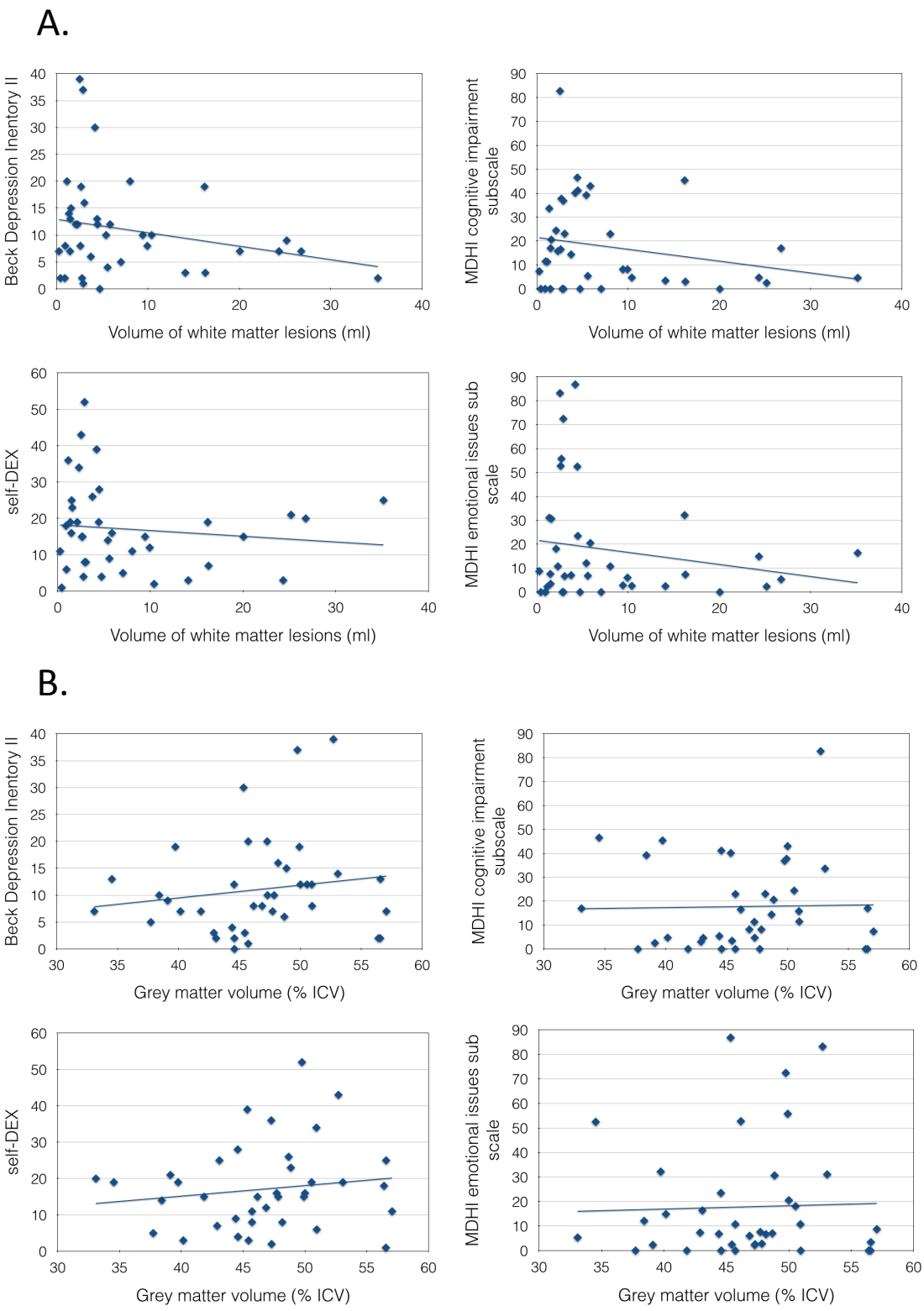

Figure 24: Scatterplots of self-reported CNS symptoms against (A) volume of white matter lesions and (B) grey matter volume

In contrast, executive dysfunction rated by a friend, carer or relative (independent-DEX) did show a significant positive correlation with VWMLs ($p < 0.001$, Adj $R^2 = 0.278$). An inverse correlation with GMV was also observed, although this relationship was no longer significant after correction for multiple comparison ($p = 0.029$, Adj $R^2 = 0.104$. Figure 25).

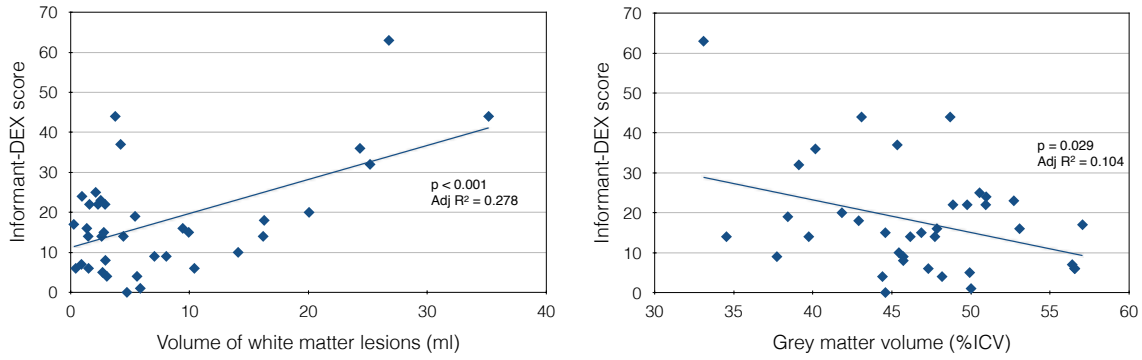

Figure 25: Executive symptoms reported by a proxy correlate positively with volume of white matter hyperintensities (A) and inversely with global grey matter volume (B)

5.3.4.6 Global MRI measures and cognitive performance

Grey matter volume alone did not significantly correlate with performance in neuropsychology assessments, except the Stroop colour and word tasks (Adj $R^2 = 0.171$ and 0.316 respectively). Given the observed sex differences in GMV, and that our cohort was highly heterogeneous in age, we repeated linear regression analyses including sex and age as covariates, which improved correlations with several measures, although some could not be considered significant after correction for multiple comparisons (Table 13).

Table 13: Linear regression analyses of GMV against cognitive performance in DM1-affected subjects only

	Age Adj R^2	**GMV** Adj R^2	**GMV + sex** Adj R^2	**GMV + sex + age**
Stroop test (T-score)				
Word task	ns	0.171	0.200	p = 0.003 Adj R^2 = 0.280
Colour task	ns	0.316	0.367	p < 0.001 Adj R^2 = 0.399
Colour-word task	ns	(0.101)	0.287	p < 0.001 Adj R^2 = 0.353
Interference	ns	ns	0.275	p = 0.006 Adj R^2 = 0.257
D-KEFS™ Trail Making (scaled score)				
1. Number scanning	ns	ns	Ns	p = 0.013 (Adj R^2 = 0.199)
2. Number sequencing	ns	ns	(0.173)	p = 0.007 Adj R^2 = 0.232
3. Letter sequencing	ns	(0.089)	0.232	p = 0.002 Adj R^2 = 0.276
4. Number-letter sequencing	ns	ns	(0.135)	p = 0.043 (Adj R^2 = 0.137)
5. Motor	ns	ns	ns	ns
Motor contrast score	ns	ns	ns	ns
FAS word association				
Number of words	ns	ns	ns	p = 0.006 Adj R^2 = 0.234
WASI-II Block Design				
Standard score (T-score)	ns	ns	(0.162)	p = 0.041 (Adj R^2 = 0.140)
Non-adjusted score	0.140	(0.105)	0.225	p = 0.009 (Adj R^2 = 0.214)
ECAS				
Language	ns	ns	ns	ns
Verbal fluency	(0.097)	ns	ns	p = 0.006 Adj R^2 = 0.232
Executive	ns	(0.124)	(0.126)	ns
Memory	(0.070)	ns	ns	ns
Visospatial	ns	ns	ns	ns
Total score	ns	ns	ns	ns

ns = not significant ($p > 0.05$) before correction for multiple comparisons. Adj R^2 values in brackets were no longer significant after Benjamini-Hochberg correction.

In the previous chapter, the question was raised as to whether primary muscle weakness might influence performance in traditional cognitive assessments. To explore the relative effects of GMV and muscle impairment on cognitive performance, while controlling for other factors, DM1-ActivC© score was added to this multivariate model, to give: score ~ age + sex + GMV + DM1-ActivC© (Table 14). Addition of DM1-ActivC© improved the fit of the model further, reflected by an increase in Adj R^2, for the Stroop colour and word tasks, Trails 1 to 5 of the D-KEFS Trail Making Tests and both the Block Design standard and non-adjusted scores. The contribution of DM1-ActivC© score to the model reached statistical significance (at $p < 0.05$ without correction for multiple testing) in the D-KEFS number scanning and motor trails, as well as the Block Design standard score.

Table 14: Standardised beta coefficients and significance of predictor variable in the model Score ~ age + sex + GMV + DM1-ActivC©

	Age	Sex	GMV	DM1-ActivC©	Whole model Adj R^2 (p)
Stroop test (T-score)					
Word task	p = 0.023 Beta = 0.521	p = 0.196 Beta = 0.272	p = 0.001 Beta = 0.862	p = 0.074 Beta = 0.272	0.327 (p = 0.002)
Colour task	p = 0.080 Beta = 0.361	p = 0.100 Beta = -0.244	p < 0.001 Beta = 0.884	p = 0.123 Beta = 0.219	0.425 (p < 0.001)
Colour-word task	p = 0.048 Beta = 0.461	p = 0.002 Beta = -0.514	p = 0.001 Beta = 0.868	p = 0.903 Beta = 0.018	0.333 (p = 0.002)
Interference	p =0.560 Beta = 0.110	p = 0.002 Beta = 0.566	p = 0.050 Beta = 0.517	p = 0.668 Beta = -0.069	0.238 (p = 0.014)
D-KEFS™ Trail Making (scaled score)					
1. Number scanning	p = 0.001 Beta = 0.747	p = 0.849 Beta = -0.028	p = 0.001 Beta = 0.506	p = 0.001 Beta = 0.506	0.410 (p < 0.001)
2. Number sequencing	p = 0.040 Beta = 0.497	p = 0.101 Beta = -0.434	p = 0.013 Beta = 0.653	p = 0.089 Beta = 0.267	0.274 (p = 0.005)
3. Letter sequencing	p = 0.061 Beta = 0.444	p = 0.015 Beta = -0.405	p = 0.003 Beta = 0.782	p = 0.147 Beta = 0.223	0.300 (p = 0.003)
4. Number-letter sequencing	p = 0.269 Beta = 0.286	p = 0.109 Beta = -0.288	p = 0.032 Beta = 0.609	p = 0.364 Beta = 0.154	0.143 (p = 0.064)
5. Motor	p = 0.309 Beta = 0.237	p = 0.731 Beta = -0.055	p = 0.461 Beta = 0.182	p < 0.001 Beta = 0.603	0.299 (p = 0.003)
Motor contrast score	p = 0.714 Beta = 0.097	p = 0.156 Beta = -0.261	p = 0.074 Beta = 0.514	p = 0.053 Beta = -0.343	0.088 (p = 0.130)
FAS word association					
Number of words	p = 0.001 Beta = 0.917	p = 0.419 Beta = -0.136	p = 0.004 Beta = 0.805	p = 0.604 Beta = 0.083	0.218 (p = 0.014)
WASI-II Block Design					
Standard score (T-score)	p = 0.650 Beta = 0.111	p = 0.079 Beta = 0.111	p = 0.162 Beta = 0.162	p = 0.047 Beta = 0.328	0.213 (p = 0.015)
Non-adjusted score	p = 0.589 Beta = -0.127	p = 0.099 Beta = -0.271	p = 0.266 Beta = 0.280	p = 0.052 Beta = 0.307	0.277 (p = 0.004)
ECAS					
Language	p = 0.181 Beta = 0.364	p = 0.094 Beta = 0.316	p = 0.627 Beta = 0.140	p = 0.226 Beta = 0.215	0.048 (p = 0.229)
Verbal fluency	p = 0.002 Beta = 0.824	p = 0.559 Beta = 0.098	p = 0.032 Beta = 0.580	p = 0.696 Beta = -0.063	0.213 (p = 0.016)
Executive	p = 0.254 Beta = 0.259	p = 0.265 Beta = 0.198	p = 0.074 Beta = 0.500	p = 0.273 Beta = 0.186	0.135 (p = 0.062)
Memory	p = 0.203 Beta = -0.352	p = 0.270 Beta = 0.209	p = 0.475 Beta = -0.209	p = 0.300 Beta = 0.187	0.021 (p = 0.328)
Visuospatial	p = 0.871 Beta = 0.046	p = 0.521 Beta = -0.125	p = 0.335 Beta = 0.292	p = 0.883 Beta = 0.027	0.048 (p = 0.687)
Total score	p = 0.146 Beta = 0.386	p = 0.169 Beta = 0.250	p = 0.113 Beta = 0.449	p = 0.294 Beta = 0.181	0.104 (p = 0.102)

VWML did not correlate with performance in any of the neuropsychology assessments tested.

5.3.5 Voxelwise statistical analysis

Voxelwise statistical modelling was used to identify regional differences in grey matter volume.

First, a group comparison was made between DM1 subjects and controls. The model included age and sex as covariates, and correction for family-wise error (FWE) was set at a false discovery rate of 0.05. Applying contrast for grey matter loss in the DM1-group, a strong signal was seen in subcortical grey matter, with smaller, widespread foci in frontal, temporal and occipital lobes (Figure 26).

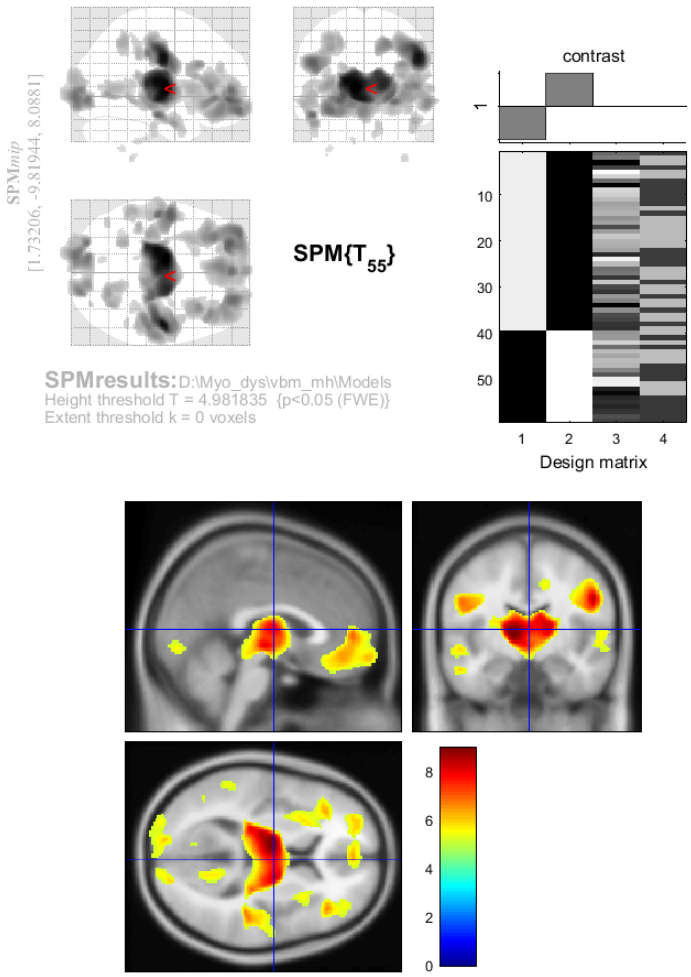

Figure 26: Results of VBM group comparison between DM1-affected subjects and controls. Age and sex were included as covariates. Contrast demonstrates T scores of voxels with increased GM loss in DM1 subjects compared with controls, after correction for family-wise error at a false discovery rate of 0.05.

A linear regression model with logPAL was then explored in the DM1-affected group only, in order to identify regions in which grey matter loss was most strongly driven by CTG repeat length. Age and sex were again included as covariates. This model showed a similar strong signal from subcortical grey matter structures before correction for FWE, with smaller foci in frontal, temporal and occipital lobes (Figure 27). No cluster remained significant after correction for FWE however.

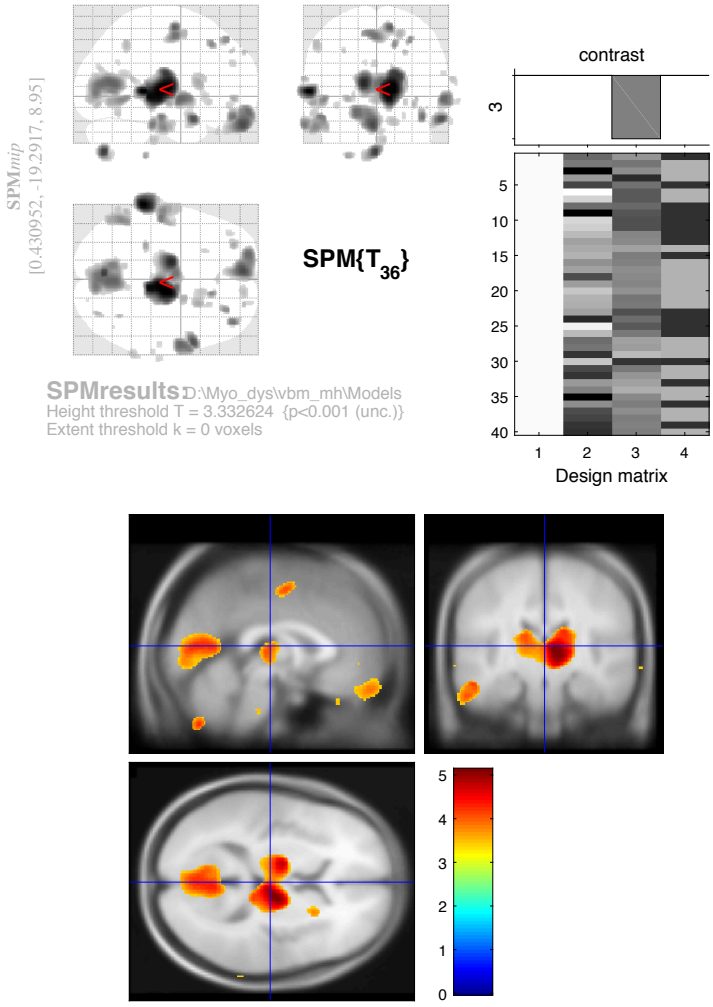

Figure 27: Voxelwise multiple regression analysis in DM1-affected subjects, with contrast representing an inverse association between grey matter volume and logPAL, before correction for FWE.
Age and sex are included as covariates.

5.4 Discussion

5.4.1 Validity of clinical outcome measures

Comparison of self-reported outcomes with imaging demonstrated that subjects' perception of their own CNS-related symptoms do not closely reflect the severity of global structural brain changes seen on MRI. In the preceding chapters, it was shown that DM1 patients with lower mood generally reported more somatic symptoms, particularly relating to cognition and social performance. Perhaps surprisingly, when compared with MRI measures, there was a clear trend towards greater reporting of depression and central symptoms in those with milder white matter change, which is the inverse of what might be predicted. This finding is, however, consistent with data from previous imaging studies, which also observed a tendency for greater reporting of depression and fatigue in DM1 subjects with milder white matter change [166,256]. The reason why participants with more widespread structural brain change are less inclined to rate their symptoms as severe is not clear from the data available. One possibility is that this reflects acceptance of symptoms over in those with more longstanding disease. Impaired disease awareness is, however, a recognised component of the DM1 central phenotype [202], hence impairment of insight with regard to cognition or social performance may also be relevant.

These results add further evidence that self-reported symptom measures often do not accurately reflect the primary CNS disease process in DM1, hence may represent a poor choice for use as clinical trial outcome measures in isolation. In contrast, executive symptoms rated by a relative or carer did show a positive relationship with the severity of MRI changes, highlighting a possible role for proxy measures as part of global CNS assessment.

In the previous chapter, it was discussed that impaired performance in elements of the Stroop and Trail Making tests is influenced by a limitation of basic speed. The relative contribution of central factors and peripheral muscle weakness to this speed limitation was unclear from the data available. Analysis of imaging data shows significant correlations of performance in key components of the Stroop, Trail Making Tests and Block Design subtest with GMV after accounting for age and sex, suggesting structural brain changes are major modifiers of

performance. However, inclusion of DM1-ActivC© score in a multivariate model supported the hypothesis that muscle impairment accounts for some of the residual variation in performance in the Stroop Colour and Word tasks, as well as all elements of the Trail Making Tests. Perhaps unsurprisingly, the effects of muscle were most pronounced in the number scanning and motor components of the Trail Making Tests. Overall, these results suggest that further work to develop and validate cognitive assessments that are not excessively influenced by manual dexterity or dysarthria, perhaps utilising assistive technology, would be a highly useful step towards clinical trial readiness.

5.4.2 Characteristics of global brain changes

Segmentation of tissue class volumes demonstrated lower intracranial volume and increased proportion of CSF in DM1-affected subjects. Reduced brain volume was primarily driven by grey matter loss, and greater grey matter loss was associated with increasing age, male sex and increasing ePAL. The volume of white matter lesions was markedly greater in DM1-affected subjects compared with controls, and increased in an age-dependent manner.

Selection criteria for the present study excluded participants with clear onset of symptoms before the age of 16, or those with learning disability in childhood. It was, therefore, somewhat surprising to observe lower mean ICV in the DM1-affected group. Since ICV is fixed after fusion of the skull sutures, irrespective of further changes in the brain parenchyma, intracranial volume is generally held to represent a marker of maximal brain growth during development and maturation to adolescence [257]. In adult-onset DM1, CNS deficits are generally regarded as representing a neurodegenerative process. However, this finding in relation to ICV suggests that even in adult-onset populations, there is evidence of a neurodevelopmental process restricting brain growth long before the overt onset of clinical features. The effects of the CTG repeat expansion on brain growth, and hence ICV, are likely to be highly complex. However elements of the mTOR signalling pathway could be speculated to be relevant, since mTOR signalling is shown to be dysregulated in muscle of mouse models of DM1 [258] and variants in this pathway are known to influence intracranial volume in humans [259].

It should also be acknowledged that, although participants denied onset of symptoms before 16 years at recruitment, and were considered to have a phenotype consistent with adult onset disease by the recruiting clinician, a few subsequently reported their first symptom at an earlier age in a study questionnaire. Inconsistency in self-reporting of age at onset, as well as the presence of reduced ICV in individuals considered to have an adult-onset phenotype, also highlights the somewhat subjective and arbitrary distinction between juvenile, adult and late-onset forms of DM1, and would rather support these phenotypes being regarded as a clinical continuum.

It was interesting to note an apparent sex effect on GMV in this cohort. Inclusion of sex in a multivariate model improved both correlations of GMV with age, and with performance in several neuropsychology assessments. To our knowledge, sex-specific differences in grey matter atrophy have not specifically been explored in DM1, but in the general population, a marginally greater rate of grey matter atrophy in males is observed [260]. Therefore, given that several features of DM1 show a sex bias in penetrance [261], it is plausible that a sex effect on grey matter atrophy might exist in DM1. This finding highlights sex as an important cofactor to include in subsequent analyses, and for future studies aiming to identify imaging biomarkers.

Unlike global GMV, VWML did not correlate well with cognitive impairment measured by neuropsychology assessments, or with CTG repeat length in this study. White matter lesions in the general population may be influenced by vascular risk factors [254], hence it is probable that additional environmental and/or genetic factors could also influence the severity of VWMLs in DM1, which may limit their potential for use as a disease-specific biomarker.

5.4.3 Regional brain differences

Group comparison of grey matter in DM1 subjects versus controls suggests that marked loss occurs in subcortical regions in affected individuals, as well as patchy cortical regions including frontal, temporal and occipital lobes. Volume loss in similar regions also correlated with CTG repeat length (logPAL) in a linear model, although no clusters were significant after stringent correction for multiple comparisons. The finding of volume loss affecting subcortical grey

matter is particularly intriguing, since it offers plausible links to several aspects of phenotype seen in human subjects with DM1. In the general population, decreasing volume of thalamus is associated with decline in processing speed as part of cognitive aging [262]. Slowing of basic processing speed was a prominent feature of the deficits detected by neuropsychology testing in DM1, both in the present study and in previous work [112,115,239,241]. The thalamus also plays a role in sleep and wakefulness, forming both an important neural relay in the ascending arousal system [150], as well as generating the sleep spindles that characterise stage 2 sleep [263]. Impaired thalamic function therefore could contribute to the excessive daytime somnolence, and reduction in stage 2 sleep previously described in DM1 [95], hence further characterisation of subcortical structures should form a key component of further exploration of structure-phenotype relationships.

5.5 Conclusions

In summary, this chapter explored clinical correlates of global MRI measures in 39 individuals with DM1 and 20 controls. An additional individual with a premutation-range allele was also included in correlations with CTG repeat length. Individuals with DM1 had, on average, smaller ICV and increased atrophy of whole-brain, which was primarily driven by grey matter loss. Lesions of white matter that were hyperintense on T2 imaging were increased in DM1-affected subjects, and correlated positively with executive problems reported by a proxy, while self-reported depression and cognitive problems were more common in those with mild white matter change. Grey matter was inversely correlated with age, and greater loss was associated with male sex and increasing CTG repeat length. A voxel-wise group comparison suggests there is an excess of volume loss affecting subcortical structures in DM1-affected subjects compared with controls, which could plausibly be associated with features of impaired processing speed and excessive sleepiness.

In light of these observations, it was important to further characterise the regional structural changes observed in DM1-affected patients, particularly in relation to subcortical grey matter structures. The Monckton Laboratory group had an existing collaboration with a team at the University of Iowa led by Prof Peg Nopoulous. This group have a strong background in research pertaining to

structural MRI imaging in Huntington disease, and more recently had commenced a longitudinal imaging study in DM1, for which the Monckton group were undertaking DNA analysis of study participants. At the outset of the Glasgow study, it had been anticipated that combining MRI data from both cohorts could hold considerable value in improving power to detect both group differences, and correlations with CTG repeat length, and so efforts were therefore made to ensure comparable sequences were obtained. The following chapter therefore describes harmonisation and imaging analysis of the pooled Glasgow and Iowa cohort.

6 Magnetic resonance imaging: Glasgow-Iowa pooled cohort

6.1 Summary

This chapter describes the harmonisation and analysis of imaging datasets from the Glasgow DM1 cohort, and a comparable group recruited by our collaborators based at the University of Iowa. The pooled cohort contained 79 individuals with adult-onset DM1, and 58 controls.

Using a volumetric joint label fusion approach, subjects with DM1 were again found to have reduced ICV compared with controls, with a greater effect size seen in males. After correction for ICV, age and sex, DM1-affected subjects had significantly reduced volume in whole cerebrum, frontal lobe (both grey and white matter), parietal grey matter, cerebellar white matter, corpus callosum, putamen, accumbens and thalamus. Surprisingly, relative to ICV, hippocampus and amygdala volumes were significantly increased in the DM1-affected group.

Correlation of regional volumes with CTG repeat length was also explored. Repeat length, expressed as logPAL was inversely correlated with volume of occipital grey matter, putamen and thalamus, while a positive association was seen with volume of cerebellar white matter and amygdala. Screening for variant repeats was positive in seven individuals in the pooled cohort. There was no clear effect of the presence of variant repeats on regional volumes in this sample.

The regional volumetric changes identified offer plausible links to key features of the DM1 phenotype. For example, reduced thalamus volume could be postulated to influence speed of processing, somnolence symptoms and integrity of stage 2 sleep. Increased amygdala volume relative to frontal lobe and accumbens could be predicted to hamper regulation of negative emotions, and hence provide a substrate for social avoidance. These findings therefore highlight key candidate structures for use as CNS imaging biomarkers, as well as providing possible new insights into the mechanisms underlying CNS symptoms.

6.2 Introduction

The preceding chapters have highlighted a need to better understand the clinical significance of structural brain changes in DM1, both to aid identification of imaging biomarkers for use in clinical trials, and to identify potential targets for treatment of CNS symptoms. In a group comparison using a voxel-based morphometry approach, we observed highly significant volumetric changes in cortical and subcortical grey matter of DM1-affected participants compared with controls in our Scottish cohort. Since dysfunction of subcortical structures could plausibly make a major contribution both to excessive sleepiness symptoms [150] and cognitive dysfunction, including basic processing speed [262], this finding merits further detailed investigation.

Previous case-control imaging studies have sought to describe structural brain differences in DM1. The majority used whole-brain, voxel-based morphometry approaches [164,166-171], along with a single recent study using automated segmentation tools [161]. Broadly, these studies confirm widespread atrophy affecting all major cortical lobes and deeper grey matter structures. Thinning of the grey matter cortex is also described in parietal, temporal and occipital lobes in a single small study [171]. Results have been inconsistent, however, with respect to correlations between structural change and aspects of neuropsychological phenotype [162]. Findings are likewise inconsistent regarding the effect of CTG repeat length on structural brain change, with some studies describing an inverse correlation between repeat length and grey matter volume in motor and prefrontal cortices [163], or orbitofrontal cortices, cingulate gyrus and left precentral gyrus [168], while many others found no association [159,167,169,171]. Limitations of these studies included use of traditional methods to measure CTG repeat length, which fail to take account of age-dependent changes of somatic mosaicism, as well as the limited power of the sample sizes used, typically n = 30 to 40. Because DM1 is rare in most populations, and symptoms of the condition itself form a major barrier to participation in clinical studies, it seems unlikely that recruitment of a substantially larger cohort, particularly one representing a broad spectrum of DM1 phenotypes, would be achievable from a single neuroimaging centre. As

such, combining data from different research sites would be a positive step towards improving the power of imaging studies, and so more definitively defining the structural landscape of brain changes in DM1.

Historically, harmonisation of imaging data acquired at different sites, and thus using distinct MRI scanning platforms, has been troublesome. This is because the properties of separate scanners, even identical models from the same manufacturer, can vary with respect to properties such as field strength and linearity of the magnetic field gradient. As a result, subtle heterogeneities in voxel geometry, and contrast between tissue classes (termed 'bias field') can significantly influence results obtained by automated segmentation methods [264]. Fortunately, processing tools have been developed to correct bias field [220], with some multi-modal tools able to run bias correction, tissue classification and image registration in a synchronous, iterative way to robustly adjust for site-effects in multi-site studies [223]. Furthermore, application of a Bayes-based statistical framework to output data from MRI processing has also been shown to be an effective means of reducing scanner-dependent differences, whilst preserving true biological variation [225,226].

In light of the apparent feasibility of harmonisation of MRI data from separate sites, we set out to combine our structural MRI data with those from a similar-sized cohort, recruited to a longitudinal DM1 imaging study by our collaborators at the University of Iowa. From the resulting well-powered, case-controlled cohort, we aimed to describe in detail structural differences between the DM1-affected group and controls, using a regional volumetric approach. The effect of CTG repeat length on structural differences detected was also explored.

6.3 Results

6.3.1 Combined cohort demographics

Demographic details of the regional and pooled cohorts are summarised in Table 15. One of the original Glasgow participants (DMN-030A) was excluded from final analysis, as automated segmentation using BRAINSTools repeatedly failed quality control. The Glasgow cohort therefore constituted 38 DM1-affected individuals (17 male and 21 female), and 20 controls (12 male and 8 female), while the Iowa

group included 41 DM1-affected (12 males and 29 females) and 38 controls (17 male and 21 female). The Iowa cohort generally had less severe muscle symptoms compared with Glasgow, reflected by a lower modal MIRS score (2 versus 4, $p < 0.001$). Mean ePAL was also lower in the Iowa subgroup (170 versus 225 CTG repeats, $p = 0.007$).

Table 15: Demographic details of the pooled Glasgow and Iowa imaging cohorts

				Controls		DM1	
				Glasgow	Iowa	Glasgow	Iowa
Sample	N		Site	20	38	38	41
			Pooled	58		79	
Sex	N	Male	Site	12	17	17	12
			Pooled	29 (50%)		29 (37%)	
		Female	Site	8	21	21	29
			Pooled	29 (50%)		50 (63%)	
Age	Mean (SD)		Site	46.0 (13.1)	44.6 (13.7)	47.1 (13.2)	45.4 (11.7)
			Pooled	45.1 (13.4)		46.2 (12.4)	
MIRS	Mean (mode)		Site	-	-	3.2 (4)	2.1 (2)
			(Pooled)	-		2.6 (2)	
ePAL	Range (min to max)		Site	-	-	56 to 572	55 to 501
			Pooled	-		55 to 572	
	Mean (SD)		Site	-	-	225 (112)	170 (115)
			Pooled	-		198 (117)	

In the pooled cohort, the DM1-affected and control groups were well matched for age (mean 46.2 versus 45.1 years; $p = 0.671$). The DM1-affected group contained a greater proportion of females compared with the control group (63% versus 50%, $p = 0.161$ in Fisher's exact test).

6.3.2 Volumetric analysis

6.3.2.1 Harmonisation of data from different sites

Each MRI was acquired on one of three 3T scanners. Scans in Glasgow were all acquired on a Siemens-Prisma platform, while those in Iowa either used a Siemens-Trio (n.52) or GE Discovery scanner (n.27). Several elements of the processing pipeline sought to minimise the effect of site. Firstly, the N4 bias correction algorithm available for Advanced Normalisation Tools (ANTS;

http://stnava.github.io/ANTs/) [220] was applied to T1 weighted images prior to segmentation. Second, the BRAINSAutoWorkup (BRAINSTools) pipeline for automated segmentation itself runs an iterative process for bias correction along with tissue segmentation and registration. Finally, the raw values for ROI volumes output by BRAINSTools were further adjusted using ComBat (www.bu.edu/jlab/wp-assets/ComBat/Abstract.html), a Bayes-based statistical framework for R statistics software.

Visual inspection of corrected regional measurements (*e.g.* for cerebral grey matter in Figure 28) suggests the pipeline was effective in preparing the data for analysis as a single cohort.

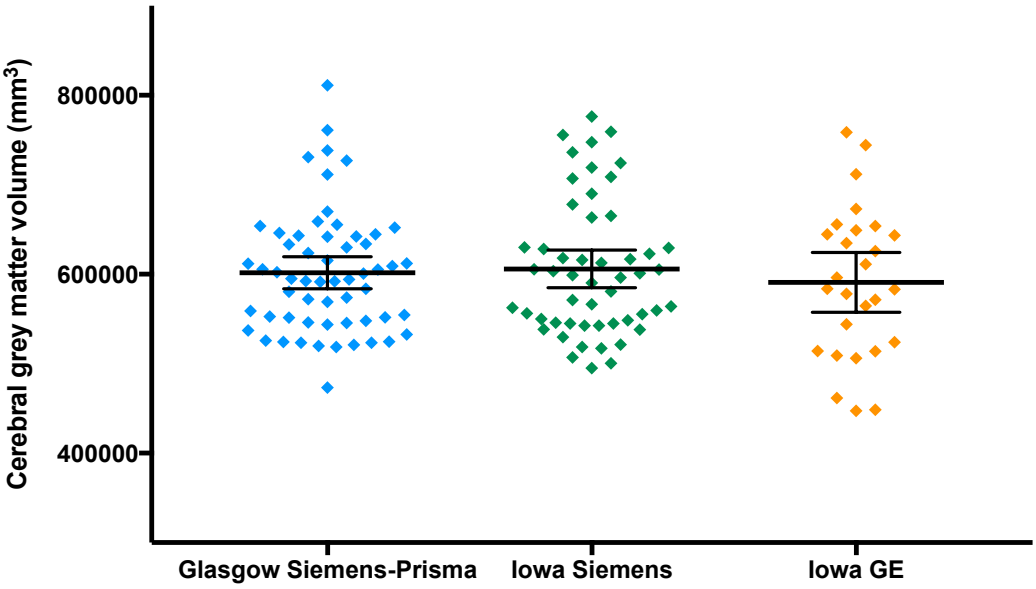

Figure 28: Segmentation volumes of cerebral grey matter derived from MRIs undertaken on separate scanners, after adjustment for the effect of site
Horizontal lines denote mean with 95% confidence interval

6.3.2.2 Intracranial volume

In both male and female participants, total ICV was significantly lower in the DM1-affected group compared with controls (Cohen's D = 0.90 and 0.52, p = 0.002 and 0.035 respectively; Figure 29).

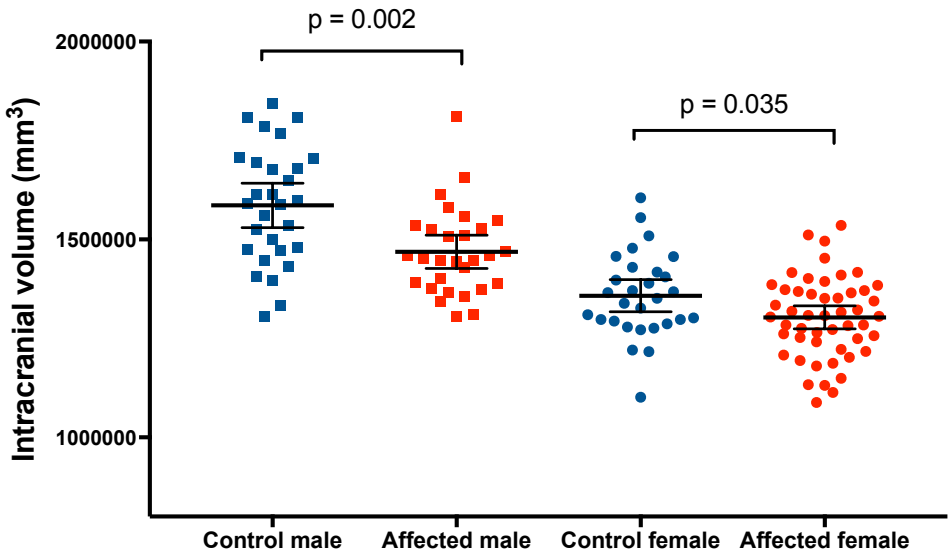

Figure 29: Intracranial volume is significantly lower in DM1-affected versus control participants in both males and females

While considering factors that might contribute to intracranial volume, it was noted that several female DM1-affected participants from both cohorts had appearances of thickening of the frontal skull vault, which was not apparent in control participants. In the Glasgow cohort, the reporting radiologist had commented on the presence of frontal thickening (hyperostosis frontalis interna; HFI) in two female DM1-affected participants. A further female recruited in Iowa had particularly marked changes (Figure 30). While the presence of skull vault thickening in some individuals could have marginally impacted internal volume of the skull case, these findings alone did not appear sufficient to entirely explain the group differences in ICV.

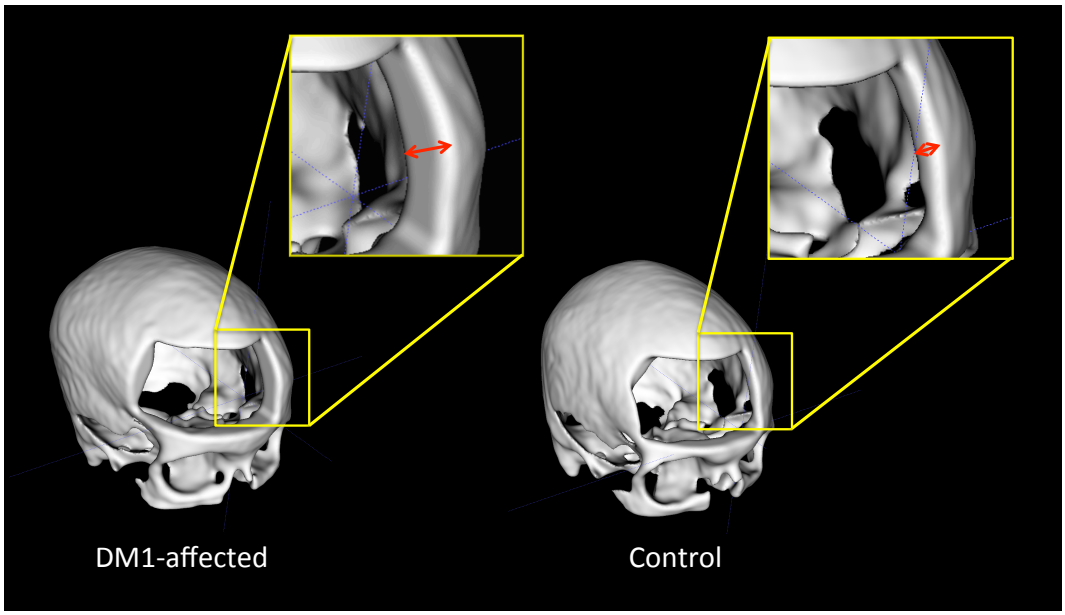

Figure 30: Skull rendering from T1 weighted imaging in a 50 year old female with DM1 (left) and a 51 year old female from the control group (right)
The frontal skull vault is markedly thicker in the DM1-affected participant compared with control (red arrows). Skull renderings produced by Dr Timothy Koscik, University of Iowa.

6.3.2.3 Regions of interest

Group differences in region of interest (ROI) volumes between the DM1-affected group and controls were explored by regression analysis. The analysis presented here was undertaken by Prof Jeffrey D. Long, University of Iowa. As described in Chapter 2, ROIs were adjusted for ICV by a power proportion method. For each ROI, ß was estimated from the non-linear regression model ROI ~ α*ICV^β. Each ROI was divided by ICV^β. Predictor variables were then explored using the following expression:

$$Z_i = \gamma_0 + \gamma_1 group_i + \gamma_2 age_i + \gamma_3 sex_i + \gamma_4 site_i + e_i,$$

In this expression, Z_i is the adjusted ROI volume for the *i*th participant, and group, sex and site were expressed as numeric factors.

The DM1-affected group had significantly reduced volume in whole cerebrum, primarily driven by grey matter loss ($p < 0.0001$). Cerebral lobes with the greatest volume difference were frontal lobe (both grey and white matter; $p < 0.0001$ and 0.05 respectively) and parietal lobe (grey matter $p < 0.0001$, white matter $p = 0.123$). In the cerebellum, only white matter volume was significantly reduced ($p < 0.01$). Additional structures with reduced volume in the DM1-

affected group were the corpus callosum ($p < 0.001$), putamen ($p < 0.05$), accumbens ($p < 0.05$) and thalamus ($p < 0.01$).

Perhaps surprisingly, hippocampus and amygdala volumes were significantly increased in the DM1-affected group compared with controls ($p < 0.05$, < 0.0001 respectively). Prior to ICV correction however, the size of these structures was closely matched between the two groups. The group effects for ICV-adjusted ROIs are summarised in Table 16 and Figure 31.

Table 16: Standardised beta coefficients, t-statistics, significance levels and confidence intervals of group differences in regional volumes, adjusted for age, sex and intracranial volume. Analysis by Prof Jeffrey D. Long, University of Iowa.

Region of interest	Standardised beta coefficient	t-statistic	p	Lower 95% confidence interval	Upper 95% confidence interval
ICV*	-0.537	-4.099	0.00007	-0.794	-0.280
CSF*	0.497	3.831	0.0002	0.243	0.751
Cerebrum*	-0.561	-4.211	0.00005	-0.823	-0.300
Cerebrum GM*	-0.498	-4.402	0.00002	-0.720	-0.276
Cerebrum WM	-0.328	-1.864	0.065	-0.673	0.017
Cerebellum	-0.297	-1.786	0.076	-0.624	0.029
Cerebellum GM	-0.195	-1.167	0.245	-0.523	0.133
Cerebellum WM*	-0.457	-2.660	0.009	-0.793	-0.120
Frontal Lobe*	-0.584	-4.309	0.00003	-0.850	-0.319
Frontal GM*	-0.505	-4.402	0.00002	-0.730	-0.280
Frontal WM*	-0.416	-2.398	0.018	-0.755	-0.076
Parietal Lobe*	-0.561	-3.653	0.0004	-0.863	-0.260
Parietal GM*	-0.562	-4.227	0.00004	-0.823	-0.302
Parietal WM	-0.274	-1.551	0.123	-0.619	0.072
Temporal Lobe	-0.184	-1.111	0.269	-0.510	0.141
Temporal GM	-0.131	-0.818	0.415	-0.446	0.183
Temporal WM	-0.199	-1.120	0.265	-0.548	0.149
Occipital Lobe	-0.002	-0.011	0.991	-0.345	0.341
Occipital GM	-0.150	-0.888	0.376	-0.480	0.181
Occipital WM	0.304	1.739	0.084	-0.039	0.646
Basal Ganglia	-0.146	-0.921	0.359	-0.457	0.165
Caudate	0.159	0.919	0.360	-0.180	0.497
Putamen*	-0.330	-2.060	0.041	-0.643	-0.016
Pallidum	-0.033	-0.207	0.836	-0.347	0.281
Accumbens*	-0.377	-2.259	0.026	-0.704	-0.050
Thalamus*	-0.382	-2.791	0.006	-0.651	-0.114
Hippocampus*	0.422	2.413	0.017	0.079	0.765
Amygdala*	0.858	5.373	0.0000003	0.545	1.171
Corpus Callosum*	-0.586	-3.533	0.0006	-0.911	-0.261
Hypothalamus	0.032	0.184	0.854	-0.309	0.373

ICV = intracranial volume, CSF = cerebrospinal fluid, GM = grey matter, WM = white matter. ROIs in which the group difference was significant at $p < 0.05$ are marked *.

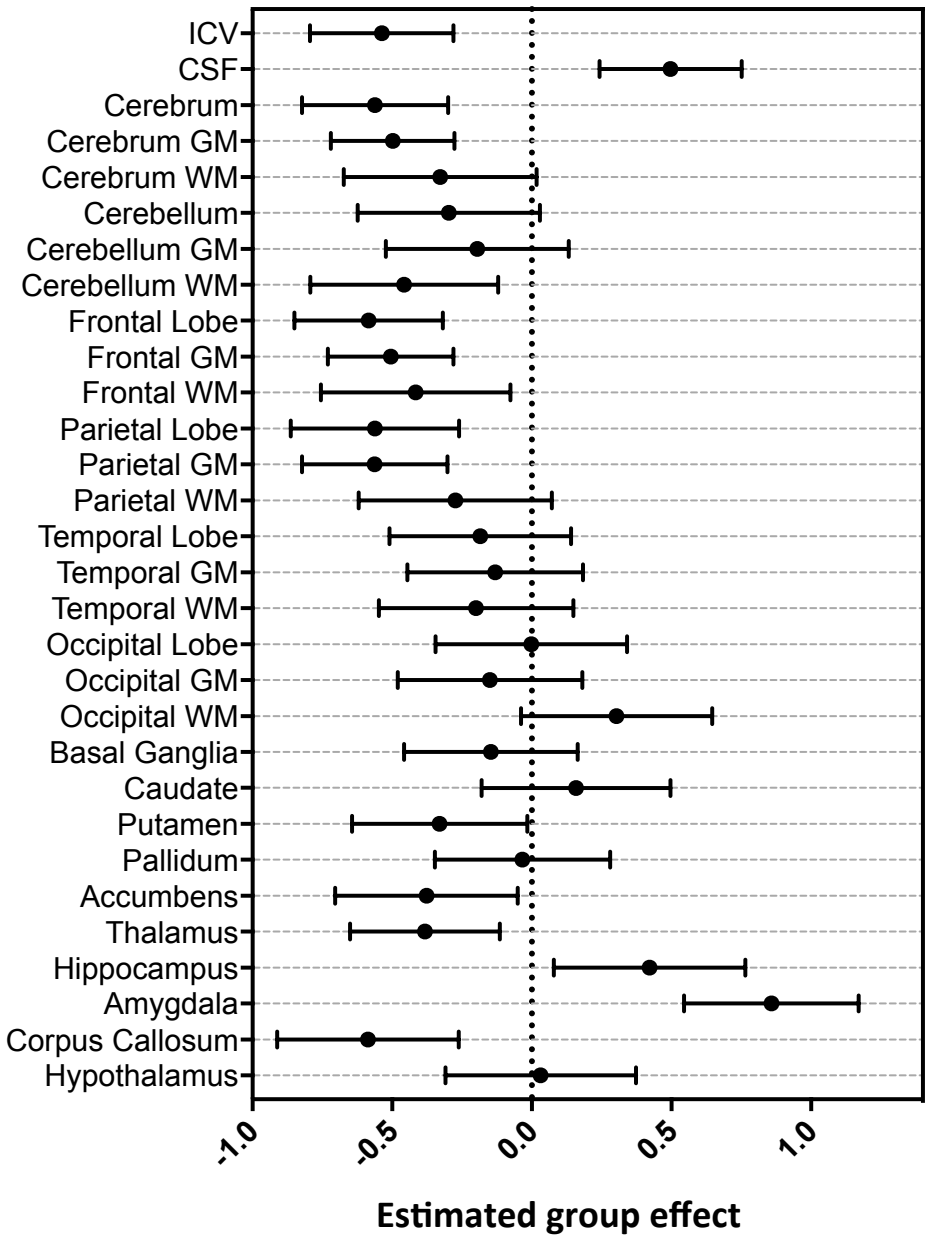

Figure 31: Estimated effect of group (DM1 versus controls) in the measured regions of interest, adjusted for age, sex and intracranial volume. Error bars represent the 95% confidence interval.

The effect of CTG repeat length on volume of ROIs was also explored, using the following expression:

$$Z_i = \gamma_0 + \gamma_1 ePAL_i + \gamma_2 age_i + \gamma_3 sex_i + \gamma_4 site_i + e_i,$$

This demonstrated that ePAL was inversely correlated with occipital grey matter ($p < 0.05$) and putamen volume ($p < 0.01$). An inverse correlation with thalamus volume approached statistical significance ($p = 0.054$). ePAL was positively correlated with volume of amygdala ($p < 0.05$) and cerebellar white matter ($p = 0.052$).

Table 17: Standardised beta coefficients, t-statistics, significance level and 95% confidence intervals for effect of ePAL against region of interest volumes, adjusted for age, sex and ICV. Analysis by Prof Jeffrey D. Long, Universiy of Iowa.

Region of interest	Standardised beta coefficient	t-statistic	p	Lower 95% confidence interval	Upper 95% confidence interval
ICV	0.00092	1.227	0.224	-0.00101	0.00285
CSF	-0.00049	-0.527	0.600	-0.00289	0.00191
Cerebrum	-0.00025	-0.266	0.791	-0.00270	0.00219
Cerebrum GM	-0.00130	-1.618	0.110	-0.00337	0.00077
Cerebrum WM	0.00189	1.511	0.135	-0.00133	0.00511
Cerebellum	0.00037	0.337	0.737	-0.00246	0.00320
Cerebellum GM	0.00021	0.191	0.849	-0.00265	0.00307
Cerebellum WM	0.00211	1.976	0.052	-0.00064	0.00487
Frontal Lobe	-0.00009	-0.100	0.920	-0.00246	0.00227
Frontal GM	-0.00109	-1.344	0.183	-0.00318	0.00100
Frontal WM	0.00151	1.290	0.201	-0.00151	0.00454
Parietal Lobe	0.00096	0.917	0.362	-0.00174	0.00366
Parietal GM	-0.00004	-0.045	0.964	-0.00234	0.00226
Parietal WM	0.00224	1.818	0.073	-0.00093	0.00541
Temporal Lobe	0.00004	0.044	0.965	-0.00249	0.00258
Temporal GM	-0.00056	-0.575	0.567	-0.00308	0.00195
Temporal WM	0.00146	1.285	0.203	-0.00147	0.00439
Occipital Lobe	-0.00212	-1.817	0.073	-0.00512	0.00088
Occipital GM*	-0.00281	-2.518	0.014	-0.00569	0.00006
Occipital WM	-0.00010	-0.083	0.934	-0.00314	0.00295
Basal Ganglia	-0.00168	-1.567	0.121	-0.00445	0.00108
Caudate	0.00007	0.056	0.955	-0.00304	0.00317
Putamen*	-0.00302	-2.899	0.005	-0.00571	-0.00034
Pallidum	0.00010	0.096	0.924	-0.00267	0.00287
Accumbens	-0.00013	-0.119	0.906	-0.00284	0.00259
Thalamus	-0.00163	-1.959	0.054	-0.00377	0.00051
Hippocampus	-0.00018	-0.159	0.874	-0.00307	0.00272
Amygdala*	0.00226	2.178	0.033	-0.00041	0.00493
Corpus Callosum	-0.00103	-0.952	0.344	-0.00382	0.00176
Hypothalamus	0.00112	0.954	0.343	-0.00190	0.00413

ROIs in which the effect of ePAL was significant at $p < 0.05$ are marked *.

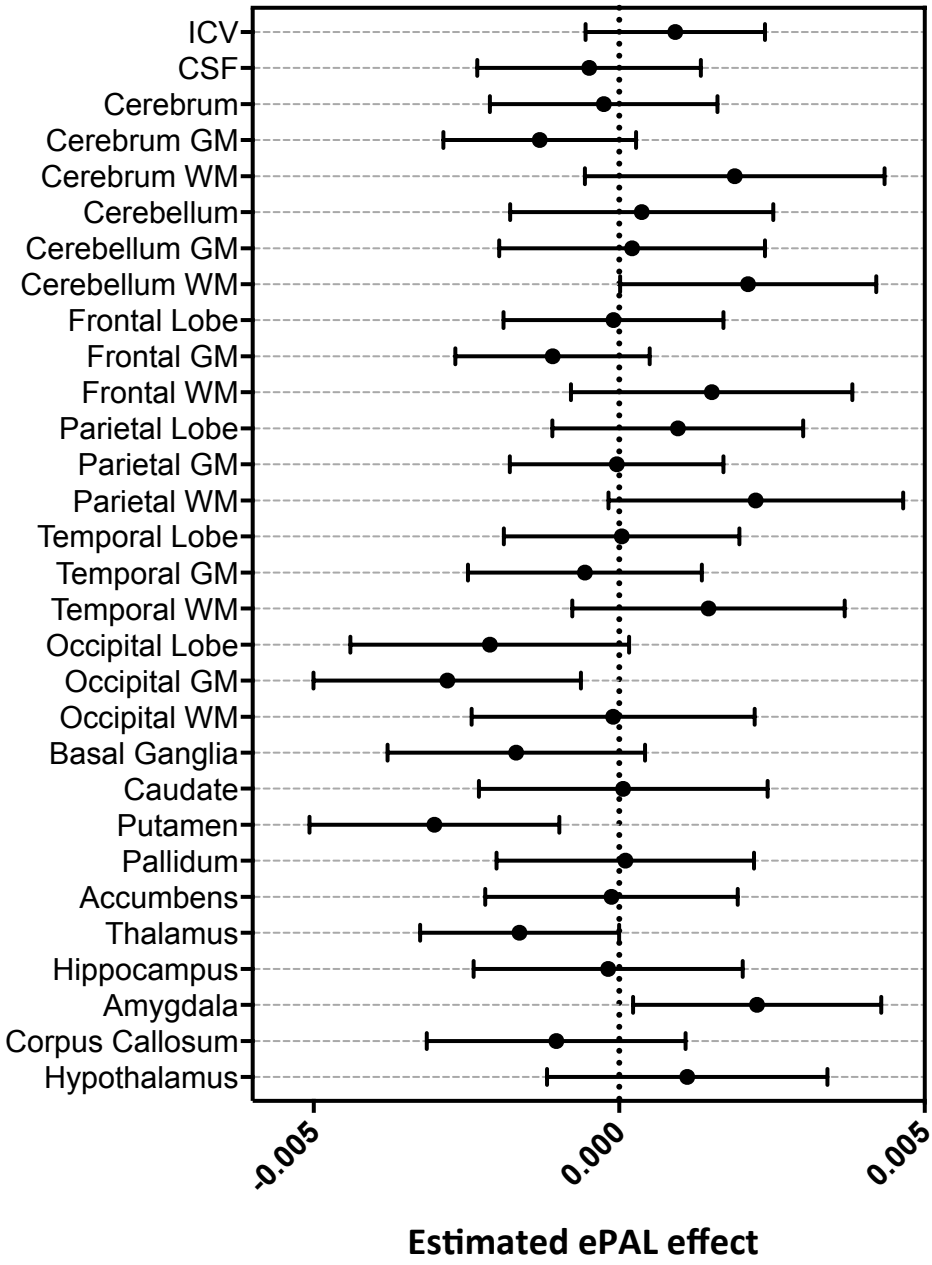

Figure 32: Estimated effect of ePAL on volume of regions of interest, adjusted for age, sex and intracranial volume. Error bars represent the 95% confidence interval.

6.3.3 Effect of variant repeats

Seven individuals were identified as likely carrying variant repeats, either due to positive AciI enzyme digest, or repeated failure of the expanded allele to amplify by SP-PCR. These were the three previously identified in the Glasgow group, and four from Iowa – giving a total incidence in our pooled cohort of 8.9%. For the purposes of the following analyses, an approximation of ePAL was estimated for the individuals in whom SP-PCR failed from the lower boundary of the smear from Southern blotting of restriction digested genomic DNA.

In the group as a whole, logPAL was inversely correlated with age at onset of symptoms ($p < 0.001$, Adj $R^2 = 0.322$). Two individuals with variant repeats denied symptoms of DM1. In the remaining five, there was no clear effect of the presence of variant repeats on subject's self-reported age at onset (Figure 33).

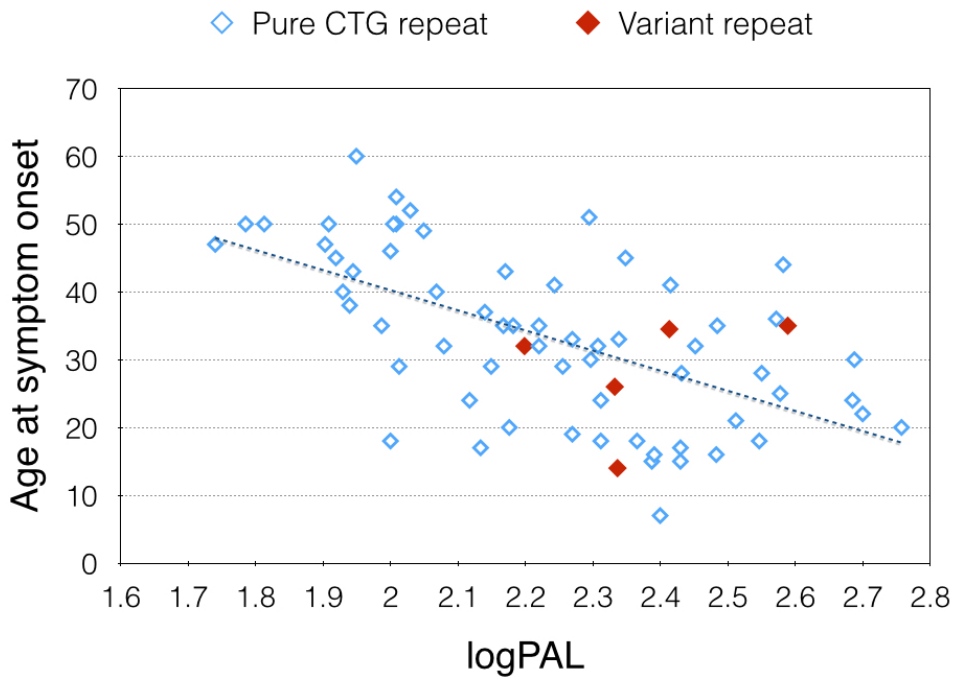

Figure 33: Age at onset of symptoms plotted against logPAL in the pooled cohort. Individuals with variant repeats are highlighted in red.

To further explore the effects of variant repeats, individuals carrying variant alleles were paired with pure CTG repeat-carrying subjects of the same sex, and who were as closely matched for age and ePAL as possible (Table 18).

Table 18: matching of individuals with variant repeats with pure CTG repeat-carrying subjects of the same sex, and similar with respect of age and ePAL

	Variant repeat subject	Matched pure CTG repeat subject
Study ID sex; age (years); ePAL; cohort	**006A** M; 36; 158; GLA	**032A** M; 31; 150; GLA
	052A F; 22; 251; GLA	**004A** F; 23; 269; GLA
	059A M; 33; 217; GLA	**736** M; 34; 270; IOA
	917 M; 38; 259; IOA	**172** M; 37; 246; IOA
	871 F; 50; 215; IOA	**021A** F; 51; 218; GLA
	697 M; 21; 276; IOA	**839** M; 19; 152; IOA
	890 F; 50; 388; IOA	**018A** F; 55; 382; GLA

M = male, F = female, GLA = Glasgow cohort, IOA = Iowa cohort

The level of somatic instability in blood leukocytes at the time of sampling (ΔCTG) was estimated by subtracting the ePAL from MAL. This was not possible for the two subjects in whom SP-PCR had failed, as an estimate for MAL was not available. With the exception of one subject with a small expansion of 158 repeats, the variant repeats were consistently associated with lower ΔCTG Table 19).

Table 19: Somatic instability in blood leukocytes at time of sampling

Variant repeat subject				Matched pure CTG repeat subject			
ID	ePAL	MAL	Δ CTG	**ID**	ePAL	MAL	Δ CTG
006A	158	196	38	**032A**	150	178	28
052A	251	276	25	**004A**	269	435	166
059A	217	249	32	**736**	270	552	282
917	259	329	70	**172**	246	502	256
871	215	ND	ND	**021A**	218	418	200
697	276	ND	ND	**839**	152	196	44
890	388	516	128	**018A**	382	998	616

ND = no data

Volumetric measures between the two groups were compared. Mean ICV was fractionally larger in the group with variant repeats (1.415 litres versus 1.397 litres; p = 0.749). Differences in ROI volumes were explored using ICV-adjusted values (ROI / ICV^ β). There was no clear effect of presence of variant repeats on ICV-adjusted ROI volumes (Figure 34).

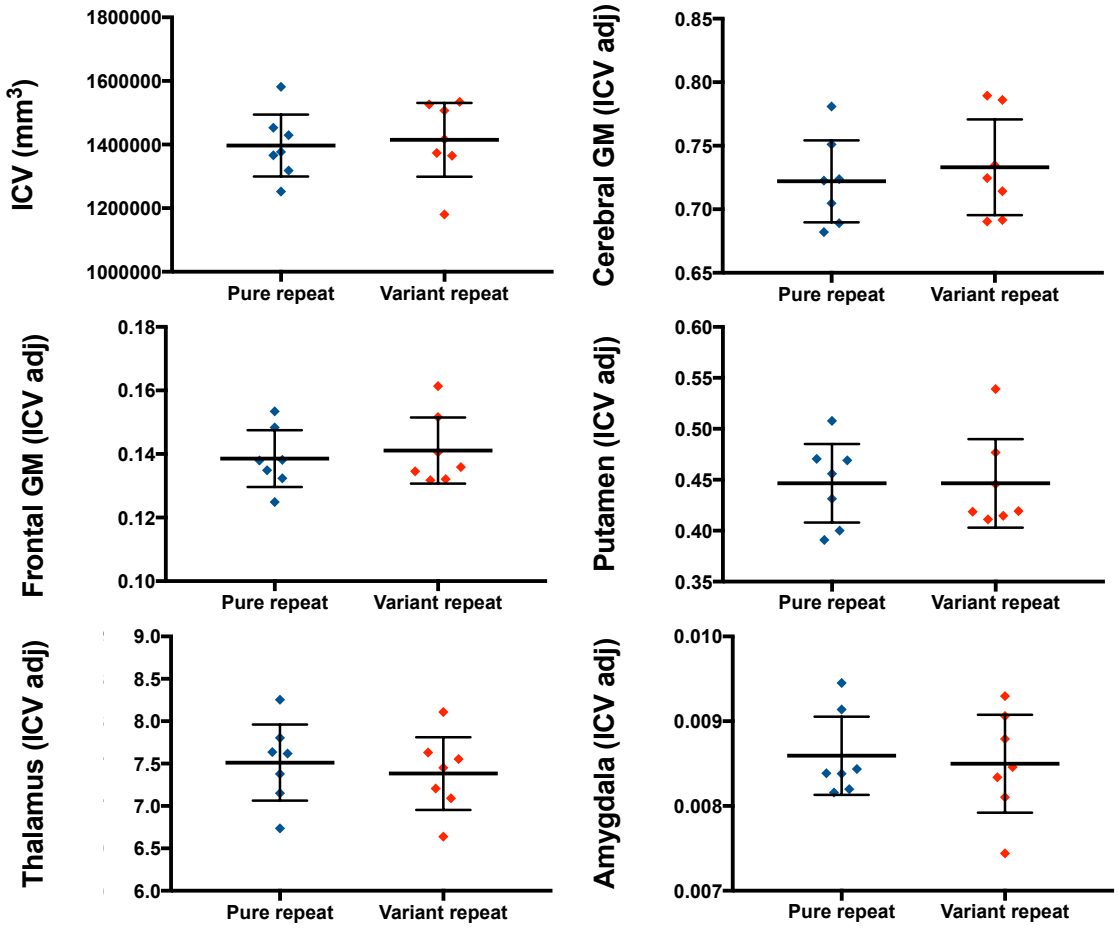

Figure 34: Comparison of intracranial volume and regional brain volumes in individuals with variant repeats, compared with age, sex and ePAL-matched participants with pure CTG repeats

GM = grey matter, ICV Adj = adjusted for intracranial volume. Black bars indicate mean and 95% confidence intervals.

6.4 Discussion

In this chapter, brain imaging data were harmonised from two geographically distinct, case-controlled DM1 populations, enabling exploration of structural brain features in almost 80 affected individuals; the largest DM1 imaging cohort reported to date. Using a joint-label fusion approach, the results confirm group differences in regional volumetric measures that are driven by CTG repeat length, and provide a plausible link to clinical features observed in individuals affected by DM1.

Firstly, consistent with our findings in the Glasgow cohort alone, we found total ICV was reduced in the DM1-affected group compared with controls. This adds further evidence for a possible effect of DM1 on early brain development, even in individuals whose manifest symptoms of DM1 were not apparent until adulthood.

While considering factors that might influence intracranial volume, it was noted that several DM1-affected female individuals in both cohorts had appearances of frontal skull thickening, consistent with hyperostosis frontalis interna (HFI). Previous authors have highlighted thickening of the calvarium, in particular affecting the frontal region, as a feature of DM1 [265]. The mechanisms underlying these skull changes are not well understood. In the general population, HFI is associated with increasing age and female sex, occurring most frequently in females after the age of menopause. It is rarely encountered in males, except in the context of androgen deprivation, either by iatrogenic androgen blockade [266] or surgical castration [267]. As such, its pathogenesis is thought to be related to endocrine changes, possibly driven by hypogonadism [268]. DM1 recapitulates risk factors for HFI as part of the disease process, since affected men commonly exhibiting low testosterone levels with testicular atrophy [269], and early menopause and subfertility reported in females [270].

Useful further work within our own dataset would include more detailed exploration of skull morphological changes in DM1 compared with controls, including sex effects and quantifying the contribution of HFI to reduced intracranial volume. Further, while HFI in itself is usually not thought to be of direct clinical consequence, rare case reports do exist of severe HFI impinging on

the brain to cause neuropsychological sequelae [271,272], and so correlations with functional measures could also be explored.

Cortical volume reduction in DM1 patients was driven predominantly by grey matter loss affecting frontal and parietal lobes. Impaired frontal lobe function provides a plausible substrate for a number of clinical features described in DM1, including apathy [130], impaired emotional regulation [125], deficiencies of theory of mind [126], and deficits in higher cognitive functioning [236]. The association between parietal volume loss and clinical features is less immediately clear, since cardinal features of parietal lobe syndromes such as impaired tactile localization, neglect syndromes, eye movement disorders or dyspraxia are not considered core features of the DM1 phenotype. Individuals with DM1 do however perform poorly in tests of visuospatial cognition, such as the block design test [112], and lack of disease awareness consistent with anosognosia is also well described [202], both of which could be postulated to arise from parietal lobe dysfunction.

Among subcortical structures, greatest volume loss was seen in thalamus, putamen and nucleus accumbens. In the general population, decreasing thalamic volume is associated with decline in processing speed as part of cognitive aging [262]. It is therefore interesting to note slowing of basic processing is a key feature of DM1, evidenced by poorer performance in Trail Making Test A [112,115,241], and the word card of the Stroop colour-word test [239]. The thalamus also plays a role in sleep and wakefulness, forming both an important neural relay in the ascending arousal system [150], as well as generating the sleep spindles that characterise stage 2 sleep [263]. Impaired thalamic function therefore could contribute to the excessive daytime somnolence, and reduction in stage 2 sleep described in DM1 [95]. The putamen has complex connections with basal ganglia and cortical structures, and has a role in movement, learning and higher cognitive processes. In particular, the putamen is implicated in the control and speed of motor tasks [273]. This gives the insight that some impairment of motor function in DM1 could be related to central deficits as opposed to primary muscle disease.

Loss of volume in the nucleus accumbens may provide a link to mood disorder and apathy in DM1. The nucleus accumbens is broadly considered a key player in

the reward system, and so plays a role modulating motivation and goal-directed behaviour [274]. Smaller volume of nucleus accumbens [275,276], and reduced activation on fMRI [277] have been linked to symptoms of depression, apathy and anhedonia. Social withdrawal in DM1 therefore could be heightened by a blunted ability to gain reward and enjoyment from participation.

A particularly surprising, and apparently novel finding in this study was of increased relative volume of some brain structures in DM1 subjects compared with controls; specifically the hippocampus and amygdala. Whether these changes might represent a loss of mechanical growth restriction due to atrophy of adjacent structures, abnormal growth driven by the molecular pathology of DM1, or a compensatory change to serve a more specific functional purpose is unclear. The similarity in volume of these structures between DM1 subjects and controls before ICV correction might even suggest simply a relative sparing of these structures from disease-specific atrophy. The hippocampus is strongly implicated in memory formation, and verbal memory appears relatively preserved until late in the disease course of DM1 [123]. Interestingly, the dentate gyrus of the hippocampus is also one of the few regions in the adult brain where new neuron cells can be generated from progenitor cells [278], and so this structure could be capable of compensatory growth if somehow spared from the primary disease process in DM1.

The amygdala is involved in the generation of negative emotional states including fear and anxiety [279], as well as judgment of facial expressions [280]. In the general population, increasing amygdala volume is positively associated with size and complexity of social networks [281], contrasting with the classical DM1 neuropsychological phenotype of social avoidance. Pathological over activity of this structure, however, has been linked to social anxiety disorders [282] and borderline personality disorder [283], in which affected individuals have impaired ability to regulate emotional responses. In normal neurophysiology, a process of cognitive reappraisal can modulate negative emotional responses. This process involves an individual seeking to alter their interpretation of an experience or stimulus to reduce its emotional impact. Successful reappraisal involves recruitment of additional brain regions, including the prefrontal cortex and ventral striatum, including nucleus accumbens

[284,285]. It could therefore be postulated that, because these structures show atrophy in the DM1 brain, there may be a tendency towards failure of reappraisal, and hence greater inclination to associate external stimuli with negative emotion. In turn, this could further perpetuate a tendency towards avoidant behaviours.

We observed an inverse correlation between CTG repeat size and volume in occipital grey matter, putamen and thalamus, and a positive correlation with amygdala volume. The processes driving structural brain changes in DM1 are relatively poorly understood, and are likely to be complex, including the impact of dysregulated alternative splicing on a range of critical pathways. One example is the shift in alternative splicing of tau protein seen to occur in DM1-affected neurons, which favours fetal isoforms. These forms have poorer affinity to bind and stabilise the microtubular cytoskeleton, and may impact axonal transport [286]. The presence of neurofibrillary tangles on histological examination of DM1 brains supports a role of tau dysfunction in CNS pathogenesis [287]. Cell signalling within the CNS is also likely to be impacted, due to abnormal splicing of a range of receptors and signalling molecules. For example, *NMDAR1* is a heteromeric glutamate-gated ion channel with a key role in synaptic transmission, which is known to be subject to alternative splicing in the presence of increased activity of CUG-BP due to DM1 [288]. The functional impact of this observation has not yet been explored.

Our observation of a possible effect of DM1 on neural development, as reflected by reduced ICV, adds a further perspective to pathogenesis in brain. In a related neurodegenerative disorder, Huntington disease, it has been postulated that abnormal neural development, occurring long before overt onset of symptoms, might render some populations of neurons more susceptible to environmental or endogenous stressors over time, thus contributing to the apparent neurodegenerative phenotype [289]. This hypothesis highlights understanding the neurodevelopmental impact of DM1, even in adult-onset populations, as a major research priority.

Somatic instability of the CTG repeat in DM1 varies between tissues, generally being higher in the tissues most affected such as muscle [27], supporting this somatic instability as a significant modifier of disease progression. Somatic

instability also varies within the CNS, for example being much higher in frontal cortex compared with cerebellum [28]. Detailed topography of instability in different brain tissues has not been widely explored, but we hypothesise that greatest structural change is likely to occur in regions with the greatest somatic instability, and hence more severe dysregulation of alternative splicing. Further exploration of this hypothesis is currently limited by availability of human DM1-affected brain tissue for research, but if confirmed, this observation would further highlight somatic instability as a promising target for therapeutic intervention within the CNS.

Given our hypothesis that somatic expansions are a key modifier of CNS pathology in DM1, we anticipated that variant repeats showing increased stability in blood leukocytes would be associated with milder structural brain changes. Comparison of key regional volumes between variant-carrying subjects and pure CTG subjects, matched for age, sex and ePAL, did not show a clear effect however. The seven individuals carrying variant repeats were comparatively young, (mean age 36 years; range 21 to 50), and so one possible explanation for this is that any modifying effect of variant repeats on age-dependent volume loss may be less apparent in younger subjects. Second, a small sample of only seven individuals is susceptible to sampling error. Smoking status of the Iowa subjects was not known, but two individuals in the Scottish cohort were current smokers, which has been linked to diffuse grey matter volume loss [290]. Finally, volumetric measures show considerable background variation in general population subjects, and volume alone gives little information with regard to structural integrity or function. Hence exploration of further imaging modalities such as DTI and fMRI may reveal more marked modifying effects of variant repeats.

6.5 Conclusions

In conclusion, this portion of the study demonstrates feasibility and value in the harmonisation of imaging datasets from different sites, to achieve larger sample sizes for DM1 research. Our data confirm regional volumetric changes in cortical and subcortical structures, some of which correlate with CTG repeat length, and provide potential links to symptoms experienced by patients. This underlines the importance of CNS disease as a target for new therapy development in DM1, and

identifies structures representing strong candidates for use as imaging biomarkers in the context of clinical evaluation and drug trials. Further longitudinal studies are required to identify markers that show progression within the timescale of a clinical trial. Additional limitations of the current study to be addressed by future work include correlation of volumetric changes with clinical measures, and application of additional MRI modalities such as DTI to evaluate integrity of white matter tracts, and fMRI to explore functional networks linking the affected regions.

7 Sleep

7.1 Summary

This chapter describes the results from domiciliary polysomnography and modified maintenance of wakefulness tests, applied to the Scottish DM1-affected cohort. Compared with normative data, results confirm altered sleep architecture in DM1, characterised by an increased proportion of slow-wave and rapid eye movement sleep. Sleep efficiency was also reduced, and correlated inversely with age.

Analysis of respiratory events showed a high prevalence of sleep-disordered breathing, present in 86% of participants by standard criteria. Over half had moderate or severe sleep-disordered breathing. In linear regression analysis, frequency of respiratory events was positively associated with age and male sex. Individuals with moderate to severe sleep-disordered breathing, on average, reported greater muscle weakness, more sleepiness or fatigue symptoms, and had higher pCO_2 on capillary blood gas sampling. However, no single measure had strong power to distinguish those with clinically significant disordered breathing from those without. Sleep latency measured by the modified maintenance of wakefulness test was not strongly associated with self-reported sleepiness, although shorter latencies weakly correlated with longer CTG repeat, higher MIRS score and greater Epworth score.

Structural MRI data demonstrated an association between sleep-disordered breathing and white matter volume loss affecting frontal and parietal lobes. One possible interpretation of this findings is that it represents evidence that sleep-disordered breathing has the potential to cause end-organ damage in DM1, and hence would support a proactive approach to identifying and treating nocturnal hypoxia. Reduced grey matter volume was associated with an increased percentage of rapid eye movement sleep in widespread cortical and subcortical regions, and shorter sleep latency was associated with volume loss in several cortical regions, as well as the anterior cingulate. The latter finding demonstrates a potential direct link between structural damage to the ascending arousal system and excessive somnolence symptoms.

7.2 Introduction

7.2.1 Excessive daytime sleepiness and sleep disorder in DM1

Excessive daytime sleepiness (EDS) is an extremely common symptom in DM1, impacting quality of life in up to 90% of affected individuals [132]. Patients describe a persistent feeling of sleepiness, that is not erased by overnight sleep or daytime naps [134]. There may be a tendency to doze off throughout the daytime, particularly if attention is not held [133], which in turn may significantly hamper ability to meet social or family obligations [104,137]. Despite its ubiquity however, the mechanisms underlying EDS are poorly understood, and as a result treatment options and consensus guidance for clinicians are lacking with respect to this disabling symptom [149].

Assessment of sleep by polysomnography (PSG) reveals that sleep-disordered breathing (SDB) is prevalent in DM1 [134,140], occurring more frequently in this condition even in comparison to other neuromuscular disorders with a similar pattern of weakness [148]. Since obstructive sleep aponea is a common cause of daytime sleepiness in the general population [291], it is generally held that DM1 patients with EDS should be screened for sleep disordered breathing, and treatment with overnight positive airway pressure (PAP) therapy initiated if this is present [149]. Screening for SDB is not straightforward in DM1 however, since the gold standard method to diagnose SDB in the clinical setting traditionally involves inpatient PSG [292], an approach that is financially expensive for health services, and potentially burdensome for patients with the physical and cognitive difficulties associated with DM1. Furthermore, because SDB may be present in DM1 patients with normal daytime respiratory assessments [145-147] or those who deny EDS symptoms [146], clinicians currently lack clear guidance on which individuals should be referred for further investigation of sleep.

Although SDB is a common finding in DM1, evidence suggests that sleep fragmentation due to apnoeas is not the principal driver of daytime sleepiness symptoms. EDS may be present in individuals with normal respiratory parameters during sleep [134,140,142], and treatment with PAP therapy or non-invasive

ventilation may have little or no impact on EDS symptoms in some individuals [97,293]. These observations suggest that central factors make a major contribution to subjective sleepiness symptoms. Further evidence for a central sleep disorder includes the observation that sleep architecture is altered in DM1 independently of SDB, including an increased total sleep time, greater fragmentation of sleep [138,139] and longer periods spent in slow wave and REM stages of sleep [95,140]. In addition, patterns of breathing consistent with a central dysregulation of respiratory drive occur, as well as the obstructive apnoeas considered more typical of neuromuscular disorders [145,146]. Again however, no clear relationship has been yet described between CNS involvement in DM1 and sleep disturbance or EDS symptoms (reviewed in [149]).

7.2.2 Objective measures of excessive sleepiness

Given the limitations of self-reported symptom questionnaires in DM1 demonstrated in the preceding chapters, an objective measure of EDS is desirable for use in both clinical and research contexts. Traditionally, objective measurement of EDS is achieved by use of the multiple sleep latency test (MSLT). A typical MSLT protocol includes at least four tests at 2 hourly intervals, during which the subject is monitored by electroencephalogram (EEG). The subject is asked to assume a comfortable position in a bed within a quiet, temperature-controlled room. The lights are dimmed, and the subject is then given the instruction “please lie quietly, keep your eyes closed, and try to fall asleep”. The test is typically terminated after 20 minutes if no sleep has occurred [294]. The MSLT aims to measure the “manifest sleep tendency”; the physiological ability to transition into sleep in the absence of alerting factors. The MSLT has some limitations however, in that it is not thought to be sensitive to variation among individuals at the severe end of the sleepiness scale, and the 20-minute time limit introduces a ceiling effect in those more mildly affected [295].

To address some of these limitations, the Maintenance of Wakefulness Test (MWT) was developed in 1982 [296]. In the MWT, the subject is asked to sit up in a quiet, dimly lit room. Crucially, the instructions given ask the subject to try to remain *awake*, rather than to try to fall asleep. Four or five tests are undertaken at 2 hourly intervals. The test therefore measures the time the subject is able to

volitionally resist sleep in soporific conditions, which was reasoned to represent a more ecologically relevant measure for workplace or driving safety compared with the MSLT [295].

The MSLT, but not to our knowledge the MWT, has been applied in several clinical studies of DM1, demonstrating significantly shorter sleep latencies compared with controls [95,138]. Very short sleep latencies (less than 8 minutes) [95,138] and sleep onset REM periods (SOREMP; presence of REM sleep within 15 minutes of sleep onset) [95,140-142], were generally rare. Sleep latencies measured by MSLT do not appear to correlate well with self-reported sleepiness scores [95,139].

In the present study, we chose to apply a version of the MWT, since inability to resist sleep despite the volition to do so (*e.g.* dozing off during a favourite television programme) appears to best represent the 'real world' impact of EDS symptoms in DM1 [104,137]. As was the case for PSG, we felt that uptake and adherence to the study protocol would be severely compromised by lengthy in-patient studies, hence a modified maintenance of wakefulness test (mMWT) protocol was devised. Novel features of our mMWT protocol included the setting, in that it was undertaken in the patient's own home rather than a controlled sleep lab. One to three nap opportunities were performed, while a typical mMWT would involve at least four. Finally, the test was not monitored in real-time, hence could not be terminated immediately upon the subject falling asleep. This feature did however offer the advantage of characterising any sleep that did occur during the test, including detecting onset of REM sleep, which might constitute a SOREMP.

7.2.3 Aims of the present study

Given the clear area of need to better understand sleep in DM1, we sought to evaluate the prevalence and nature of sleep disorders in our clinically and radiologically well-characterised DM1 cohort. In so doing, we aimed to evaluate an unattended, domiciliary Type II PSG system [297], and domiciliary mMWT protocol for use in DM1 patients. While unattended PSG systems bring benefit in terms of cost and convenience, these benefits are offset by an increased potential failure rate, since the recording is not monitored in real-time, and no

technician is present overnight to reposition detached leads. If well tolerated and technically adequate, domiciliary type II PSG would represent a viable alternative to inpatient studies for DM1 patients.

Second, we aimed to explore whether specific clinical parameters can be identified that place individuals at increased risk of SDB. Understanding such risk factors would aid risk stratification in the clinic setting, as well as potentially offering better insight into the causes of SDB in DM1.

Recent evidence suggests that compliance with home ventilation improves survival in patients with DM1 [298]. The mechanisms by which the improved survival is mediated, however, is unclear. Having identified which subjects in our cohort have clinically significant sleep disordered breathing, we aimed to assess whether these patients showed increased evidence of end-organ dysfunction compared with other DM1-affected subjects.

Finally, we sought to describe the relationship between structural brain changes and sleep disorders.

Specific hypotheses were as follows:

- Presence of SDB may be poorly predicted by self-reported somnolence symptoms. Current Scottish guidelines for management of DM1 advise criteria of worsening dyspnoea, morning headaches, recurrent respiratory infections or Epworth Sleepiness Score greater than 12 as triggers for referral to a respiratory physician [299]. These triggers may be confounded by impaired disease awareness, leading to under-ascertainment of sleep disorders in this cohort.

- Presence of moderate to severe SDB may be associated with more severe end-organ effects of DM1 in general, including structural brain change, cardiac and endocrine markers, independent of differences in age, repeat size and BMI.

- Further, we speculated that changes involving the respiratory pacemaker regions of the medulla [205] may be linked to frequency of central apnoea

events. We also hypothesised that volume loss affecting the thalamus could be the substrate for decreased stage 2 sleep seen in DM1, and that excessive daytime sleepiness (measured as shortened sleep latency in mMWT) would be linked to structural changes affecting structures of the ascending arousal system, including thalamus and hypothalamus.

7.3 Results

7.3.1 Recruitment and adequacy of PSG and mMWT data

All DM1-affected participants in the original imaging study (n.46) were invited to undertake domiciliary polysomnography and mMWT tests. This included the subject subsequently found to carry a premutation allele. Three individuals declined, two did not respond to invitations and two were excluded as they were consistently using nocturnal ventilation or PAP therapy.

Domiciliary polysomnography (PSG) was therefore attempted in 39 participants. The quality of four studies were not adequate for analysis; one because EEG failed entirely, one because EEG failed after 140 minutes (capturing only 90 minutes of sleep), one suffered battery failure of the PSG device, and a fourth subject was unable to tolerate facial electrodes and nasal cannulae. Thirty-five of 39 (89.7%) PSGs were therefore technically valid on the first attempt. PSG was repeated in the subject in whom there was a battery failure, meaning satisfactory PSG data were eventually available for 36 subjects. Of these, 32 had undergone MRI of brain as part of the study (Figure 35).

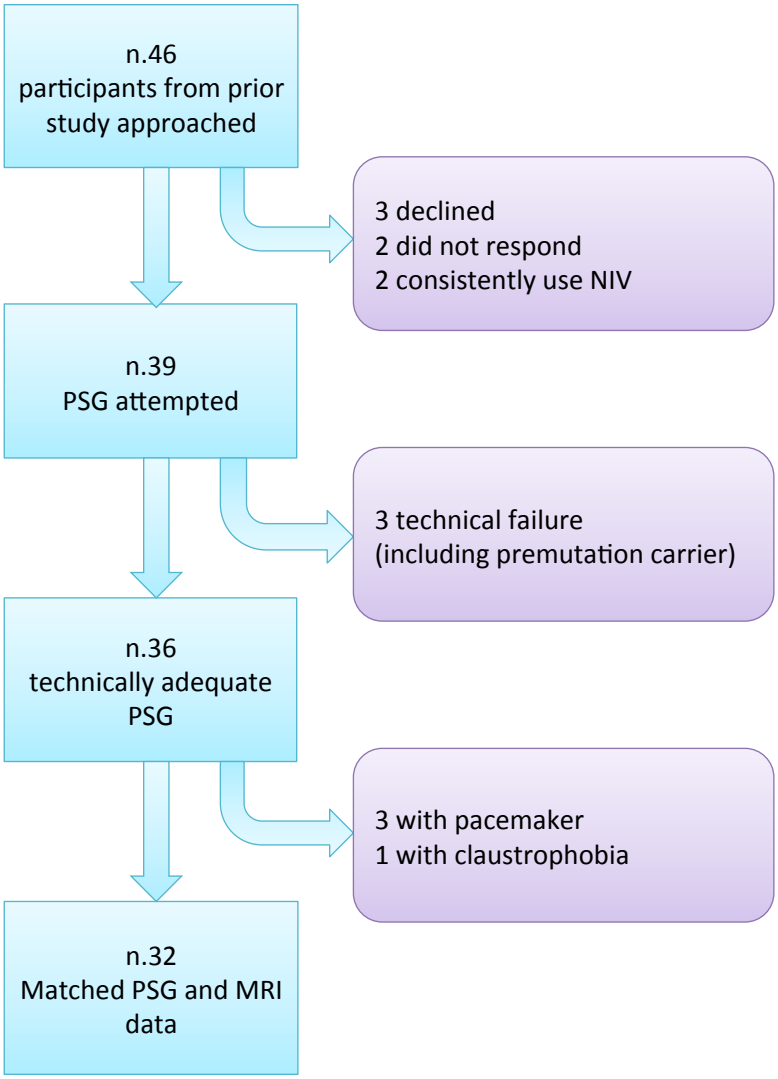

Figure 35: Yield of technically adequate PSG studies

All participants in whom PSG was attempted (n.39) were also asked to undertake mMWT the following morning. One participant declined, as she had found the equipment too uncomfortable overnight. Of the 38 remaining individuals, at least one adequate mMWT trial was recorded in 37. The remaining participant could not tolerate facial or chin electrodes, and so all of her studies were considered inadequate by the reporting clinician (Dr Atalaia). Excluding the premutation carrier, thirty-two of those with MWT data had previously undergone MRI (Figure 36).

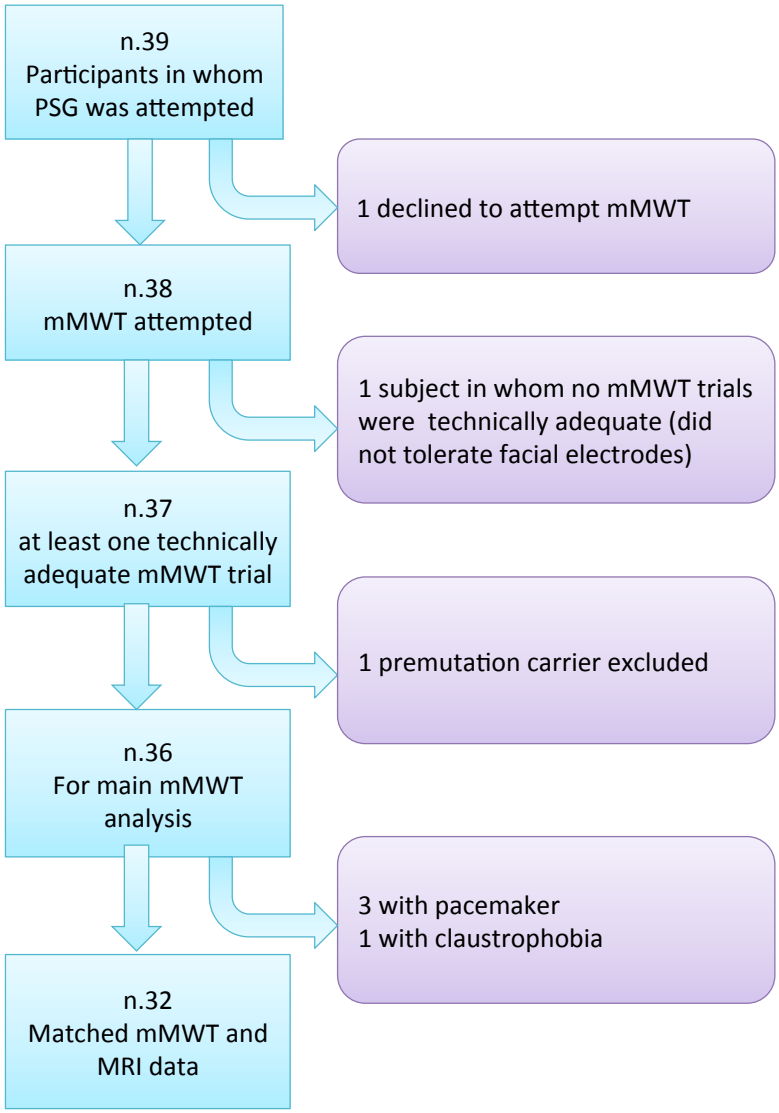

Figure 36: Yield of technically adequate mMWT

The completeness of mMWT data was hampered by both technical failure and subject's willingness to complete three trials (Figure 37). Eleven participants declined to complete all three trials. Reasons for this included family commitments, discomfort due to the equipment, or simply finding the 40-minute trials excessively long and dull. Of all of the mMWT trials, data from the first (MWT1) was most complete, available for 36 participants.

Data pertaining to the subject found to be a premutation carrier were withdrawn from analysis. PSG data for this subject (DMN-005A) was not suitable for analysis in any case, since EEG had failed after 140 minutes, although the quality of her three mMWTs had been adequate.

Study number	PSG	MWT1	MWT2	MWT3
DMN-001A				
DMN-003A				
DMN-004A				
DMN-005A	EEG failed after 140 minutes	Withdrawn (premutation carrier)		
DMN-007A				
DMN-008A				
DMN-009A		Inadequate EEG	Inadequate EEG	
DMN-010A	Battery fail – study repeated			
DMN-013A	Inadequate EEG			
DMN-014A				
DMN-016A				
DMN-017A				Inadequate EEG
DMN-020A				
DMN-021A				
DMN-023A				
DMN-024A			Inadequate EEG	
DMN-026A				
DMN-027A				
DMN-028A				
DMN-029A			Inadequate EEG	
DMN-030A				
DMN-031A				
DMN-032A				
DMN-033A				
DMN-035A				
DMN-036A				
DMN-037A				
DMN-038A				
DMN-039A				
DMN-040A				
DMN-041A				
DMN-043A				
DMN-046A				
DMN-047A				
DMN-048A				
DMN-052A				
DMN-053A				
DMN-055A	Subject could not tolerate face electrodes or nasal cannulae			
DMN-067A				

Figure 37: Summary of completeness of PSG and mMWT data
Blue: technically adequate; Red: technical fail; Green: battery failure first attempt, second attempt satisfactory; Black: patient declined; Yellow: technically data adequate but withdrawn from analysis

7.3.2 Cohort demographics

Demographic details of patients with satisfactory PSG and mMWT data are summarised in Table 20 and Table 21 respectively. The mean age of participants who underwent sleep investigation is slightly higher than the original study cohort, possibly reflecting younger subjects in full-time employment being less likely to volunteer for this time-consuming portion of the study. The cohorts

remain diverse however with respect to age, muscle impairment (MIRS score) and CTG repeat length.

Table 20: Demographic details of DM1-affected subjects with PSG data

Total with satisfactory PSG (n.)	36
Female: n. (%)	22 (61.1)
Age: mean (SD)	49.0 (12.1)
MIRS: Ratio 1:2:3:4:5	3:6:7:19:1
BMI: mean (SD)	26.6 (4.6)
ePAL in CTG repeats: mean (SD)	237 (124)
MAL in CTG repeats: mean (SD)	481 (238)

Table 21: Demographic details of DM1-affected subjects with mMWT data

Total with at least one adequate mMWT trial (n.)	36
Female: n. (%)	21 (58.3)
Age: mean (SD)	49.4 (12.4)
MIRS: Ratio 1:2:3:4:5	3:6:8:18:1
BMI: mean (SD)	26.5 (4.64)
ePAL in CTG repeats: mean (SD)	225 (119)
MAL in CTG repeats: mean (SD)	458 (233)

7.3.3 Sleep efficiency and sleep architecture

Sleep efficiency describes the total time spent asleep (total sleep time; TST) as a proportion of the total time spent in bed. In the general population, sleep efficiency decreases with age, from an average of around 95% at age 20 years, to around 80% at age 75 years. The rate of decline is slightly greater in females compared with males [300]. In our DM1 cohort, sleep efficiency also reduced with age ($p = 0.005$, Adj $R^2 = 0.187$). This model improved with inclusion of sex in a multivariate model ($p = 0.006$, Adj $R^2 = 0.223$), with the effect of age appearing greater in males (Figure 38). Body mass index and CTG repeat size (log PAL or MAL) did not improve the model further. Our data suggest a sharper decline in sleep efficiency with age in DM1-affected subjects compared with normative data [300], although a lack of internal controls means that the

contribution of the ‘first night’ effect, due to presence of polysomnography equipment, cannot be quantified.

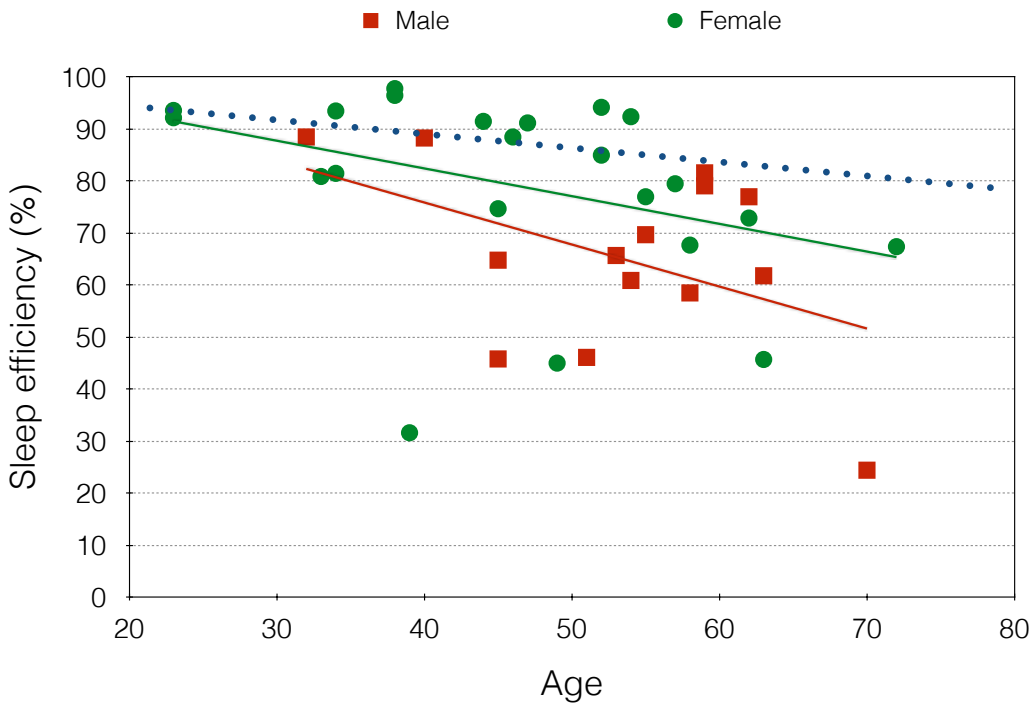

Figure 38: Scatterplot of sleep efficiency against age in DM1-affected subjects
Blue dotted line represents the general population mean, derived from meta-analysis of multiple studies (Ohayon *et al.* n.3,577) [300]

Study subjects on average spent around 7% of total sleep time (TST) in stage 1 sleep, 47% in stage 2, 21% in slow-wave sleep and 25% in REM sleep (Table 22). Compared with general population data [300], DM1 subjects spent a greater proportion of TST in slow wave and REM sleep, and less in stage 2 sleep. Percentage of stage 1 sleep was highly variable in the cohort, which is similar to findings in the general population, making comparison difficult. The trend line, however, suggests a possible mean increase in percentage of stage 1 sleep in DM1-affected individuals also (Figure 39).

Table 22: Mean percentage of sleep time in each stage of sleep in the DM1-affected cohort

Sleep stage	% of total sleep time mean (SD)
Stage 1	7.3 (4.4)
Stage 2	47.1 (11.3)
Slow wave sleep (stage 3 and 4)	20.8 (11.4)
REM sleep	24.8 (7.6)

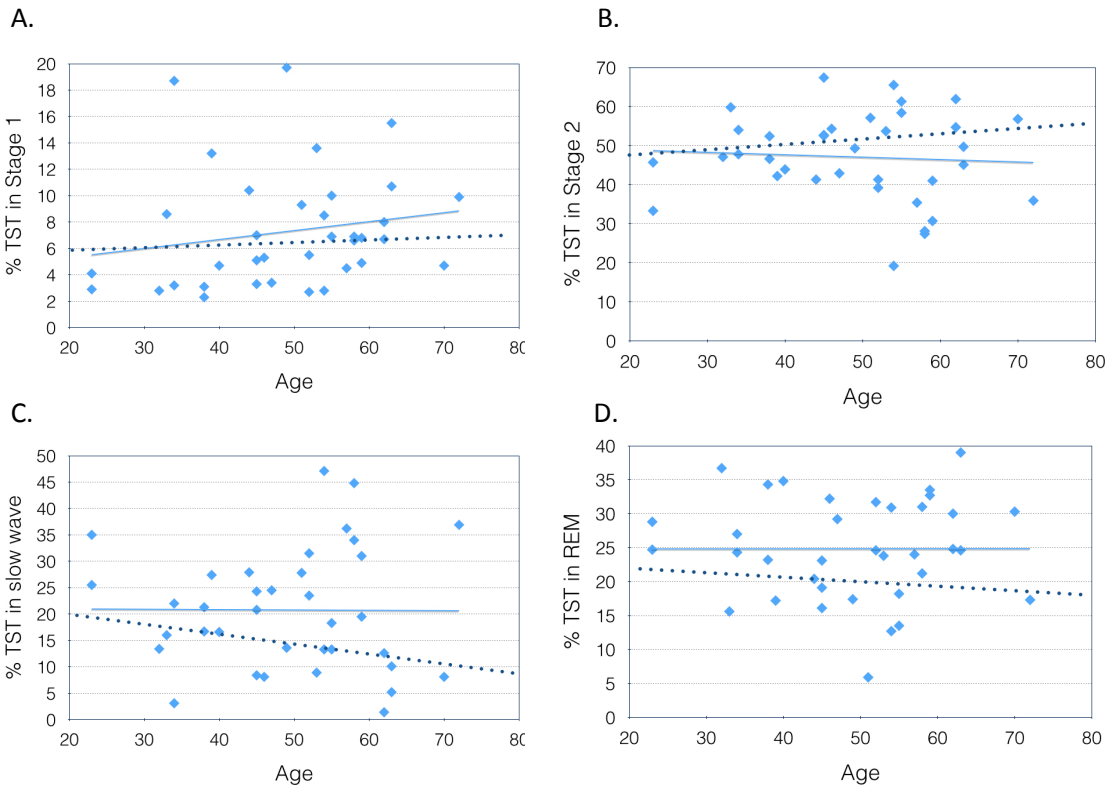

Figure 39: Percentage of total sleep time (TST) in (A) stage 1, (B) stage 2, (C) slow-wave and (D) REM sleep plotted against age in DM1-affected subjects
The solid line represents best fit of the data points shown, from DM1-affected subjects. The dotted line represents the mean from the general population (Ohayon *et al.*) [300]

Age alone did not significantly predict percentage of total sleep time spent in any given stage of sleep, either in univariate analysis or multivariate analysis including sex, contrasting the clearer age-affect seen in the general population.

7.3.4 Prevalence and severity of sleep disordered breathing

American Academy of Sleep Medicine (AASM) recommend classification of the severity of SDB according to the number of sleep-related breathing events

recorded per hour of sleep [301], referred to as the apnoea-hypopnoea-index (AHI; Table 23).

Table 23: American Academy of Sleep Medicine (AASM) classification of sleep-disordered breathing

Classification	**Apnoea-hypopnoea index** (Number of sleep-related breathing events per hour of sleep)
Normal study	Up to 5
Mild	5 to 15
Moderate	15 to 30
Severe	Greater than 30

Applying AASM criteria to our cohort, five subjects (14%) had normal studies, 11 (31%) had mild SDB, 10 (28%) had moderate SDB and 10 (28%) had severe SDB (Figure 40). This represents a marked increase from the background general population incidence SDB. In a large study conducted on middle-aged adults in the USA general population, 76% of participants had normal studies, 14% mild, 5% moderate and 4% severe SDB respectively [302].

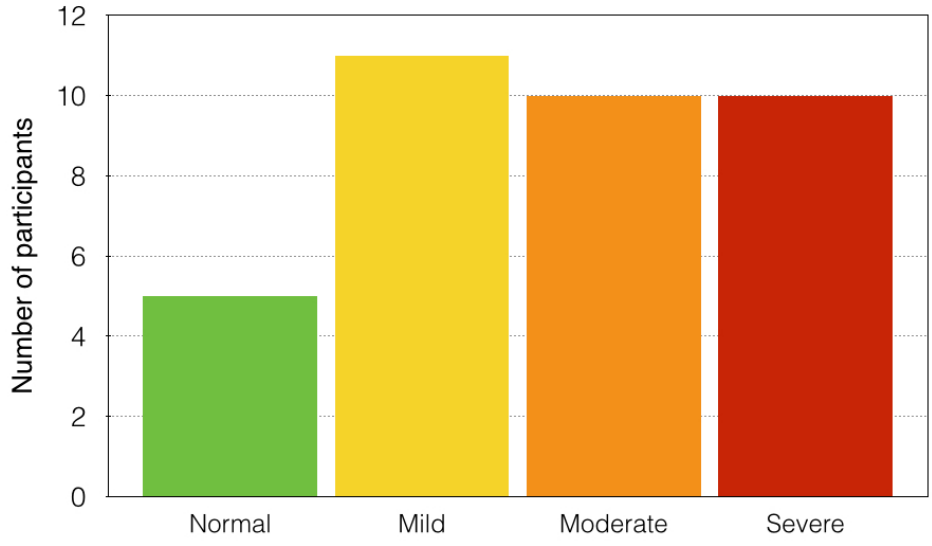

Figure 40: Prevalence of sleep disordered breathing in the DM1-affected cohort according to AASM criteria

Clinical factors contributing to the severity of SDB were explored by stepwise linear regression analysis. Because the distribution of AHI was highly skewed ($p < 0.001$ in Shapiro-Wilk test of normality), a transformation by logarithm with base 10 was undertaken, to give logAHI ($p = 0.235$ in Shapiro-Wilk). LogAHI was positively associated with age ($p = 0.001$; Adj $R^2 = 0.253$). Fit of the linear model improved with inclusion of sex ($p = 0.001$; Adj $R^2 = 0.290$), with severely abnormal studies observed more frequently in male subjects. The relationship between AHI (without log transformation) and age is shown in Figure 41.

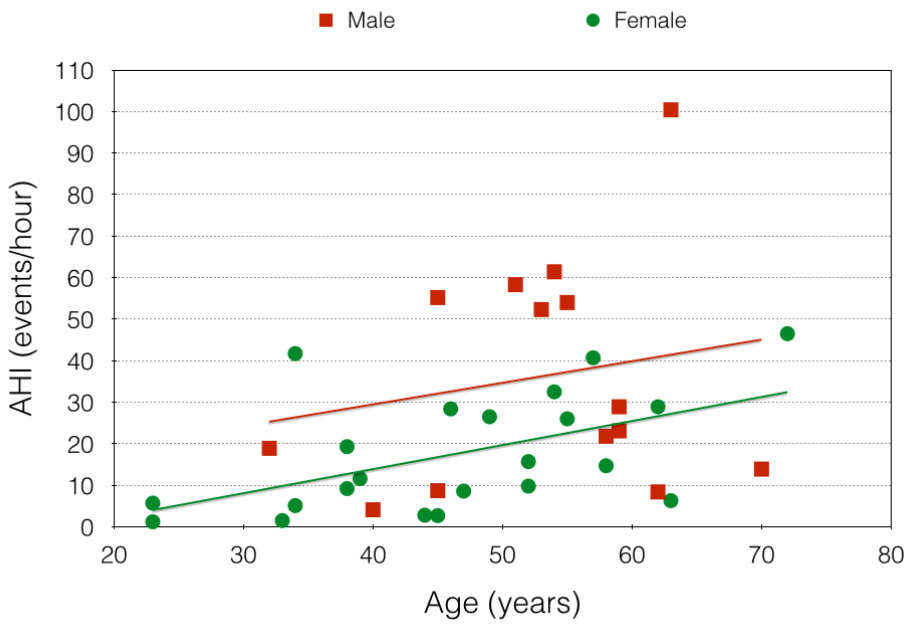

Figure 41: Scatterplot of AHI against age in DM1 subjects, with data points distinguished by sex

The contribution of additional clinical factors was explored by a stepwise linear regression analysis. Factors included were sex, muscle impairment (DM1-ActivC© score), body mass index (BMI) and logPAL. LogAHI ~ age + sex + DM1-ActivC© + BMI emerged as the model with best fit (Adj $R^2 = 0.361$). Beta coefficients and significance levels from this model are summarised in Table 24 showing that increasing age and male sex made a significant contribution to the model. There was also a trend towards a positive association with BMI and muscle weakness (reflected by decreasing DM1-ActivC© score), though these did not reach statistical significance.

Table 24: Best-fit model following stepwise linear regression analysis of logAHI against age, sex, DM1-ActivC score, BMI and logPAL

Factor	Standardised Beta coefficient	T	p
Age	0.383	2.642	0.013
Sex (female = 1; male = 0)	-0.324	-2.208	0.035
DM1-ActivC© score	-0.244	-1.716	0.096
BMI	0.154	1.043	0.305

7.3.5 Clinical correlates of moderate to severe sleep disordered breathing

In the general population, Scottish guidelines for obstructive sleep apnoea recommend use of PAP therapy for individuals with AHI ≥ 15 events per hour [303] and daytime sleepiness symptoms. In the absence of DM1-specific guidelines, respiratory physicians broadly use the same cut-off value for recommendation of treatment in patients with DM1. We therefore undertook group comparisons of patients with AHI ≥ 15 per hour, in whom nocturnal PAP might be recommended, with those who had normal or mildly abnormal studies.

First, an uncorrected comparison of means was undertaken (Table 25). Data suggest that patients with clinically significantly SDB tend to report more motor impairment on DM1-ActivC© ($p = 0.003$) and the MDHI mobility subscore ($p = 0.007$), more fatigue on FDSS ($p = 0.022$) and the MDHI fatigue subscale ($p = 0.006$), and more problems with sleep in the MDHI sleep subscale ($p = 0.027$). Mean pCO_2 was also significantly higher in the AHI ≥ 15/h group ($p = 0.021$). There was a trend towards older age, male sex, larger modal repeat size, higher Epworth scores and longer sleep latency in the AHI ≥ 15/h group that did not reach statistical significance.

Table 25: Group comparison of clinical features in DM1 subjects with normal or mildly abnormal sleep studies, versus moderate to severe sleep disordered breathing

	AHI < 15 (n.16)	AHI ≥ 15 (n.20)	*p*
Demographic			
Age (years)	44.8 (13.7)	52.4 (9.8)	0.059
Females (n.)	12 (75%)	10 (50%)	0.176¶
BMI (kg/m^2)	25.3 (3.2)	27.5 (5.4)	0.408 *
ePAL (repeats)	242 (122)	233 (129)	0.648 *
MAL (repeats)	456 (201)	500 (267)	0.584
Motor measures			
MIRS score ratio 1:2:3:4:5 (mean)	2:4:4:6:0 (2.88)	1:2:4:11:2 (3.53)	0.095 *
MDHI mobility subscale (centile)	19.8 (29.6)	51.6 (31.2)	0.007
DM1-ActivC (centile)	80.3 (16.5)	61.4 (17.8)	0.003 *
Respiratory			
pCO_2 (n.31); (kPa)	4.80 (0.64)	5.37 (0.65)	0.021
MDHI breathing subscale (centile)	9.38 (22.1)	22.5 (28.0)	0.132
Fatigue or sleepiness symptoms			
FDSS (centile)	31.6 (13.1)	43.0 (14.9)	0.022
MDHI Fatigue subscale (centile)	27.2 (29.6)	57.1 (28.7)	0.006
MDHI Sleep subscale (centile)	27.7 (23.8)	48.8 (29.9)	0.027
Epworth score	5.56 (4.0)	7.60 (3.3)	0.101
Sleep latency			
MWT1 latency (minutes)	27.6 (14.1)	33.2 (11.0)	0.372 *
Mean sleep latency (minutes)	26.3 (12.3)	30.0 (10.6)	0.612 *

Values represent the group mean score, with standard deviation in brackets unless otherwise stated. Comparison of means was undertaken by independent samples *t* test, unless data were not normally distributed in both groups (defined as $p < 0.05$ in Shapiro-Wilk test), in which case a Mann Whitney U test was applied. P values relating to a Mann Whitney U test are marked *. ¶Fishers exact test.

In order to consider which clinical factors might play an independent, causative role in the development of moderate to severe SDB, a multiple logistic

regression model was also explored comprising age, sex, BMI, and muscle function (measured by MDHI mobility score). In this model, only the effect of MDHI mobility score remained significant while controlling for other factors (Table 26). This suggests that peripheral muscle weakness is significantly associated with SDB, independent of age, sex or BMI.

Table 26: Multiple logistic regression analysis of factors associated with AHI ≥ 15

Variable	Beta coefficient	Odds ratio	95% CI	p
Age	0.024	1.025	0.939 – 1.118	0.582
Sex (male = 0, female = 1)	-1.890	0.151	0.020 – 1.124	0.065
BMI	0.175	1.191	0.946 – 1.499	0.136
MDHI Mobility subscale	0.034	1.035	1.003 – 1.068	0.030

7.3.6 Towards a predictive score for sleep-disordered breathing

Although mean values of several clinical measures were significantly different in subjects with AHI ≥ 15 compared to those with normal or mildly abnormal studies, the range of any single score showed considerable overlap (Figure 42). Therefore, in a clinical context, no single measure has strong discriminatory value for the presence of moderate to severe SDB.

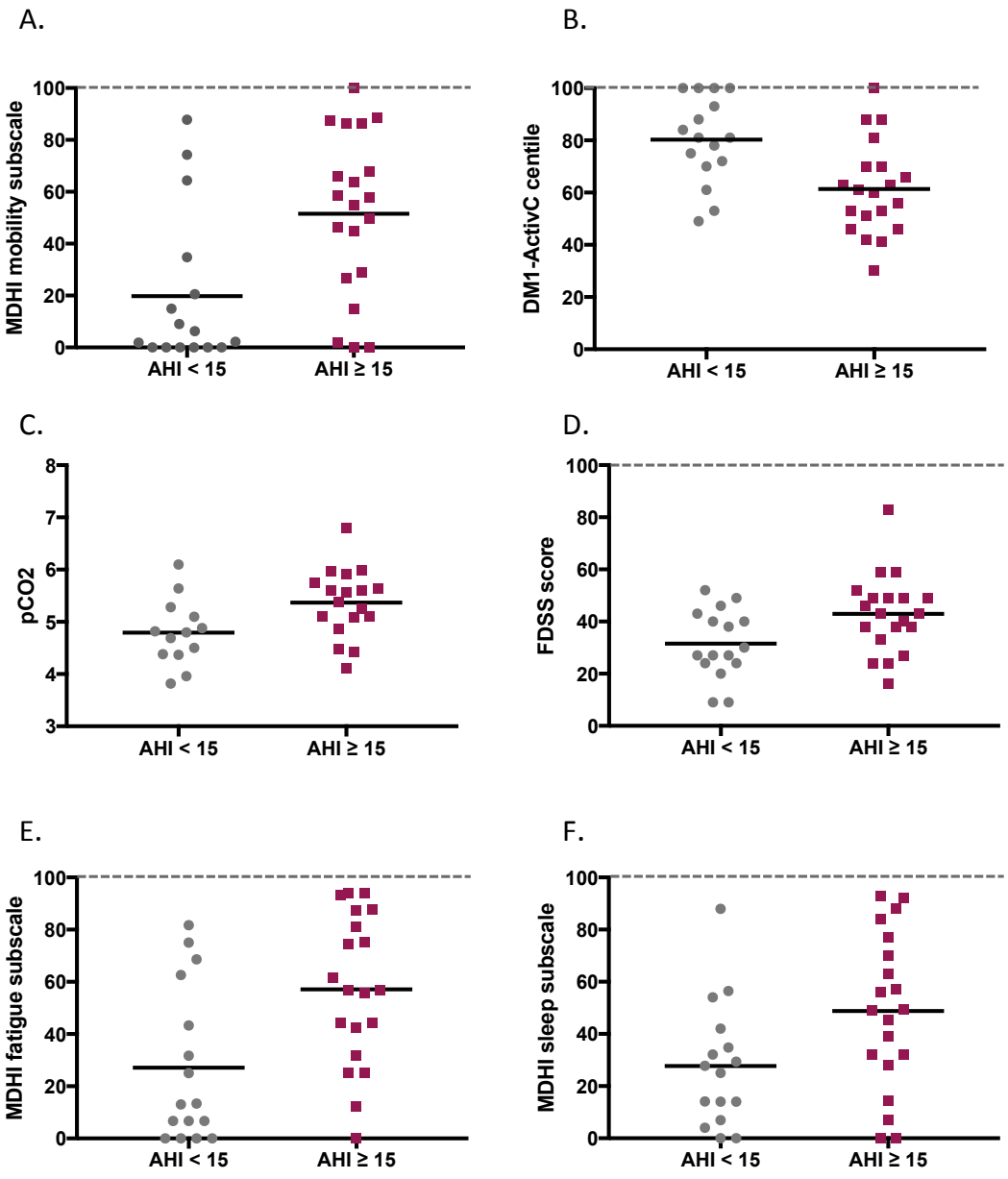

Figure 42: Scatter plots of clinical measures in the AHI < 15 group compared to subjects with AHI ≥ 15.
All measures that were considered significant by comparison of means are included: (A) the MDHI mobility score; (B) DM1-ActivC©; (C) pCO_2; (D) The fatigue and daytime sleepiness score (FDSS); (E) the MDHI fatigue subscale; (F) the MDHI sleep subscale. Dotted lines indicate the maximum possible score in each scale if relevant.

Current Scottish guidelines recommend that DM1 patients with an Epworth score of greater that 12 are referred for specialist respiratory assessment [299]. Of the 20 patients we identified with moderate or severe sleep disordered breathing, three had an Epworth score of 12, and none greater than 12 (Figure 43), suggesting this criterion is not a sensitive screen for SDB.

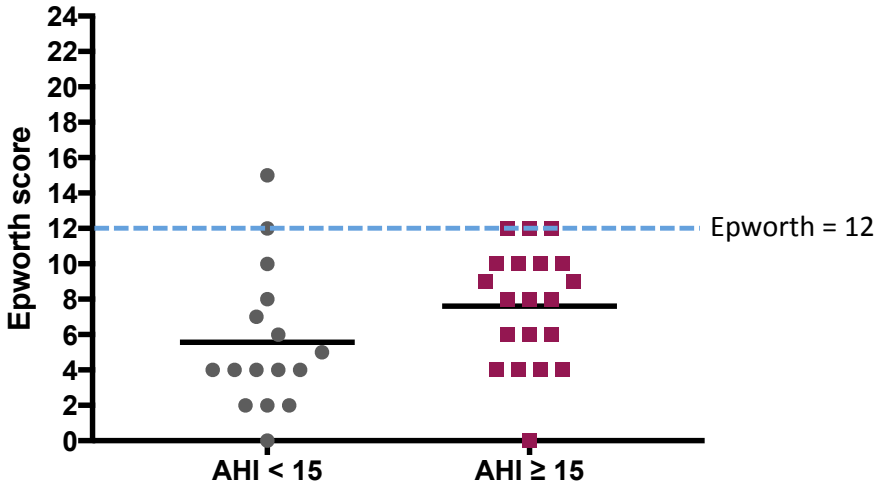

Figure 43: Epworth score of 12, currently recommended as a referral threshold by Scottish guidelines for DM1, discriminates poorly between patients with significant sleep disordered breathing and those without
Maximum possible Epworth score = 24.

To identify which outcome measures might be most useful to distinguish patients in whom a sleep study might alter clinical management, a receiver operating characteristic (ROC) curve approach was used. The discriminatory power of each outcome measures was quantified as the area under the ROC curve. The cut-off value most closely associated with a specificity of ~80% was also recorded (Table 27).

Table 27: Output from ROC curve analysis of potential predictors of AHI ≥ 15

Measure	Area under the curve	Significance (p)	Cutoff value associated with ~80% sensitivity	1 – specificity for this value
Age	0.678	0.070	45.5	0.375
BMI	0.581	0.408	23.3	0.750
ePAL	0.455	0.644	125.5	0.813
MAL	0.566	0.504	179	0.875
MDHI mobility subscale	0.759	0.008	23.6	0.250
DM1-ActivC	0.783	0.004	71.0	0.250
pCO_2 (n.31)	0.739	0.025	4.84	0.385
MDHI breathing subscale	0.648	0.130	NA	NA
FDSS centile	0.703	0.039	31.5	0.438
MDHI Fatigue subscale	0.766	0.007	28.4	0.375
MDHI Sleep subscale	0.717	0.027	27.9	0.438
Epworth	0.684	0.060	5.5	0.375

Significant area-under-the-curve values were obtained for motor outcome measures (MDHI mobility subscale and DM1-ActivC), sleepiness or fatigue measures (FDSS, MDHI sleep and fatigue subscales) and pCO_2. Using the cut-off values associated with 80% sensitivity, a composite score was trialled in which subjects were awarded one point each for significant muscle weakness (DM1-ActivC score < 71), significant fatigue (MDHI fatigue subscale > 28.4), pCO_2 in the upper range (pCO_2 > 4.8 kPa), or male sex. Using threshold of two or more points on the composite score, nine individuals without significant SDB could be excluded, with seven false positives (three of whom were male), and no false negatives (Figure 44).

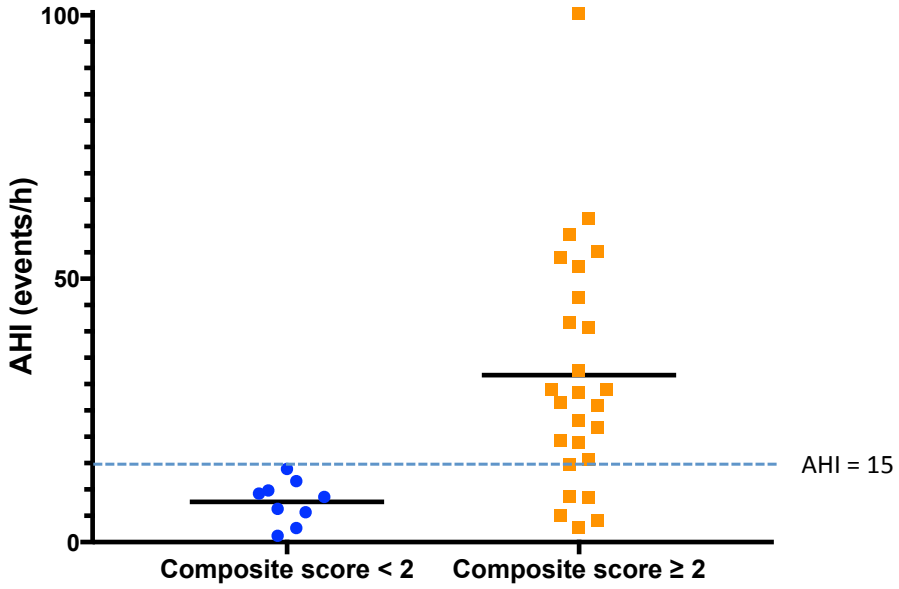

Figure 44: A composite score including sex, DM1-ActivC© score and MDHI Fatigue score is a sensitive, though not specific screen to identify patients meeting the threshold for PAP treatment in this cohort

7.3.7 Sleep disordered breathing and end-organ dysfunction

A recent longitudinal study demonstrated that, among DM1 patients who have been recommended to have home ventilation for reasons including prolonged nocturnal desaturation, compliance with therapy is associated with improved survival [298]. The mechanisms by which ventilation might improve survival are unclear, however. In order to explore the relationship between SDB and end-organ dysfunction, we compared means of a range of clinical parameters relevant to DM1 in patients with significant SDB (AHI ≥ 15) to those without Table 28). Measures were adjusted for differences in age, sex, CTG repeat size (ePAL) and BMI by one-way ANCOVA. ECGs from two participants, one from each group, were excluded from analysis as they showed pacemaker-dependent rhythms.

Only total white matter volume was significantly different between the two groups after adjustment for confounding factors, being lower in the AHI ≥ 15 group. A trend was also observed towards higher liver transaminases, higher HbA1c, and longer PR and QRS duration in the AHI ≥ 15 group.

Table 28: Comparison of means, adjusted by one-way ANCOVA for age, sex, BMI and logPAL between subjects with AHI < 15 and those with AHI ≥ 15

Measure	AHI < 15 Estimated marginal mean (95% CI)	AHI ≥ 15 Estimated marginal mean (95% CI)	p
Liver			
ALT (IU/L)	38.3 (26.3 –50.2)	47.9 (37.3 – 58.4)	0.259
AST (IU/L)	29.6 (23.0 – 36.2)	36.3 (30.4 – 42.2)	0.157
Alkaline phosphatase (IU/L)	120.3 (87.9 – 152.6)	114.1 (85.6 – 142.6)	0.785
Insulin resistance			
HbA1c (mmol/mol)	35.0 (33.4 – 36.7)	35.9 (34.3 – 37.4)	0.354
Cardiac conduction			
Pacemaker: n. (%)	3 (18.8%)	3 (15.0%)	1.000
Heart rate (bpm)	68.6 (61.0 – 76.1)	70.5 (63.8 – 77.3)	0.722
PR interval (ms)	183.8 (164.7 – 203.0)	188.6 (172.1 – 205.0)	0.726
QRS duration (ms)	94.9 (79.3 – 110.5)	103.4 (89.1 – 117.3)	0.458
MRI brain			
White matter hyperintensities (ml)	8.13 (3.2 – 13.1)	8.12 (4.2 – 12.1)	0.999
White matter volume (%ICV)	31.5 (30.0 – 33.0)	29.2 (28.0 – 30.4)	0.029
Grey matter volume (%ICV)	46.4 (44.1 – 48.7)	46.1 (44.3 – 47.9)	0.865

In order to explore the contribution of sleep disordered breathing to excessive sleepiness symptoms, further one-way ANCOVAs were carried out also adjusting for age, sex, ePAL and BMI (Table 29). After adjustment for potential confounders, self-reported fatigue and/or sleepiness remained significantly higher in the AHI ≥ 15 group (measured by the FDSS, or both MDHI fatigue and sleep subscales). This suggests that elevated AHI does make some contribution to subjective sleepiness symptoms, independent of the overall severity of DM1 disease. Objective sleepiness, measured by mMWT sleep latencies, was not significantly different between the two groups however.

Table 29: Comparison of age- and BMI-adjusted mean measures of excessive sleepiness in subjects with AHI < 15 compared with AHI ≥15.

Measure	AHI < 15 Estimated marginal mean (95%CI)	AHI ≥ 15 Estimated marginal mean (95%CI)	p
FDSS centile	30.7 (22.5 – 38.9)	43.7 (36.4 – 50.9)	0.030
MDHI Fatigue subscale	24.0 (7.4 – 40.6)	59.7 (45.1 – 74.3)	0.004
MDHI Sleep subscale	24.2 (9.2 – 39.1)	51.6 (38.5 – 64.8)	0.013
Epworth	5.4 (3.5 – 7.3)	7.8 (6.1 – 9.4)	0.083
MWT1 latency	29.0 (22. – 35.6)	32.1 (26.3 – 38.9)	0.509
Mean latency	27.3 (21.1 – 3.4)	29.2 (23.7 – 34.8)	0.655

7.3.8 Types of respiratory event

It has been proposed that the high prevalence of SDB in DM1 may be related in part to a central dysregulation of breathing during sleep, in addition to peripheral muscle weakness. To explore this hypothesis, we considered the types of respiratory event contributing to AHI in our cohort. The most common respiratory events were obstructive hypopnoeas (Figure 45), with a mean incidence of 20.4/hour. Of 36 participants, 29 (80%) had five or more obstructive hypopnoeas per hour. Central apnoeas were the next most common event (Figure 46), with a mean incidence of 3.1/hour (SD 5.8). Five individuals (14%) had frequent central apnoeas, occurring 5 or more times per hour of sleep. Other events, such as obstructive apnoeas, central hypopnoeas or mixed events were rare (Table 30).

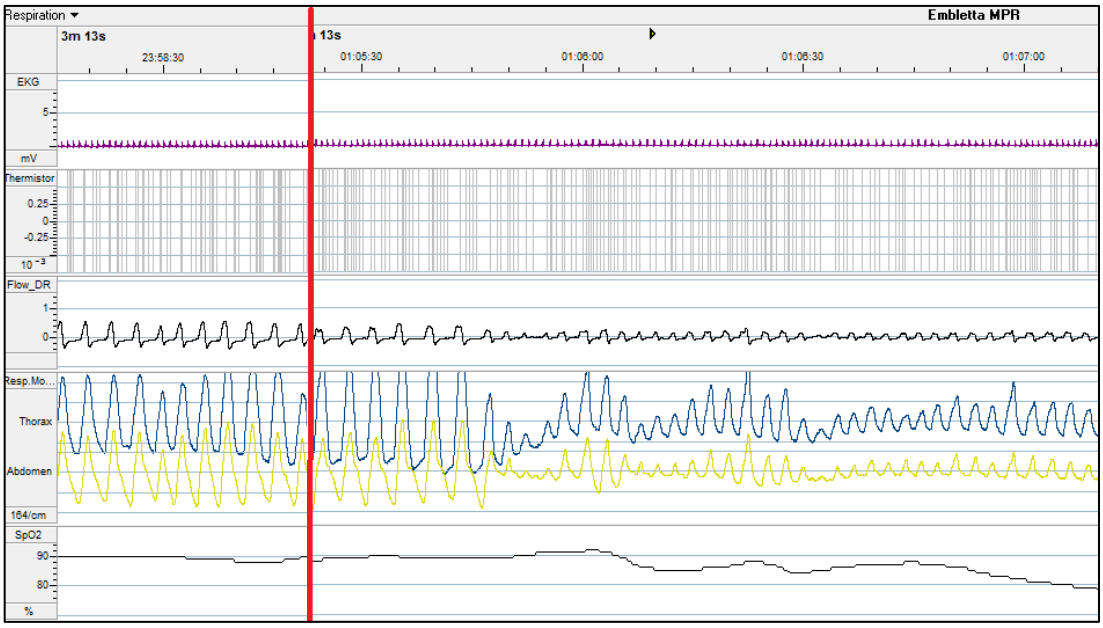

Figure 45: Example of an event scored as an obstructive hypopnoea
Traces to the left of red line demonstrate normal tidal volume (blue and yellow thorax and abdomen effort belts respectively) with maintained oxygen saturation (SpO_2; bottom trace). To the right of the red line, there is onset of an obstructive hypopnoea, with reduced amplitude of chest and abdominal effort, and consequent downward trend of SpO_2.

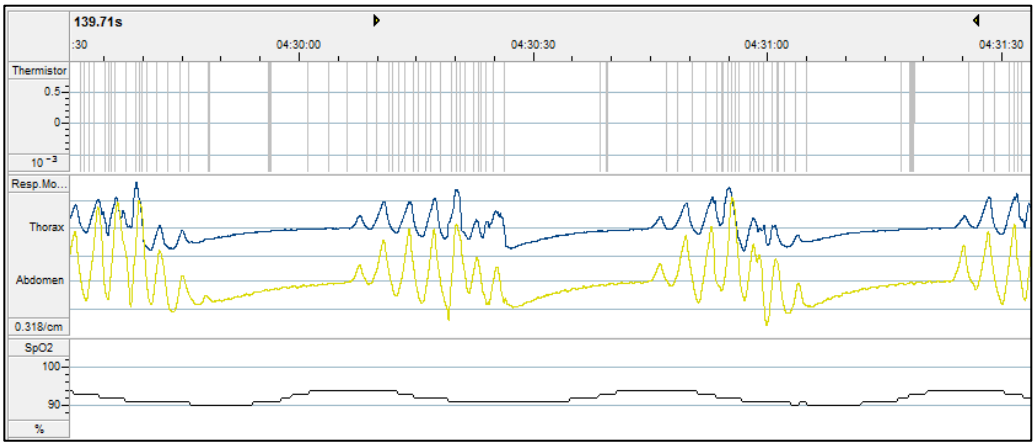

Figure 46: Example of central apnoeas
Periods of reduced respiratory excursion (detected by oronasal thermistor, top trace) accompanied by absent respiratory effort (thorax and abdomen effort traces) constitute a central apnoea. As in the example shown, central apnoeas may occur in clusters with a crescendo-decrescendo pattern, termed Cheyne-Stokes respiration. Tick marks on the horizontal axis represent 5 seconds. Each episode in this example lasts approximately 20 seconds.

Table 30: Major classes of respiratory event observed in PSG (n.36)

Class of event	Mean number of events per hour of sleep (range)	Number of patients with ≥ 5 events per hour of sleep
Obstructive apnoea	0.1 (0.0 – 0.9)	0 (0%)
Central apnoea	3.1 (0 – 24.9)	5 (13.9%)
Mixed apnoea	0.31 (0 – 4.1)	0 (0%)
Obstructive hypopnoea	20.4 (1.1 – 54.5)	31 (86.1%)
Central hypopnoea	0.6 (0.0 – 11.5)	1 (2.8%)
Mixed hypopnoea	1.5 (0 – 8.3)	4 (11.1%)

Events classed as obstructive, which would include episodes of hypoventilation, are commonly encountered in neuromuscular conditions, occurring as a consequence of bulbar and peripheral muscle weakness [144]. The presence of events classed as ‘central’, in which there are periods of absent respiratory effort, are not easily explained by primary muscle weakness, and could represent a distinct pathological process. We therefore explored factors contributing to central apnoeas separately.

7.3.8.1 Central apnoeas

Factors contributing to central apnoeas were explored by stepwise linear regression analysis. The raw number of central apnoeas per hour (CenAp/h) was used, since 10 of the 36 subjects had no central apnoeas, hence log-transformation was not possible. The best fit was for CenAp/h ~ age + DM1-ActivC© score + BMI + sex (Adj R^2 = 0.202). Only the contribution of sex to this model reached statistical significance (Table 31).

Table 31: Beta coefficients and significance level of cofactors in the model CenAp/h ~ age + sex + DM1-ActivC score + BMI

Factor	Standardised Beta coefficient	T	p
Age	0.226	1.449	0.157
Sex (female = 1; male = 0)	-0.412	-2.450	0.020
DM1-ActivC© score	0.280	1.634	0.112
BMI	0.264	1.443	0.159

A group comparison was also undertaken between patients with frequent central apnoeas (> 5 events per hour) and those without. Subjects with frequent central apnoeas were exclusively male (p = 0.005) and tended to be older (p = 0.032). No other factors reached statistical significance. Perhaps surprisingly however, there were trends towards better motor function, less self-reported sleepiness and longer sleep latencies in the group with frequent central apnoeas, supporting these events as representing a pathology distinct from obstructive hypopnoeas. None of the participants with frequent central apnoeas were on opiates or other sedating medicines. None had overt heart failure, although one had a history of mild left ventricular systolic dysfunction and another had first degree heart block and was recommended pacemaker implantation shortly afterward. None had known cerebrovascular disease, although two had treated hypertension.

Table 32: Group comparison of DM1 subjects with frequent central apnoeas and those without

	< 5 central apnoeas per hour sleep (n.31)	≥ 5 central apnoeas per hour sleep (n.5)	*p*
Demographic			
Age (years)	48 .0 (12.7)	55.2 (4.6)	0.032
Females (n.)	22 (71%)	0 (0%)	0.005¶
BMI (kg/m^2)	26.6 (4.5)	26.3 (5.8)	0.567*
ePAL (repeats)	243 (126)	200.0 (119)	0.476*
MAL (repeats)	496 (234)	387 (266)	0.352
Motor measures			
MIRS score ratio 1:2:3:4:5 (mean)	2:4:7:15:2 (3.5)	1:2:1:1:0 (2.4)	0.091*
MDHI mobility subscale (centile)	39.9 (35.0)	22.3 (24.4)	0.262*
DM1-ActivC (centile)	68.2 (19.5)	79.6 (18.0)	0.224*
Respiratory			
pCO_2 (n.31); (kPa)	5.57 (0.90)	5.04 (0.64)	0.123
MDHI breathing subscale (centile)	18.55 (27.4)	5.00 (11.18)	0.422*
Fatigue or sleepiness symptoms			
FDSS (centile)	39.0 (15.3)	30.8 (12.9)	0.263
MDHI Fatigue subscale (centile)	44.7 (32.2)	38.6 (37.0)	0.657*
MDHI Sleep subscale (centile)	42.0 (28.0)	23.2 (32.8)	0.183
Epworth score	7.06 (3.64)	4.40 (3.58)	0.137
Sleep latency			
MWT1 latency (minutes)	29.1 (13.0)	40.0 (0.0)	0.089*
Mean sleep latency (minutes)	27.0 (11.7)	36.1 (5.5)	0.086*

Values represent the group mean score, with standard deviation in brackets unless otherwise stated. Comparison of means was undertaken by independent samples *t* test, unless data were not normally distributed in both groups (defined as $p < 0.05$ in Shapiro-Wilk test), in which case a Mann Whitney U test was applied. P values relating to a Mann Whitney U test are marked *. ¶Fishers exact test.

7.3.9 Correlates of disordered breathing with sleep efficiency and architecture

To explore whether presence of apnoeas significantly altered the quality or architecture of sleep, the relationship between logAHI and sleep measures were explored by linear regression. LogAHI was not significantly correlated with sleep efficiency or REM latency from sleep onset. Higher logAHI was significantly associated with greater percentage of sleep spent in stage 1 sleep ($p = 0.036$, Adj $R^2 = 0.198$), but not with percentage of total sleep time in other stages.

7.3.10 Modified maintenance of wakefulness tests

7.3.10.1 Clinical correlations of sleep latency

A complete set of three mMWT latencies was not available for 14 (38%) of participants, either due to technical failures or subject refusal (Figure 37). Two values were therefore used for analysis from each participant; the latency in the first mMWT trial (MWT1), which was available for 35 out of 36, and the average of all latencies measured (meanLat), which available for all 36.

Because both MWT1 and meanLat were highly skewed towards an upper ceiling value of 40 minutes ($p < 0.001$ for both in Shapiro-Wilk test of normality), correlations were explored using Spearman's rank-order correlation. MWT1 did not significantly correlate with age, AHI, sleep efficiency, pCO_2, sleep efficiency, %TST in any stage of sleep, self-reported sleepiness (Epworth score, MDHI fatigue subscore, MDHI sleep subscore) or self-reported motor limitation (DM1-ActivC and MDHI mobility subscore). Shorter MWT1 latency was, however, weakly associated with larger ePAL ($p = 0.018$, $r_s = -0.397$) and greater muscle impairment expressed as MIRS ($p = 0.047$, $r_s = -0.338$).

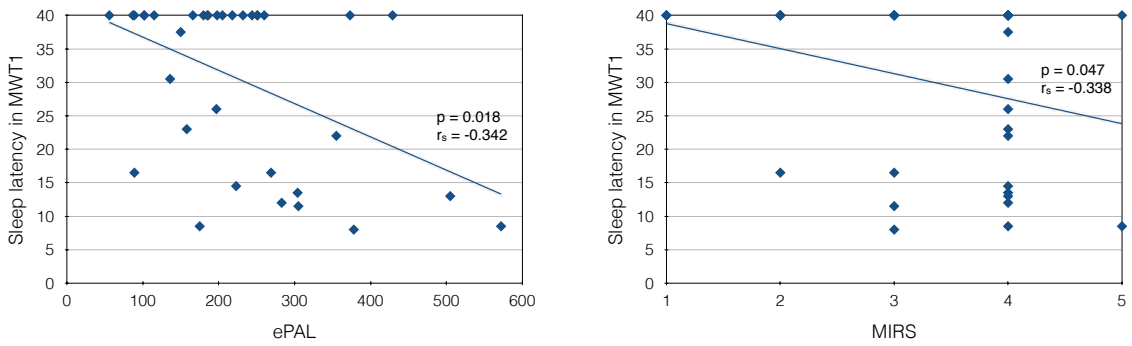

Figure 47: Scatterplot demonstrating significant inverse correlation between sleep latency in MWT1 and both estimated progenitor allele length and MIRS score

MeanLat similarly did not correlate with age, AHI, pCO_2, sleep efficiency, %TST in any stage of sleep, ePAL, MAL, DM1-ActivC, FDSS or MDHI subscores for mobility, fatigue and sleep. There was however a significant inverse association with Epworth score ($p = 0.015$, $r_s = -0.401$).

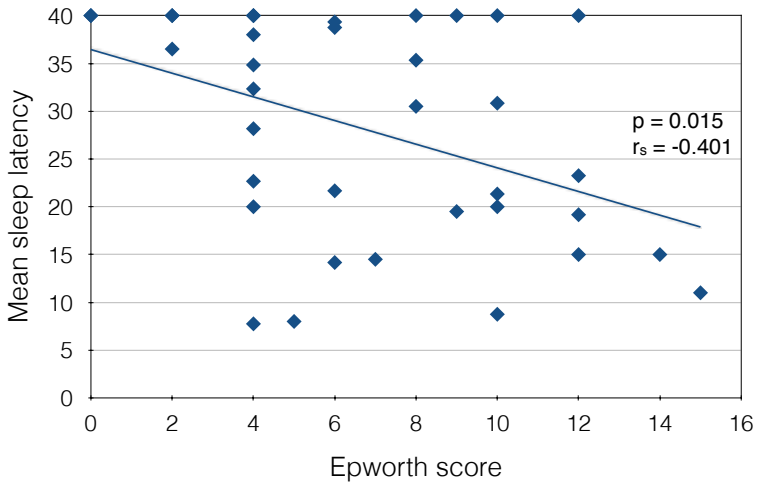

Figure 48: Scatterplot demonstrating significant correlations between mean sleep latency and Epworth score

7.3.10.2 Sleep Onset REM Periods (SOREMPS)

A single SOREMP was identified in each of three patients during MWT, and a further two during PSG. No participant had more than one SOREMP detected. In group comparison, there were no clinical features that clearly distinguished patients with SOREMPs from those without (Table 33).

Table 33: Clinical features of participants in whom a SOREMP was detected in PSG or mMWT compared with those in whom none were detected

	SOREMP absent (n.32)	SOREMP present (n.5)	*p*
Demographic			
Age (years)	49.75 (12.5)	47.2 (11.3)	0.670
Females (n.)	18 (56.3%)	4 (80%)	0.629¶
BMI (kg/m^2)	26.1 (4.2)	28.7 (6.5)	0.328*
ePAL (repeats)	222 (112)	304 (186.1)	0.505*
MAL (repeats)	453 (247)	586 (191)	0.258
Motor measures			
MIRS score ratio 1:2:3:4:5 (mean)	3:5:9:14:1 (3.14)	0:1:0:3:1 (3.80)	0.181*
MDHI mobility subscale (centile)	35.6 (33.6)	42.9 (39.7)	0.671*
DM1-ActivC (centile)	69.9 (19.2)	70.0 (21.7)	0.949*
Respiratory			
pCO_2 (n.32); (kPa)	5.14 (0.70)	5.02 (0.71)	0.730
MDHI breathing subscale (centile)	14.1 (21.9)	30.0 (44.7)	0.530*
Fatigue or sleepiness symptoms			
FDSS (centile)	37.3 (15.6)	38.6 (11.8)	0.865
MDHI Fatigue subscale (centile)	42.9 (32.1)	42.3 (39.20)	0.859*
MDHI Sleep subscale (centile)	41.4 (30.0)	36.5 (31.2)	0.738
Epworth score	6.8 (4.0)	7.2 (3.6)	0.850
Sleep latency			
MWT1 latency (n.35); (minutes)	30.8 (12.0)	27.4 (17.3)	0.657*
Mean sleep latency (n. 36); (minutes)	28.1 (11.4)	26.8 (13.1)	0.963*

Values represent the group mean score, with standard deviation in brackets unless otherwise stated. Comparison of means was undertaken by independent samples *t* test, unless data were not normally distributed in both groups (defined as $p < 0.05$ in Shapiro-Wilk test), in which case a Mann Whitney U test was applied. P values relating to a Mann Whitney U test are marked *. ¶Fishers exact test.

7.3.11 Sleep and cognition

The presence of obstructive sleep apnoea is associated with self-reported concentration difficulties, and impairments of memory and executive function in the general population [304]. We therefore explored correlations between sleep parameters and cognitive performance in our DM1 cohort.

The presence of SDB was not strongly associated with performance in any of the cognitive tasks. Increasing LogAHI was weakly associated with poorer block design non-adjusted score ($p = 0.047$, Adj $R^2 = 0.085$) only, while central apnoeas per hour showed a weak positive correlation with performance in the D-KEFS number sequencing trail ($p = 0.047$, Adj R^2 0.085).

Greater sleep efficiency was associated with higher D-KEFS motor contrast score only ($p = 0.003$, Adj $R^2 = 0.204$). Sleep latency was not correlated with performance in any of the cognitive tests (neither MWT1 nor meanLat).

Several significant correlations were observed between measures of sleep architecture and cognitive performance. Greater percentage of total sleep time in stage 1 sleep was associated with poorer performance in the D-KEFS number-letter switching trail ($p = 0.027$, Adj $R^2 = 0.110$), block design standard score ($p = 0.038$ Adj $R^2 = 0.095$), and block design non-adjusted score ($p = 0.031$, Adj $R^2 = 0.103$).

Greater percentage of stage 2 sleep was associated with better performance in the Stroop word ($p = 0.035$, Adj $R^2 = 0.101$), Stroop colour ($p = 0.009$, Adj $R^2 = 0.164$), colour-word ($p = 0.013$, Adj $R^2 = 0.148$), Stroop interference ($p = 0.044$, Adj $R^2 = 0.091$), and the D-KEFS number scanning task ($p = 0.006$, Adj $R^2 = 0.177$).

A higher percentage of total sleep time in REM sleep was associated with poorer performance in the Stroop colour ($p = 0.011$, Adj $R^2 = 0.156$), colour-word ($p = 0.033$, Adj $R^2 = 0.104$), D-KEFS number scanning ($p = 0.037$, Adj $R^2 = 0.096$), and ECAS executive ($p = 0.041$, Adj $R^2 = 0.091$) subtests.

7.3.12 Structural brain changes and sleep

An exploratory analysis of the relationship between structural brain changes and sleep study data were undertaken using a voxel-based morphometry approach. Of primary interest were relationships with regional grey matter changes, since data presented in Chapter 7 highlighted specific grey matter structures, most notably the thalamus, as potential candidates to account for differences in sleep architecture in DM1. Models were also explored using white matter segmentations, since these images give better definition of additional structures of potential importance, including corpus callosum and the brainstem.

Voxel-wise modelling was carried out on smoothed grey and white matter segmentations normalised to MNI space. None of the models presented here contained significant clusters after correction for family-wise error ($p = 0.05$), and so clusters significant at $p = 0.001$ without multiple comparison correction are shown.

7.3.12.1 Sleep disordered breathing

We hypothesised that the presence of sleep-disordered breathing might accelerate structural brain changes in DM1, particularly in regions vulnerable to hypoxia. However in a group comparison of grey matter in subjects with AHI ≥ 15/h compared to those without, controlled for age and sex, only a single tiny cluster in the left frontal lobe was significantly lower in the affected group (Figure 49). A linear model exploring regions of grey matter inversely correlated with logAHI similarly showed no differences at significance $p = 0.001$, with the exception of a single tiny cluster on the inferior surface of the left cerebellum (not shown).

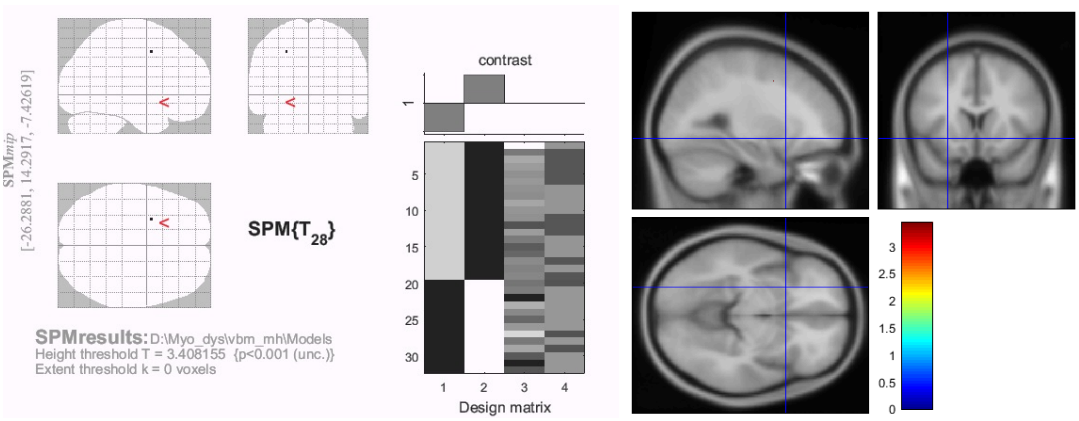

Figure 49: Voxel-wise group comparison, highlighting regions of greater grey matter volume loss in DM1-affected subjects with AHI ≥ 15/h compared to those without
Clusters significant at p = 0.001 are shown. Age and sex are included as covariates.

A group comparison for AHI > 15/h was also performed on white matter images (Figure 50). This revealed greater volume loss in several regions of subcortical white matter of frontal and parietal lobes bilaterally in subjects with significant SDB. Smaller signals were also seen at the left parahippocampal gyrus and right occipital lobe.

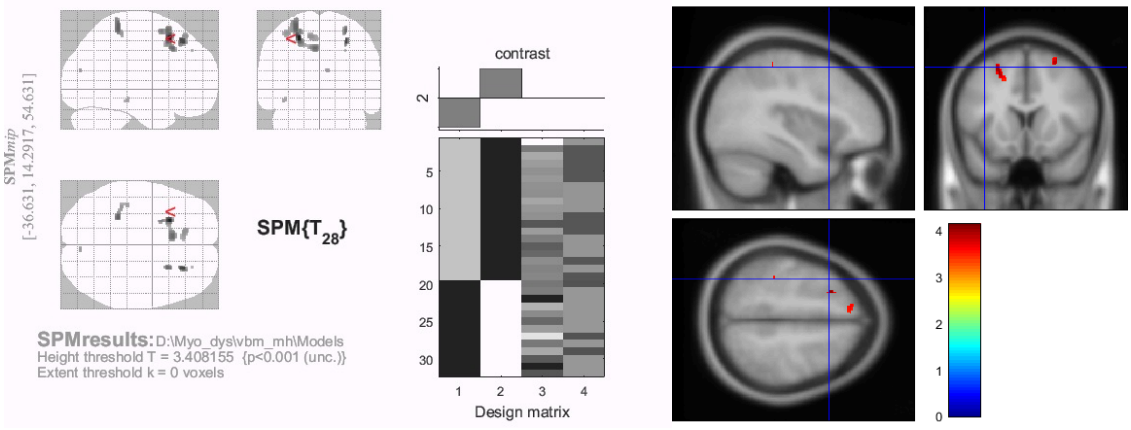

Figure 50: Group comparison, highlighting regions of greater white matter volume in DM1-affected participants with AHI ≥ 15/h compared to those without.
Clusters significant at p = 0.001 are shown. Age and sex are included as covariates. Greater white matter loss was seen in individuals with sleep-disordered breathing predominantly in frontal and parietal lobes.

Data presented earlier in this chapter suggested that the presence of central apnoeas may represent a distinct pathology in DM1, occurring due to a central dysregulation of breathing control rather than primary muscle weakness, perhaps due effects of DM1 on the brainstem. In a linear model, adjusted for age

and sex, no grey matter clusters showed a significant inverse relationship with number of central apnoeas per hour. This remained the case even when the significance threshold was reduced from p = 0.001 to p = 0.01. The same model for white matter (Figure 51) demonstrated some significant clusters in the superior frontal and parietal lobes only. Again, no signal was seen from the brainstem, even with reduction of the significance threshold to p = 0.01.

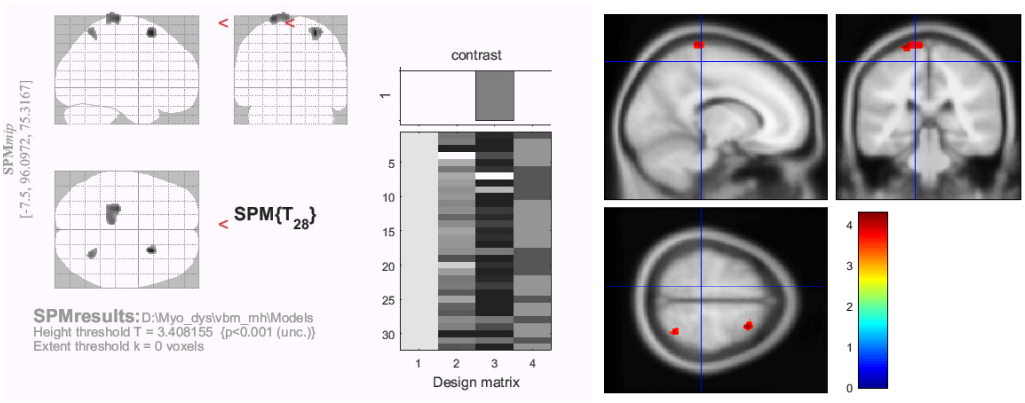

Figure 51: Voxel-wise linear model, demonstrating clusters in which white matter volume was inversely related to the frequency of central apnoeas per hour of sleep.
Clusters significant at p = 0.001 are shown. Age and sex are included as covariates.

7.3.12.2 Sleep architecture

Data from the present study, as well as previous DM1 studies, have demonstrated that changes of sleep architecture in DM1 are characterised by an increased proportion of slow wave and REM sleep, with particular loss of stage 2 sleep. We therefore used voxel-wise linear models to explore regional volumes that correlated positively with percentage of stage 1 and 2 sleep, and inversely with slow wave and REM sleep.

Grey matter models are summarised in Figure 52. Percentage of stage 1 sleep was positively associated with a single cluster in the left occipital lobe. For stage 2 sleep, we had hypothesised that loss may be related to reducing thalamus volume. Percentage of stage 2 sleep was positively correlated with two clusters in the left frontal lobe, but no signal was seen from the thalamus in this model, even with reduction of the significance threshold to p = 0.01 (not shown). Percentage of slow wave sleep was inversely related to grey matter volume at a single small cluster in the left temporal region only.

A large number of clusters were identified in which grey matter volume was inversely associated with percentage of REM sleep. Areas of greatest signal included the superior temporal gyrus of the left temporal lobe, postcentral gyrus of the parietal lobe, left caudate body and the anterior cingulate (Figure 53). Additional scattered clusters include areas of frontal, temporal and parietal lobe, as well as thalamus and superior cerebellum.

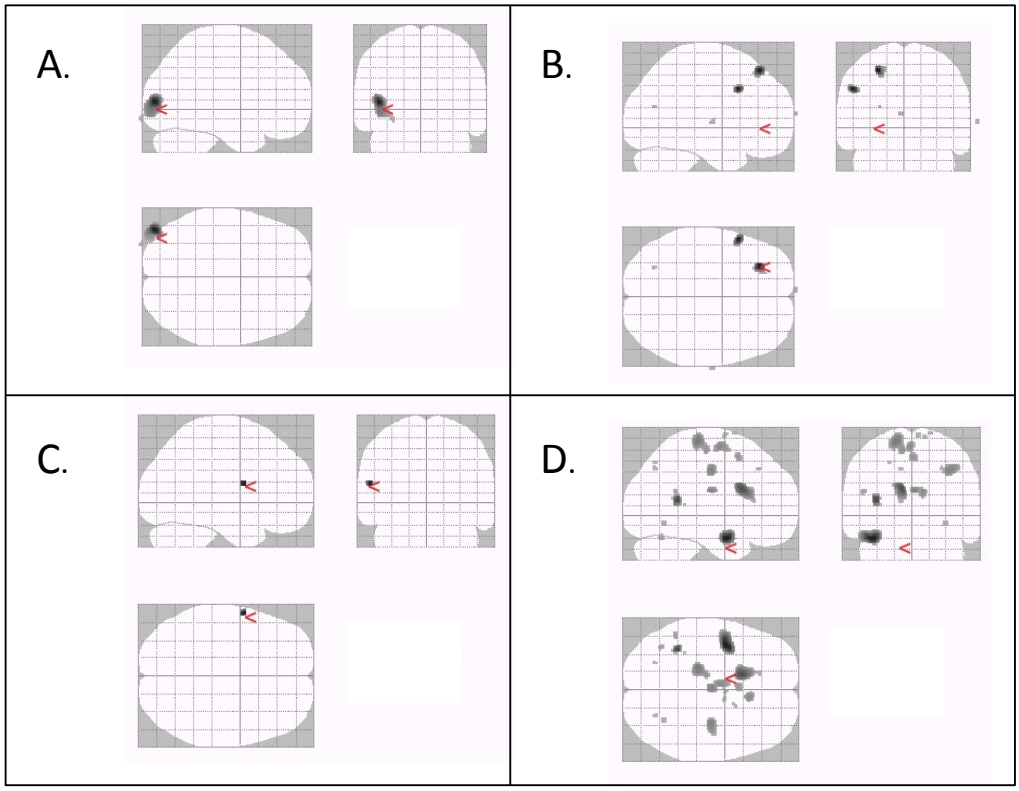

Figure 52: Glass brain view of regional grey matter volumes with a significant positive linear relationship with (A) stage 1 and (B) stage 2 sleep, and inverse relationship with (C) slow wave and (D) REM sleep
Clusters significant at p = 0.001 are shown. Age and sex are included as covariates.

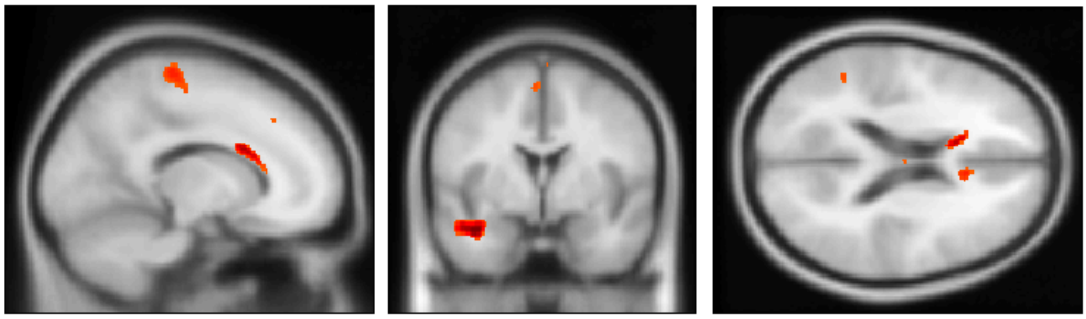

Figure 53: Regions of grey matter in which volume was inversely related to percentage of REM sleep, overlaid on an MNI canonical T1 brain image.
Areas of high signal are seen at the superior temporal gyrus of the left temporal lobe and the medial postcentral gyrus of the left parietal lobe. Of note, while some clusters appear to overlap the corpus callosum on the canonical brain, corpus callosum is not represented in segmented grey matter images. Mapping of these clusters to the closest grey matter structure using the Talairach atlas suggests they represent anterior cingulate.

The same models were explored for white matter volume, and are summarised in Figure 54. Percentage of time in stage 1 sleep was positively correlated with a large cluster of white matter at the left inferior frontal gyrus, and smaller subcortical clusters throughout the frontal, temporal and occipital lobes. Models for stage 2 and slow wave sleep revealed only a tiny, isolated subcortical voxel in each case. Percentage of REM sleep was likewise inversely related to volume of a single cluster only, localised in the left cerebellum.

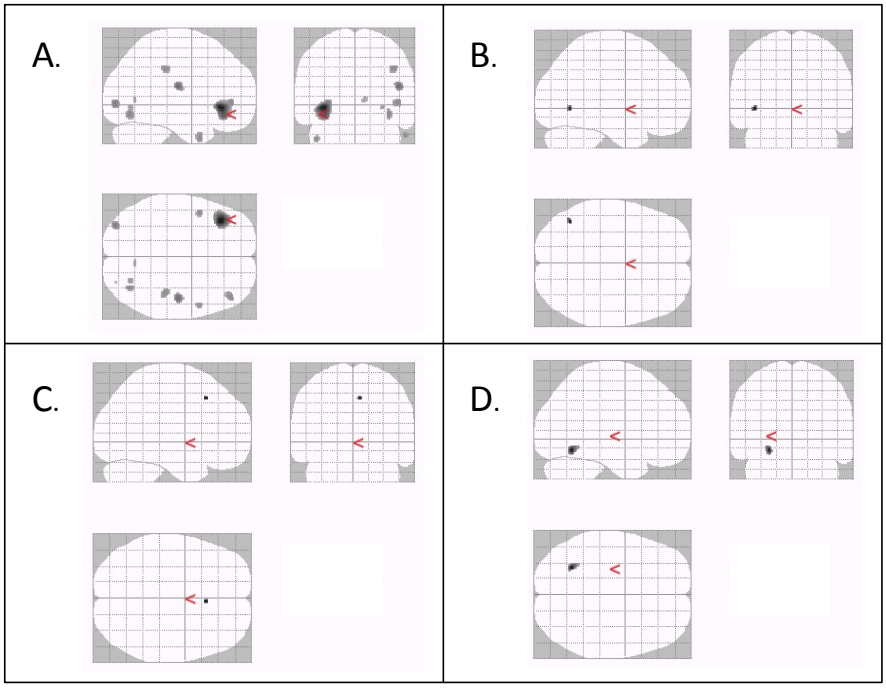

Figure 54: Glass brain view of white matter regions with a significant positive linear relationship with (A) stage 1 and (B) stage 2 sleep, and inverse relationship with (C) slow wave and (D) REM sleep
Clusters significant at p = 0.001 are shown. Age and sex are included as covariates.

7.3.12.3 Sleep latency and structural brain changes

Voxel-wise modelling was also used to identify regions in which loss of volume was associated with greater somnolence, measured by sleep latency in the mMWT. Correlations with grey matter volume were explored for both latency in mMWT1 (Figure 55A) and mean latency from all mMWTs (Figure 55B). In both models, strong signals were obtained from clusters at the right middle occipital gyrus, right middle temporal gyrus, left frontal lobe precentral gyrus, left parahippocampal gyrus and the anterior cingulate. Additional scattered clusters were observed in occipital, temporal, and parietal lobes, as well as cerebellum.

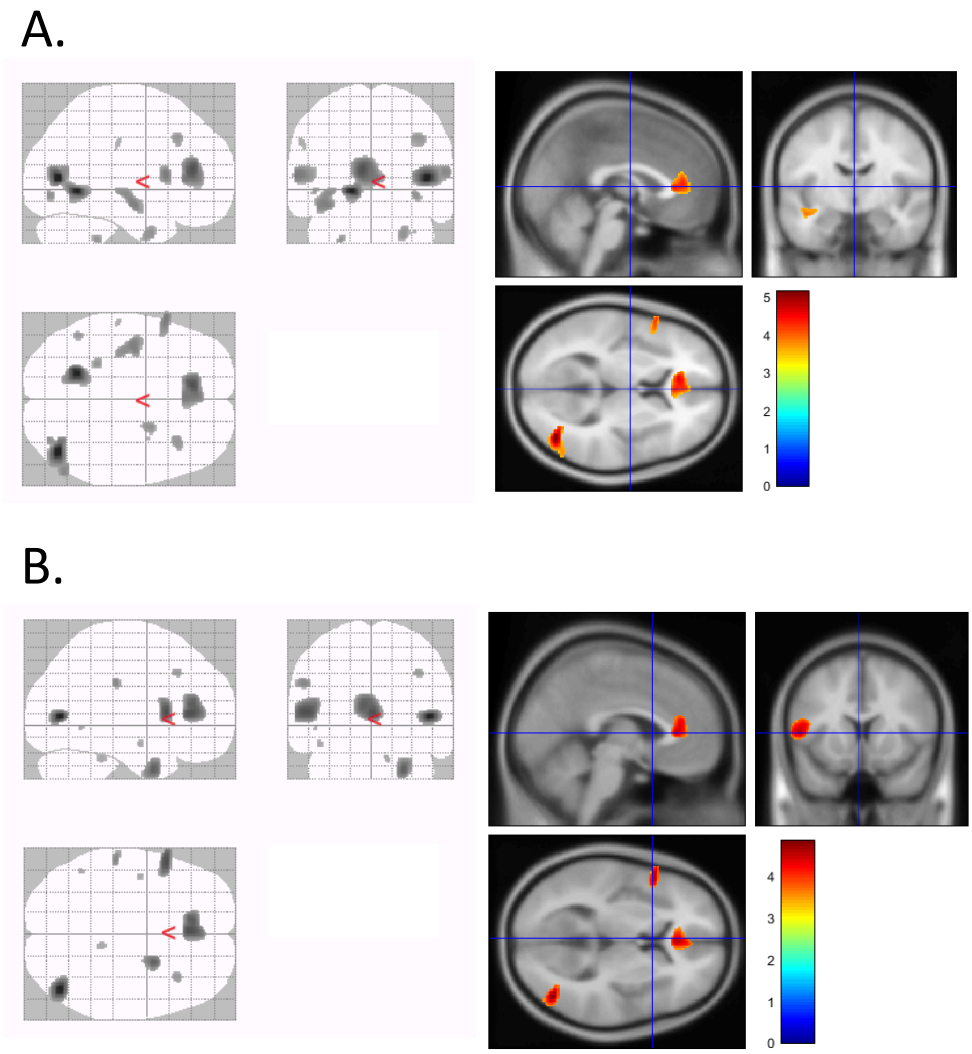

Figure 55: Areas where grey matter volume loss was significantly associated with shorter latency on (A) mMWT1 and (B) mean mMWT latency
Clusters shown were significant at threshold p = 0.001. Age and sex were included as covariates.

The same models applied to white matter images showed single significant clusters only; in the right parietal lobe for MWT1, and in the frontal lobe (meanMWT) respectively (Figure 56).

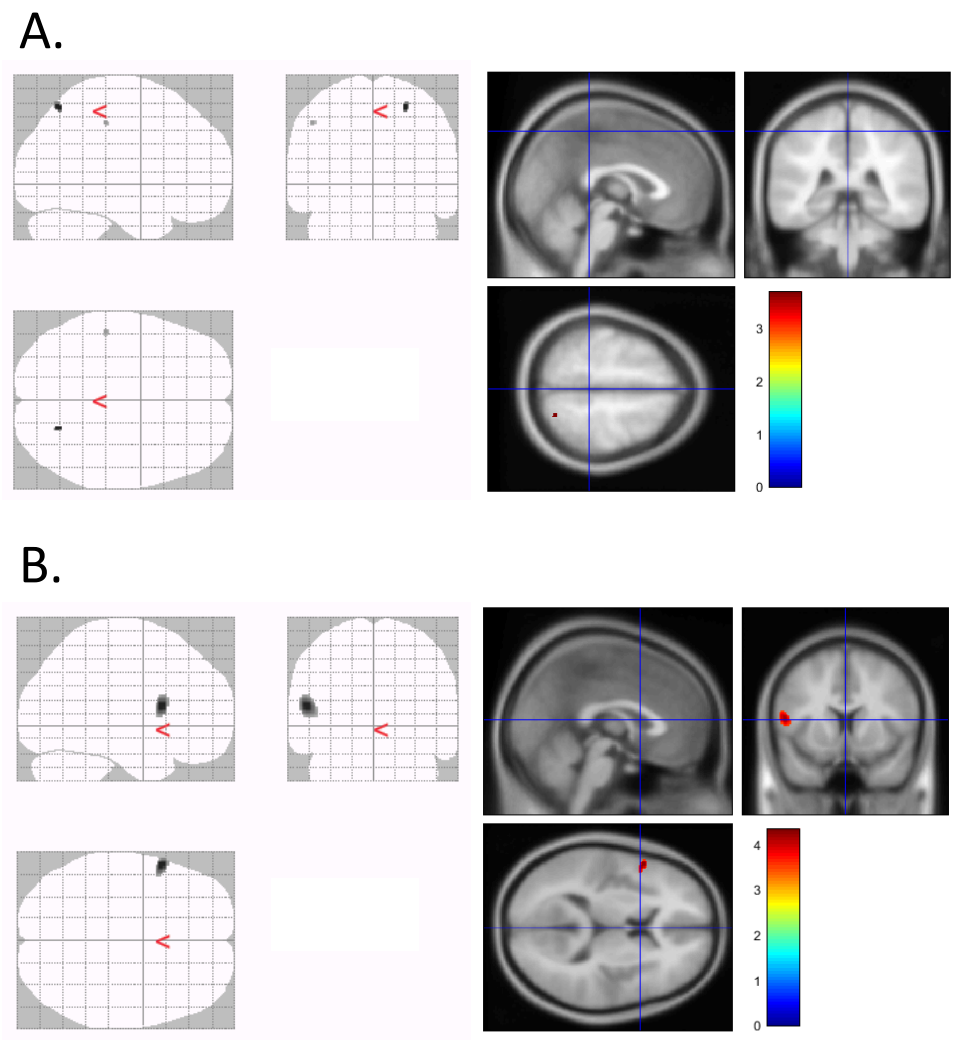

Figure 56: Areas where white matter volume loss was significantly associated with shorter latency on (A) mMWT1 and (B) mean mMWT latency
Clusters shown were significant at threshold p = 0.001. Age and sex were included as covariates.

7.4 Discussion

7.4.1 Tolerability and performance of the type II PSG and mMWT protocols

The experience of using a domiciliary type II PSG protocol for assessment of sleep in patients with DM1 was predominantly favourable. Of 46 study subjects, 39 (84.7%) were willing to attempt PSG in their own home. The morning following PSG, all participants but one were willing to continue to the mMWT, suggesting they had not found wearing the device a particularly negative experience. Just under 90% of the studies gave technically valid results on the first attempt, consistent with an acceptable technical failure rate. Of note, all sleep studies were administered by MJH, who had no experience of PSG prior to training by Dr Atalaia for the present study, therefore the failure rate could be expected to reduce further as the operator gains experience. Taking all these findings into account, we suggest that type II PSG represents a viable potential alternative to inpatient studies for selected patients with DM1.

The mMWT protocol was less well tolerated by patients however, with several failing to complete all three trials. While every effort was made to identify test days that were suitable for the subject, some were still only willing to attempt one or two trials; for example, some parents with daily childcare commitments. In other cases, the subject initially agreed to three trials, but subsequently asked to stop before all were completed, either because of discomfort due to the equipment, or boredom in relation to the lengthy periods without stimulation. It is possible that the relatively high withdrawal rate may have been compounded by the studies being carried out in the patients' own homes, where they may have felt more empowered to decline compared with a hospital setting. Since both MWT and MSLT are significantly burdensome to undertake both for clinical services and for DM1 patients in any form, this finding further emphasises that a more straightforward measure or biomarker for EDS would be highly valuable in clinical and research settings.

7.4.2 Sleep disordered breathing

The prevalence of sleep disordered breathing among adults with DM1 reported in previous studies has ranged from around 50% [145,305] to over 85% of

participants [140,146]. Results from our cohort favour the upper range of incidence, with abnormal studies found in 86.1% of subjects, and 55.6% meeting AHI criteria for consideration of nocturnal PAP therapy [303]. The incidence of SDB increased with age, and there was a male preponderance in those with severely abnormal studies. Self-reported muscle weakness emerged as a major additional risk factor, and subjects with moderate to severely abnormal studies also tended to report more sleepiness or fatigue, and tended to have higher mean capillary pCO_2. No single measure was shown to provide a specific and sensitive screen for SDB, however, and in particular self-reported sleepiness scores in isolation were poorly predictive of sleep study abnormalities. This suggests that current practice in the West of Scotland is unlikely to be effective in ascertaining patients with clinically significant SDB. A composite score, incorporating sex, muscle weakness and self-reported fatigue performed well as a stratification tool in our cohort, but further work in larger cohorts would be needed to validate such a system.

At first glance, these results seem to imply a clear need to increase efforts to actively ascertain SDB in DM1 cohorts, with the intention of initiating treatment. A valid criticism of such an approach however, would be the lack of robust evidence that treating SDB improves outcomes in DM1. This is with the exception of a very recent longitudinal study, demonstrating improved survival in patients compliant with home non-invasive ventilation, prescribed for a variety of reasons including prolonged nocturnal hypoxia [298]. Furthermore, there is current controversy with regard to whether screening for, and treatment of asymptomatic obstructive sleep apnoea (OSA) in the general population is clinically beneficial. A recent literature review by the US Preventative Services Task Force highlighted a lack of studies evaluating the impact of such a strategy on health outcomes, calling for more research in this area [306]. The review concluded that there was currently insufficient evidence to support screening or treatment of OSA in adults who are asymptomatic, or have not presented with complaints of daytime sleepiness symptoms. Similarly, in the context of DM1, treatment of SDB has traditionally been rationalised primarily as a therapy for EDS symptoms rather than with the aim of improving survival in its own right, hence the appropriateness of treating SDB in individuals who did not present with EDS is unclear.

In the general population however, it is long recognised that obstructive sleep apnoea syndrome (OSAS) - obstructive sleep apnoea accompanied by daytime sleepiness symptoms - is strongly associated with an excess of death from cardiovascular causes [307], and that this can be attenuated, at least in part, by the initiation of PAP therapy [308-310]. The exact nature of the relationship between apnoeas and vascular events is poorly understood, and investigation is confounded by the fact that the primary risk factor for OSAS - elevated BMI - is itself associated with adverse cardiovascular events. Nonetheless, OSAS has been demonstrated to be an independent risk factor for systemic hypertension [311] and coronary artery disease [312], even when controlled for BMI. Furthermore, OSAS may precipitate angina [313], and is associated with poorer prognosis in those with existing coronary artery disease [314]. Presence of sleep apnoeas has also been linked to incidence of stroke [315], and poorer recovery from the same [316]. Nocturnal apnoeas cause haemodynamic changes that increase ventricular afterload in patients with heart failure [317], and treatment with CPAP may improve systolic function [318]. Further, bradycardia during sleep may be exaggerated in those with OSAS [319], possibly reflecting increased parasympathetic tone, and may lead to higher grade atrio-ventricular blocks [320]. In some cases, initiation of PAP therapy can obviate the need for a pacemaker [321]. An association between OSAS and sudden death also implies a link to ventricular dysrhythmias [322]. Metabolic changes occur with OSAS, characterised by insulin resistance, abnormal glucose homeostasis [323] and fatty liver disease [324], occurring in excess of that predicted by background obesity alone. The precise mechanisms underlying these diverse effects are not clearly understood, but they are thought to comprise a range of mechanisms including sympathetic nervous system over activity, activation of inflammatory pathways, dysfunction of vascular endothelium, hypercoagulability of blood as well as metabolic disturbances [325].

Since cardiac causes including arrhythmia are a major cause of morbidity and mortality in DM1 [68,72,326], and findings such as fatty liver disease [86] and relative insulin insensitivity [83] bear close similarity to the metabolic phenotype described in OSAS, it seems plausible that the presence of SDB in DM1 could compound the impact of the primary disease process. Our data demonstrate a trend towards increased liver transaminases, higher HbA1c and

greater DM1-specific ECG changes in participants with SDB, as well as a reduction in total white matter volume that reached statistical significance. This highlights considerable utility in further work to understand the contribution of SDB to DM1 pathophysiology, as well as adding further evidence in favour of a proactive approach to screening for and treating SDB in DM1 populations, including strategies to overcome existing issues of low adherence to therapy [96].

7.4.3 Sleep architecture and cognition

Our findings with regard to sleep architecture are consistent with those of previous studies [95,138-140], demonstrating decreased sleep efficiency, and increased periods of slow wave and REM sleep, at the expense of stage 2 sleep. We did not identify any clear correlations between sleep architecture changes and severity of SDB, self-reported sleepiness symptoms or sleep latencies. Several correlations were observed with measures of cognition however. Broadly, greater time in stage 1 or REM sleep was associated with poorer cognitive performance, while those with a greater percentage of stage 2 sleep performed better.

Few studies have explored the relationship between sleep and cognition in DM1. Those that have, hypothesised that sleep fragmentation due to apnoeas might influence the cognitive deficits seen, but results did not support this hypothesis [241,327]. Whether the association observed in our cohort between increased stage 2 sleep and improved cognitive performance represents a cause-and-effect relationship, or independent markers of overall CNS disease burden are unclear. In normal sleep, stage 2 is characterised by the presence of sleep spindles and K-complexes. It lasts around 10 to 25 minutes in the first cycle of sleep, lengthening with each successive cycle [328]. Sleep spindles are generated in the thalamus, and are propagated to the cortex by thalamocortical projections [263]. The density of sleep spindles is increased in subjects who have performed a learning task [329], implying a role in synaptic plasticity, memory formation and learning. The closest correlations we observed were between percentage of stage 2 sleep and performance of tasks strongly influenced by basic processing speed and attention (specifically, all elements of the Stroop test, D-KEFS™ number scanning task). Since thalamus atrophy has been linked to slowing of

processing speed in normal cognitive aging [262], it could be hypothesised that the co-linearity of both measures could be explained by the severity of DM1-related changes affecting the thalamus or its projecting fibres.

7.4.4 Structural brain changes

Previous studies in the general population have described an association between obstructive sleep apnoea and structural brain change, involving regional losses of cortical grey matter [330,331]. The largest general population study to date (n.681) suggests that the structural change most closely related to SDB is reduced white matter volume, with greatest change occurring in the parietal lobe [332]. In our DM1-affected cohort, we did not see any major effect of SDB on grey matter, but a significant association with reduced white matter volume was detected, which was localised predominantly to the frontal and parietal lobes. While cause and effect relationships cannot be inferred from our data, similar structural brain changes in the general population with OSA have been held to likely represent the sequelae of hypoxic damage [332]. This finding therefore adds further evidence to support proactive treatment of SDB in DM1.

Structural findings with respect to sleep architecture and latency are more challenging to interpret. In normal sleep physiology, the transition from wakefulness to stage 1 sleep is primarily initiated by cells located in the preoptic area of the hypothalamus. These nuclei receive input from the suprachiasmatic nucleus (SCN), which in turn integrates information from the circadian system to co-ordinate sleep with the environmental light-dark cycle [328]. Activation of these nuclei inhibits propagation of signals along the arousal system, leading to a gradual change in thalamic firing from tonic to a phasic pattern. Entry into stage 2 sleep is defined by the thalamus beginning to generate sleep spindles, which propagate outwards to the cortex and are thought to be involved in memory consolidation [263]. In SWS, there is a widespread reduction in activity of multiple brain regions, including dorsal pons, the mesencephalon, cerebellum, thalamus, basal forebrain, prefrontal cortex, anterior cingulate, precuneus and the medial aspect of the temporal lobe [333]. This in turn hyperpolarises the thalamus, which then generates slow wave oscillations.

Entry into REM sleep is characterised by an increase in activity of several brain regions including pontine tegmentum, thalamus, the limbic system and the posterior cortices, with corresponding reduced activity in dorsolateral prefrontal cortex, parietal cortex, posterior cingulate and precuneus [333]. Muscle atonia occurs in REM sleep, both through active inhibition of spinal motor neurons by neurons of the magnocerebellar reticular formation (MCRF; located in the medulla oblongata), as well as reduced drive from brainstem locomotor generators. The MCRF is influenced by a number of additional structures within the brainstem, as well as receiving input from forebrain structures including substantia nigra, hypothalamus, thalamus, basal forebrain and frontal cortex [334]. Exit from REM sleep is usually marked by activation of the wakefulness system, since REM sleep usually ends with an arousal.

Given the key role of structures within the brainstem, the hypothalamus and thalamus to wake-sleep regulation and sleep architecture, it was surprising that our data revealed no clear signals from these structures (with the exception of a small cluster of grey matter loss detected in thalamus in relation to increased REM sleep). There could be several explanations for this. Firstly, segmentation of tissue class volumes may represent a relatively crude measure of the integrity of the complex network of tracts and nuclei involved in sleep, particularly those within the brainstem. Hence further detailed analysis of additional modalities obtained within our study, most notably DTI sequences, remains highly desirable.

Secondly, the regulation of sleep and wakefulness, sleep architecture, and the transition between stages is considerably more complex than the summary above might imply. Each of the key neuroanatomical structures exist within highly complex neural networks, and their drive is in turn modulated by input from diffuse brain regions including subcortical and cortical structures [335]. Given that DM1 does not affect brain structures symmetrically, with rate of atrophy considerably greater in some structures compared with others, it could be hypothesised that altered sleep architecture is the result of an ‘unbalancing’ of the normal regulatory systems, rather than a simple loss-of-function model. Improved understanding of this process could highlight targets for therapy, since if pathology can be attributed to a relative increase of certain neurotransmitter

systems compared with others, then this could lend itself to pharmacological dampening of the relevant pathways.

The observation that volume loss affecting the anterior cingulate was associated with shorter sleep latency is interesting. In patients with normal pressure hydrocephalus, reduced relative blood flow to the anterior cingulate was associated with impaired wakefulness [336], and in positron emission tomography (PET) studies, blood flow in the anterior cingulate was seen to co-vary with that in the thalamus during transition from wake to sleep, and alertness to relaxed waking, implying that it forms part of the ascending arousal complex [337]. This therefore represents the first direct evidence of an association between excessive somnolence and structural change in the ascending arousal system.

7.5 Conclusions

Type II PSG was well tolerated by adults with DM1, and confirms SDB as a common finding in this group. Risk factors for SDB include increasing age, male sex and self-reported muscle weakness, although no single measure consistently distinguishes patients with moderate to severe SDB from those without. Presence of moderate to severe SDB was associated with white matter loss affecting frontal and parietal lobes, adding further evidence to support a proactive approach to detecting and treating SDB in DM1. Analysis by voxel-based morphometry did not support volumetric changes in the brainstem, thalamus or hypothalamus as a marker of excessive sleepiness or altered sleep architecture, although an association between sleep latency and volume loss in the anterior cingulate highlights a possible link between excessive sleepiness and structural change in the ascending arousal system. Further exploration of these findings, including analysis of the DTI and fMRI sequences already acquired in this cohort, is clearly indicated.

8 Discussion

The work presented in this thesis was undertaken in the context of continued progress towards potential disease-modifying therapies for DM1, and with the anticipation of further clinical trials involving DM1 patients in the imminent future. Against this backdrop, we set out to evaluate the validity of currently recommended clinical outcome measures for CNS evaluation in DM1, in order to help inform the robust design of future trials. We sought to describe the relationship between CTG repeat length and key symptoms, to explore the suitability of genetic measures as a basis for patient stratification and prognostication. From structural imaging analysis, we aimed to explore the volumetric brain changes in DM1 most closely associated with CTG repeat length, and in so doing identify candidate structures to act as imaging biomarkers for longitudinal research. Finally, through robust phenotyping of CNS involvement including sleep in a moderate-sized cohort, we sought to gain novel insights into the mechanisms underlying key features of this disease.

The study was broadly successful in meeting its primary aims, and its findings make both a significant contribution to understanding of CNS involvement in DM1, and provide insights that are highly relevant to the planning of future DM1 clinical trials. The final conclusions of the study, their implications for current practice and potential future directions are discussed in detail as follows.

8.1 Recruitment

The present study recruited 46 adults with DM1 and a single premutation carrier from patients attending the West of Scotland myotonic dystrophy service. Despite a comparatively demanding protocol, dropout rates were low and a robust dataset was successfully obtained for analysis. This finding in itself is significant for clinical trial planning, as it demonstrates that sample sizes suitable for early stage trials can be achieved from a single, well-integrated regional centre in the UK. Moreover, given that concerns have been raised regarding the UK's current capacity to host clinical trials in neuromuscular disorders [231], our experience should raise the profile of the West of Scotland as a potential collaborating site for future DM1 studies.

One of the strengths of our study was its relatively unbiased recruitment strategy. Steps were taken to minimise selection bias, including approaching all suitable candidates attending the annual review service, and offering home visits and transport by taxi free of charge to willing participants. Comparing demographics of our cohort with that of collaborating colleagues at the University of Iowa, we found that our subjects had on average more severe muscle symptoms (modal MIRS score = 4 versus 2), and larger CTG repeat sizes. The Iowa cohort was recruited by advertising via the Myotonic Dystrophy Foundation, with many participants recruited in person at national conferences, and subjects were typically required to take lengthy journeys within the USA to participate. The demographic differences therefore likely reflect a stronger selection bias for more highly motivated, mildly affected individuals at the Iowa site. While the recruitment strategy in Scotland could not eliminate this bias altogether, participation would have been more feasible for patients with severe symptoms. Hence we would speculate that the Scottish sample is a more representative cross-section of all individuals with adult-onset DM1 living in the community.

For future DM1 studies, this observation provides evidence that recruitment strategy can greatly influence the characteristics of a study sample. Where large samples representing the full spectrum of DM1 phenotypes are desirable, pooling of common data from multiple sites may be the optimal strategy, compared with recruitment to a single centre from a wide geographical range.

8.2 Self-reported symptoms

Analysis of self-reported symptom measures confirmed, as expected, that DM1-affected adults frequently experience fatigue, daytime somnolence, cognitive difficulties and impaired social performance. Low mood was also common, with just under 1/3 (29%) meeting the clinical threshold for depression on the BDI II. Participants' mood scores showed strong co-linearity with several self-reported somatic symptoms. Crucially, these were often not concordant with objective assessment of disease severity by clinical evaluation, MR imaging or rating by a proxy.

This adds to existing evidence for impaired disease awareness in DM1 [202], and so urges caution against the use of questionnaire-based tools as the sole outcome measures for use in future clinical trials, particularly those targeted towards CNS symptoms. As such, a straightforward, minimally invasive means to quantify brain involvement remains a significant unmet need for DM1 research. Aside from imaging measures, possible avenues to address this need include further refinement of neuropsychological evaluation, to target the most informative domains in DM1 and minimise interference from peripheral muscle weakness, perhaps through use of assistive technology. Specific circulating micro RNAs (miRNAs) have shown promise as serum biomarkers of overall disease burden and muscle involvement in DM1, though their relationship with CNS involvement has not yet been specifically explored [338-340]. Since future clinical trials may include intrathecal administration of a test drug, CSF biomarkers represent a further important, and currently under-explored avenue to identify biochemical markers of CNS involvement [341,342].

Our data in relation to mood and self-reported symptoms also highlight an important potential avenue for therapeutic intervention in DM1. It has long been recognised that perception of physical symptoms – particularly those of pain or fatigue - may be heightened by adverse psychological factors, such as negative thoughts or depressed mood [343]. In addition, negative social experiences and isolation can act as triggers for depression, which in turn may perpetuate a cycle of further avoidant behaviour [344]. In DM1, physical impairments in combination with excessive somnolence symptoms inevitably impact individuals' ability to participate in employment and leisure activities [233]. This means that many individuals spend lengthy periods at home with relatively low levels of physical activity and social interaction. Those interactions that do occur may be prone to misunderstanding or conflict, due to impaired social cognition [126-128], or communication hampered by dysarthria and poverty of facial expression. As such, a cycle of increasing social withdrawal and loss of confidence might be predicted, in keeping with the classical personality portrait of DM1 which has been described as "a private, introverted individual with little self-esteem, burdened by fatigue and low energy" [124].

A talking-therapy approach, termed cognitive behavioural therapy (CBT) has been applied in a range of contexts where symptoms such pain or fatigue may be perpetuated by low mood and behavioural factors. The basic premise of CBT is that individuals sometimes adopt fixed patterns of thinking that result in behaviours that are maladaptive to their situation, and serve to maintain their emotional distress [345]. Using the core model of CBT, an example of a hypothetical pattern that might be encountered in DM1 is given in Figure 57.

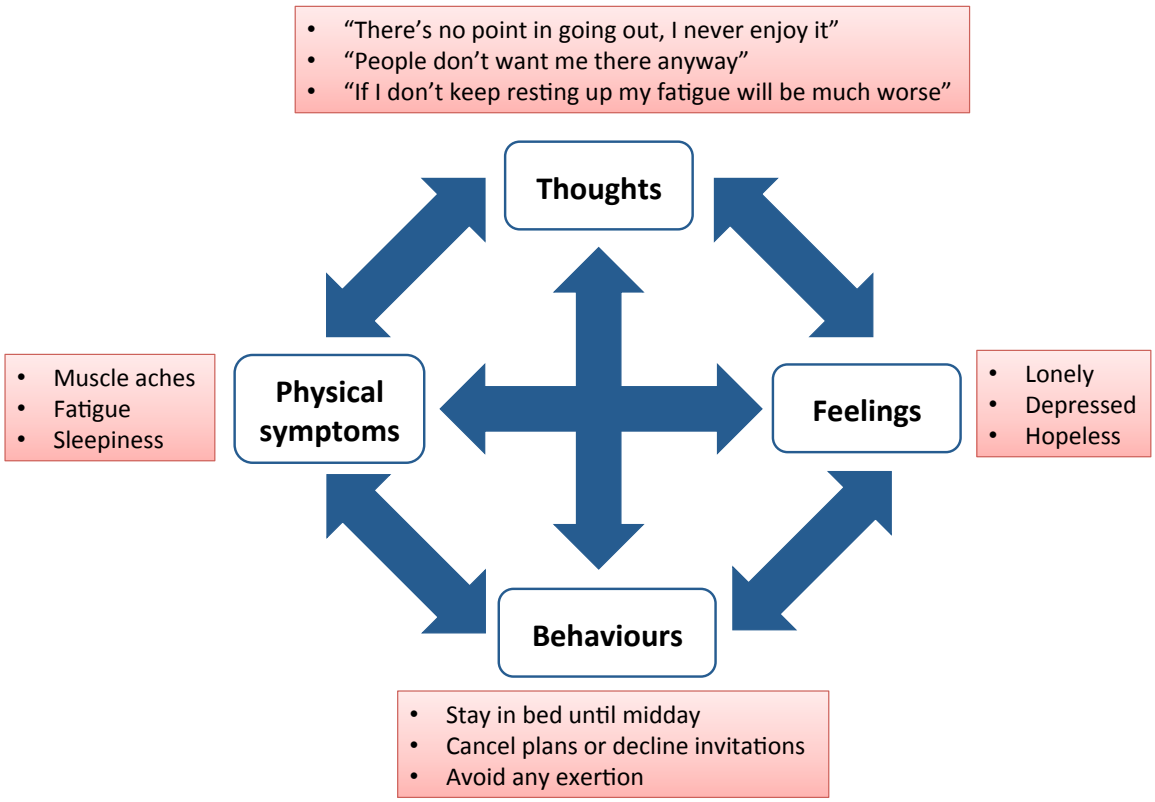

Figure 57: The core model of cognitive behavioural therapy, showing a fictional example of maladaptive patterns of thinking and behaviour that could be encountered in DM1

The aim of CBT is, through working with a psychologist or counsellor, to challenge these fixed thoughts, and replace them with more helpful patterns of thinking, thus encouraging an individual to undertake behaviours that will impact their thoughts and feelings in a more positive way [346]. This approach has demonstrated success in improving fatigue symptoms in a range of clinical contexts, including multiple sclerosis [347], post-chemotherapy fatigue [348] and in facioscapulohumeral muscular dystrophy [188].

Recently the first randomised control trial of CBT in DM1 was undertaken; the OPTIMISTIC study. CBT modules were tailored to each individual, but a common core aim was to increase physical activity levels. The intervention was associated with improvement in the primary outcome measure of DM1-ActivC© score (held to represent capacity for activity and social participation), as well as increased physical activity measured by accelerometer and reduced fatigue scores [189]. Perhaps surprisingly, apathy and depression scores did not significantly improve with the OPTIMISTIC protocol. This study therefore provides highly encouraging evidence that symptoms of DM1 can be meaningfully improved by psychological intervention and physical exercise. However the results also highlight that more work is needed to fully elucidate the basis of low mood occurring in DM1, and longer follow-up of CBT cohorts may be required to confirm whether, as would be expected, improved social participation does ultimately have a positive impact on mood in this group.

The persistence of apathy scores despite treatment in the OPTIMISTIC cohort also raises concern that new exercise habits may not be maintained on completion of the intervention. Dysregulation of a number of pathways are thought to contribute to loss of muscle mass in DM1, including inhibition of anabolic pathways, such as the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway, alongside activation of pathways that induce autophagy and apoptosis [349,350]. Together with the endocrine phenotype of increased insulin resistance and fatty liver disease, parallels have been drawn between the DM1 phenotype in muscle, and the sarcopenia of aging [351]. Undoubtedly, however, physical inactivity compounds the primary muscle disease in many individuals with DM1, further accelerating muscle atrophy. In both normal aging and other neuromuscular disorders, the benefits of physical exercise to preserve muscle function are well established [188,352,353]. Therefore improved understanding of the barriers to sustained physical exercise in DM1 - which may include psychological factors, lack of tailored guidance and coaching, or access to appropriate facilities – is a further area of research need. Indeed, it could be argued that addressing central symptoms such as apathy is a necessary prerequisite to undertaking trials of drugs intended to act on peripheral muscle, since physical inactivity may blunt to any response to treatment.

These emerging insights into the important interplay between mood and physical symptoms can help to shape the current practice of clinicians looking after patients with DM1. Specifically, they suggest healthcare professionals should be vigilant for signs of mood disorder, and have a low threshold to facilitate access to clinical psychology services where issues are identified. Furthermore, opportunities can be taken in the clinic to introduce discussions around the benefits of physical activity and social participation. This may be particularly effective if other family members are also present, who can be encouraged to take on a 'coaching' role, similar to that of the CBT counsellor.

8.3 Genotype-phenotype correlations

We did not detect any correlation between CTG repeat length and CNS symptoms of fatigue, low mood, EDS or self-reported cognitive or social difficulties. With respect to cognitive performance, only a relatively weak inverse correlation with visuospatial performance (Block Design score) was observed. A limitation of the study in detecting genotype-phenotype correlations was undoubtedly the relatively small sample size of less than 50 individuals, which could be subject to sampling error. Furthermore, active exclusion of childhood-onset forms of DM1, hence larger repeat sizes, would also have limited the power to detect correlations.

A positive correlation was however detected between CTG repeat size and self-reported mobility problems, suggesting muscle phenotype in DM1 may be more strongly related to CTG repeat length compared with CNS symptoms or cognitive performance. This hypothesis is supported by data from a separate DM1 cohort recruited in Saguenay-Lac-Saint-Jean, Canada in which genetic analysis by our group revealed strong inverse correlations between ePAL and numerous measures of muscle power and pulmonary function after correction for sex, age and height (Overend *et al*. manuscript in preparation).

Allowing for the limitations of sample size, this observation adds further evidence that factors distinct from the primary disease process are major modifiers of cognition and the perceived impact of CNS symptoms. These factors might include biological variation, including inherited variants in genes other than *DPMK* that influence premorbid intelligence, as well as personality traits

such as resilience, motivation and susceptibility to depression. In addition, myriad environmental factors including presence of a support network, educational background, impact of DM1 on employment, and past experience of relatives affected by DM1 also shape an individual's emotional response to their condition, in turn influencing perception of symptoms. As such, absence of a strong correlation with repeat length can be viewed as offering hope that additional, potentially modifiable factors play a major role in the pathogenesis of CNS symptoms. Further work to elucidate specific risk factors for fatigue, low mood, somnolence, cognitive impairment and social difficulties is therefore warranted, since this may help to guide strategies to support individuals in maintaining quality of life despite the physical impacts of DM1.

We identified three individuals with variant repeats within the Scottish cohort. All reported relatively mild muscle symptoms on DM1-ActivC©, but otherwise were not clear outliers in other markers of disease severity, such as cognitive performance or structural brain measures. Four further individuals were identified in the Iowa cohort, and similarly no clear effect of variant repeat status was observed on volumetric brain measures. Again acknowledging the small sample size and potential for sampling error, this observation was perhaps surprising. Evidence continues to grow in the published literature that variant repeat expansions are often associated with unusually mild or even absent clinical signs in DM1 [33-36,234,354,355]. It should be noted however that most individuals with variant repeats in the pooled Glasgow-Iowa cohort were comparatively young, with a mean age of 36; hence any modifying effect on progressive phenotypes may less apparent in this age group. Furthermore, the structure of the variant repeat alleles in the Iowa subjects have not yet been investigated. It is likely that the number and pattern of variant repeat interruptions, and their location within the CTG array modify their effect on somatic instability and symptomatology, although the nature of this relationship is currently not well understood. It is even conceivable that presence of variants in the sequence flanking the CTG repeat could result in a false positive result in screening by AciI enzyme digest. Therefore characterisation of these alleles, using next-generation sequencing approaches as previously described [355], is an important extension of the present study.

These cases also highlight the challenges currently facing clinicians seeking to provide genetic counselling to patients who carry variant repeats. At present, the information that can be offered to individuals with such alleles regarding their own prognosis, or likely outcome of future pregnancy, is very limited. For example, while variant alleles are generally described as more stable in the germline than pure repeats of similar length, our group recently described a variant allele containing CTG-CCG hexamers at the 3'- end that underwent surprisingly large expansions in the female germline [355]. Continuing to gather phenotypic data from large numbers of individuals with variant repeats, including examples of transmission within families, is therefore highly desirable. Routine screening for the presence of variant repeats in future natural history studies or clinical trials, and development of semi-automated methods to characterise variant allele structures would be useful steps towards this end.

8.4 Imaging biomarkers

Work in collaboration with the University of Iowa enabled us to explore structural brain changes in the largest case-controlled DM1 imaging cohort reported to date. Using a joint label fusion approach, we described the structural landscape of volumetric changes in the DM1 brain both in comparison to controls, and in relation to CTG repeat length. The successful harmonisation and analysis of MRI data as a pooled cohort demonstrates the feasibility of multi-site imaging studies in DM1, offering a solution to the problem of limited power in previous single-site imaging studies.

Imaging analysis was undertaken in the wider context of efforts to identify and validate imaging biomarkers for CNS involvement in DM1, highlighted as a major priority for clinical trial readiness by expert working groups [195,196]. We reasoned that structures with strong potential for use as imaging biomarkers would be those in which large changes are seen in DM1 individuals compared with controls, and those in which change was most directly driven by the primary genetic lesion. Furthermore, an ideal structural biomarker would have direct relevance to key symptoms of DM1, and would demonstrate deterioration within the typical timescale of a clinical trial.

With respect to these criteria, we showed that volume of whole-cerebrum grey matter, occipital grey matter, putamen and thalamus were inversely related to ePAL length. A positive association was also observed between ePAL and volume of cerebellar white matter and amygdala.

Several of these structural observations offer possible links to key symptoms in DM1. We hypothesised that increased volume of amygdala in the DM1-affected brain, combined with reduced volume of structures involved in emotional regulation and motivation (frontal lobe and nucleus accumbens respectively), could cause individuals with DM1 to be more inclined to associate external stimuli with negative emotions. This could offer some insight into the avoidant traits and social withdrawal that are frequently observed [115]. Further exploration of the association between structural changes, or activity measured by fMRI, in amygdala, frontal lobe and accumbens with clinical measures of anxiety, depression and apathy would be useful to test this hypothesis. Specific tests of cognitive reappraisal – the capacity to modify perspective on a negative stimulus, such as an unpleasant image, to modulate its emotional impact – would be particularly relevant to the function of these structures [284].

Findings in relation to the thalamus were also of particular interest, since this structure plays a key role both in architecture of sleep, and in cognitive processing [262,263]. This hypothesis was further supported by mutual correlations between severity of DM1-specific changes in sleep architecture and cognitive performance. The *a priori* hypothesis in relation to thalamus volume and loss of stage 2 sleep was not supported by results from preliminary voxel-based morphometry analysis. Nonetheless, an important extension of the work presented here will be to explore further functional correlations of the structural brain findings, including self-reported symptoms and cognitive performance. Since some common neuropsychological and symptom data are available between the Glasgow and Iowa cohorts, some of this analysis could be undertaken as a combined cohort.

The Scottish imaging study was cross-sectional in design, and so no data were obtained relating to the rate of structural brain change over time. Longitudinal imaging studies are generally lacking from the DM1 published literature, with only a single small case series describing progression of white matter lesions and

global atrophy [159]. Fortunately, participants recruited in Iowa will be followed up and re-imaged at one and two years from baseline, but at the time of writing no such plans are in place for the Scottish cohort. Therefore it remains to be seen whether volumetric changes in subcortical structures such as thalamus show sufficient change over time to be useful as clinical trial biomarkers.

The predicted effect of therapy might also be relevant to imaging biomarker selection. For example, effective treatment might be expected to cause a slowing or plateau of grey matter volume loss. However, the capacity of the brain to regenerate lost neurons (and hence potentially demonstrate volume increase in response to treatment) is unclear, but is likely to be extremely limited [356-358]. DTI metrics such as FA, on the other hand, which are held to reflect the integrity of white matter tracts in DM1 [359], may have capacity to improve with treatment. This has been demonstrated in Wilson disease, in which improvement in DTI measures may be seen following de-coppering treatment [360]. For this reason, it is important that longitudinal changes include not only on conventional structural MRI, but also a range of modalities including DTI.

8.5 Sleep-disordered breathing

The most striking finding from analysis of PSG data was the high incidence of SDB in our DM1-affected subjects. Abnormal respiratory studies were obtained from 86% of participants, and 56% had moderate or severe SDB.

Participants with clinically significant SDB had increased white matter loss in frontal and parietal regions, similar to patterns seen in the general population in association with obstructive sleep apnoea [332], which is held to represent hypoxic damage. There was also a trend towards increased liver transaminases, HbA1c and abnormal ECG parameters in this group after adjustment for differences of sex and BMI, although these did not reach statistical significance.

These preliminary observations suggest proactive treatment of SDB could serve to attenuate some end-organ effects of DM1. This hypothesis is supported by a recent longitudinal study, which demonstrated that compliance with home ventilation (prescribed for a range of reasons, including prolonged nocturnal hypoxia) was associated with improved survival in DM1 patients [298]. As

discussed in Chapter 7, SDB has potential to impact several systems relevant to DM1, including cardiovascular, CNS and endocrine systems, and so further studies in larger cohorts would be useful to attempt to quantify the relative benefit of therapy for specific DM1 sequelae. Since current evidence is considered insufficient to support treatment of asymptomatic OSA in the general population [306], it would also important to explore whether any clinical benefit is present in DM1 patients who deny EDS symptoms.

Reviewing current practice in the West of Scotland in light of the study findings, our data suggest current thresholds for respiratory referral ascertain only a small proportion of individuals with moderate to severe SDB. On the other hand, the lack of evidence to support treatment of SDB in the absence of somnolence symptoms means that local respiratory physicians are unlikely to support systematic screening of all individuals with a diagnosis of DM1 (personal communication, Dr Eric Livingston). A key component of appropriate referral must therefore be the presence of EDS symptoms in some form. Given the recognised issues of impaired disease awareness in DM1 [202], there is a major role for clinical acumen, ideally guided by collateral history from relatives, in ascertaining whether significant EDS symptoms are present. Our data demonstrated that the Epworth Sleepiness Scale had particularly poor discriminatory power for EDS, in keeping with the observations of previous studies, which found weak internal consistency of the Epworth in DM1 [212]. The reasons for poor performance of the Epworth are unclear, but it has been speculated that, while the majority of questions in the Epworth enquire about somnolence during specific activities, the characteristic phenotype of DM1 is tendency to doze off when attention is not held. We demonstrated that the FDSS, a tool developed specifically for DM1 by Rasch analysis of existing questionnaires including the Epworth [201], had greater discriminatory power for SDB. These results would therefore support use of the FDSS in place of the Epworth where a scalar measure is desirable to record somnolence symptoms. Finally, awareness of the risk factors for SDB identified in the study cohort – namely, increasing age, male sex and increasing muscle weakness – may also be useful to guide the decision of whether to refer for sleep studies in borderline cases.

In keeping with reports from other centres [96], anecdotes are frequently encountered in the DM1 clinic of patients for whom PAP therapy or ventilation has been recommended, but who declined treatment or gave up after a short trial. Increased ascertainment of SDB will therefore make little impact if adherence to therapy remains poor, and so strategies to support compliance would also be needed to achieve a clinical benefit. Scottish audit data for 2013 [361] showed adherence rates for PAP or non-invasive ventilation in DM1 patients were considerably higher in the South East of Scotland compared with other regions. This was postulated to reflect the nurse-led ventilation service available in this region, which enabled patients starting to use a device to have frequent home visits from a specialist nurse, who was able to help overcome initial problems such as ill-fitting masks or skin irritation. The feasibility of implementing such a service in other regional centres is therefore worthy of exploration, although justification of high cost for a small number of patients is likely to be a major barrier.

An additional strategy, at no significant cost, would be for DM1 clinicians to use annual review appointments as an opportunity to introduce the idea of PAP and ventilation therapies, and provide education to patients and their families regarding the rationale of these. Ideally, this should be introduced early, before any diagnosis of SDB has been made, so that key points can be reinforced over several years of annual appointments. In particular, the potential for prognostic benefit, even in the absence of symptomatic improvement, would be useful to emphasise. In this way, a patient who and has been given the opportunity to become habituated to the notion that wearing a mask overnight may form part of their treatment in the future, and is clear on the reasons for its recommendation, would presumably be more likely to accept and persevere with a trial of therapy should it subsequently be recommended.

8.6 Towards understanding sleep disorders and excessive daytime somnolence

Our sleep studies confirmed reduced sleep efficiency and altered sleep architecture in DM1, consistent with an increased proportion of slow wave and REM sleep, and reduced stage 2 sleep. Sleep efficiency was not related to AHI, suggesting respiratory events were not the primary cause of sleep

fragmentation. Nor was reduced sleep efficiency associated with objective or subjective measures of excessive sleepiness symptoms, suggesting disruption of sleep alone is not the cause of EDS.

We had hypothesised that structural brain changes secondary to neuronal loss would account for the alterations in sleep architecture. However, explorations of volumetric changes by voxel-based morphometry did not reveal changes in structures with a clear mechanistic link to sleep neurophysiology. Since control of the sleep-wake cycle and transition between stages of sleep is largely dependent on nuclei within the brainstem, further exploration of the study data using imaging modalities more informative for the integrity of brainstem structures might be valuable. An MR tractography approach, derived from DTI sequences, would be particularly effective for this purpose [362].

The correlations we observed between sleep architecture and cognitive performance were particularly interesting, and may help to refine our hypotheses. Better cognitive performance was associated with preservation of stage 2 sleep, while poorer performance was seen in those with increased percentage of stage 1 and REM sleep. While further analysis of sleep data is hampered by a relatively small sample size, analysis of cognitive performance in the combined Glasgow-Iowa cohort may highlight structures with a dual role in both cognition and sleep.

Our mMWT protocol was itself a novel approach, aiming to capture an ecologically valid measure of subjects' ability to volitionally resist sleep in their home environment. These data are inevitably confounded by the inherent variability of a domiciliary setting, such as temperature, ambient noise and ability to achieve dimmed lighting. In addition, several subjects found this portion of the protocol burdensome and were unwilling to complete all three nap opportunities. We did not detect strong correlations between sleep latency measured in this way and any other clinical measures, although trends were seen between shorter latency in MWT1 and increasing ePAL length, and increasing MIRS score. Shorter mean latency in all mMWT trials was loosely associated with increasing Epworth score. Perhaps the most significant finding in relation to sleep latency was that shorter latency was associated with greater grey matter volume loss in several regions of brain, most notably in the anterior cingulate.

This constitutes the first direct evidence of an association between excessive somnolence and volume loss affecting the ascending arousal system. Further detailed analysis of imaging data in relation to the ascending arousal system may therefore yield further candidate biomarkers for excessive somnolence.

This finding does not, however, refute theories for additional mechanisms that may contribute to EDS in DM1. The neuroendocrine systems involved in control of circadian rhythm have not been comprehensively studied in DM1, although the available evidence suggests normal diurnal patterns are substantially disrupted. Abnormal circadian variation of cortisol and thyroid-stimulating hormone secretion [363], and failure to produce the expected growth hormone elevations during slow wave sleep are described [364], which together imply dysregulation of the hypothalamus-pituitary-endocrine axis. The normal diurnal variation of daytime sleepiness also appears altered in DM1, and sleep architecture has been likened to that seen in a "free-running" system, usually seen in individuals who are isolated from the regulating influence of a 24 hour light/dark cycle [365]. Orexin levels in CSF also appear reduced, although not to the degree typically associated with narcolepsy [366]. The endocrine phenotype of DM1 is further characterised by an increase in pro-inflammatory cytokines including IL-6 and TNF-α [367], relative insulin resistance, and susceptibility to fatty liver disease [86]. In the general population, the same features have been linked to poor quality of sleep and excessive daytime sleepiness through unclear mechanisms [368,369]. Useful additional work in DM1 therefore might include a longitudinal study of sleep, including measures of diurnal variation in hormone levels, and ideally levels of daytime activity and sleep patterns.

8.7 Wider strategies for research in DM1

Our experience with the present study has underlined the value of combining data from multiple centres, to achieve large cohorts that represent a valid cross-section of the total DM1 population. Previous barriers to harmonised, multi-centre research, such as regional variation in clinical measures used, have been lessened by the development of consensus-recommended, often disease-specific clinical outcome measures [194,195]. Likewise, technological advances have enabled the effective harmonisation of imaging data acquired from multiple study sites [223,225,226].

Patient registry approaches have been used to powerful effect in other disorders, to gain large volumes of natural history data from multiple sites, as well as acting as a platform to facilitate additional clinical studies and trials. For example, the Enroll-HD study (www.enroll-hd.org) is on course to recruit over 20,000 individuals worldwide with Huntington disease, collecting common clinical data longitudinally, as well as improving clinical trial readiness by ascertaining and characterising an extremely large pool of willing study subjects [370].

For myotonic dystrophy, a number of national patient registries also exist, which are encouraged to collect a comparable minimum dataset as outlined in the Treat-NMD Registries Toolkit [371]. At the time of writing, 778 patients were registered in the UK database (https://www.dm-registry.org/uk/). Further work to integrate these national databases, and expand the core dataset would be a highly useful step towards clinical trial readiness. While funding for collaborating centres to undertake detailed phenotyping work will undoubtedly represent a barrier, technological solutions could be utilised to minimise the burden on clinical services. For example, self-reporting of symptoms, as well as neuropsychological assessment through puzzle-based games could be achievable by developing downloadable applications for mobile phones or other devices [372]. Wrist-worn devices have shown acceptable concordance with full PSG in distinguishing sleep and wakefulness [373]. While these systems would not give detailed data regarding sleep architecture, measures such as total sleep time, sleep efficiency and timing of sleep could be easily derived. Such a device would be far more acceptable to patients than inpatient PSG, be much less likely to induce first-night effects, and could also gather data relating to sleep and physical activity patterns by actigraphy over several days, and so give insight into circadian rhythms and the influence of exercise. Costs would also be minimal compared with inpatient studies, particularly since patients may even already own commercial devices suitable for this purpose.

An additional strategy, which could be more formally implemented in concert with the patient registry, is the routine use of tissue biobanks. Biobanks, such as the Newcastle MRC Centre Biobank for Rare and Neuromuscular Disease, aim to facilitate more straightforward access to patient samples for selected

biomedical researchers; samples which are otherwise rare, and may require lengthy approval and recruitment processes for each individual project [228]. Excess serum and whole blood samples can be readily obtained at routine outpatient appointments, and additional tissues may be obtained from patients undergoing planned procedures or surgery. Anecdotally, many people with DM1 also report that they would like to bequeath their bodies for research work after death, but no clear pathway currently exists to facilitate this within the UK. Post-mortem brain tissue would represent a valuable resource to verify the regional structural differences observed on MRI scanning, to explore the histological correlates of white matter lesions and grey matter volume loss, and to explore regional variation in somatic instability of the CTG repeat. Feasibility of a mechanism to record patients' wishes in this regard, and to sensitively and efficiently retrieve remains after death, should therefore be explored.

8.8 Pharmacological targets for CNS symptoms

Hypothetical targets for pharmacological treatment of CNS symptoms in DM1 might include the DNA repeat expansion itself, its toxic mRNA product, proteins implicated in or affected by dysregulation of alternative splicing, or specific neurotransmitter pathways whose function is modulated by structural brain changes. Potential targets highlighted by our findings are discussed as follows.

Several lines of evidence point to somatic instability of the CTG repeat as a major modifier of general disease progression in DM1. Evidence includes the fact that the largest expansions occur in tissues that are most severely affected [27,28], that milder symptoms are seen in individuals with variant repeats associated with reduced somatic expansion [36,355], and that somatic instability accounts for some of the residual variation in age at onset of symptoms after accounting for CTG repeat length [26]. The relevance of somatic instability specifically to CNS features of DM1 is not clear, although our observation that much greater volume loss occurs in cerebral cortex, in which the repeat is highly unstable, compared with cerebellum in which the CTG repeat is comparatively stable [28], would support a direct association with structural brain changes. Further work involving SP-PCR on dissected post-mortem brain tissue would be a useful to explore the regional topography of somatic instability further. The mechanisms that drive expansion-biased somatic instability are not fully

understood, although they are thought to include errors introduced during processes of DNA maintenance, in particular during mismatch repair [29,30]. Genome-wide association studies to identify trans-acting genetic modifiers of instability may be helpful to further shed light on this process, and may highlight specific therapeutic targets. A notable challenge for development of therapies acting on DNA mismatch repair would be to avoid any associated increased risk of malignancy. This is particularly relevant given mounting evidence that DM1 itself is associated with an increased risk of neoplasia, including cutaneous melanoma [89].

After highly encouraging results in animal models [185], IONIS Pharmaceuticals™ recently undertook a phase 1/2a trial of peripherally administered ASO, directed against toxic CUG repeat mRNA. Results announced in January 2017 revealed penetration of muscle by the ASO had not reached the intended target for therapeutic efficacy, and so IONIS elected to return to research and development of the molecule to improve delivery, rather than persevere with the drug in its present form [186]. ASOs administered peripherally are not thought to cross the blood-brain barrier in significant quantities [185]; hence effects on CNS features would not have been anticipated in this study. Since commencement of the DM1 ASO trial, the safety administration of disease-specific ASOs by the intrathecal route has been demonstrated in both Huntington disease [374] and spinal muscular atrophy [375], with striking clinical efficacy also demonstrated in the latter. As such, it would seem highly appropriate to proceed with studies of the same approach in DM1 animal models, working towards consideration of a clinical trial of intrathecal ASO administration in DM1 patients in the near future.

Improved understanding of neuroanatomical correlates with CNS symptoms may highlight specific neurotransmitter systems that are amenable to pharmacological modulation. Our observation that the volume of the amygdala, a structure implicated in the generation of negative emotional states, is increased relative to ICV in DM1-affected subjects provides one such example. Considerable efforts are currently being made to understand neurotransmission mediated by this particular structure, in order to identify targets for anti-anxiety therapy for use in the general population [376]. Inhibition of anxiety

circuitry is readily achievable with the benzodiazepine class of GABA receptor agonists, although the associated risks of excessive sedation, respiratory depression and dependence make this class of drugs unsuitable for long-term use, particularly in patients with DM1. Further work is therefore required to elucidate signalling pathways within the amygdala, which may reveal potential targets to modulate the emotional factors driving avoidant behaviours in DM1.

Methylphenidate (Ritalin™) is a psychostimulant drug, thought to improve concentration by enhancing task-dependent dopaminergic and norepinephrine signalling, while decreasing background 'noise' [377,378]. It is also considered a wakefulness-promoting drug, due to effects on dopaminergic tracts of the upper brainstem which form part of the ascending arousal system [150]. In the MBNL2 knock-out mouse model of DM1, long-term treatment with methylphenidate was recently shown to be associated with a reduction in depression-like behaviours, improved cognitive function and a reduction in pro-inflammatory microglia in the medial prefrontal cortex. To our knowledge, only a single small randomised, double-blind, placebo controlled study has explored treatment with methylphenidate in adults with DM1 (n.24) [379]. Treatment was associated with an increase in mean self-rated sleepiness scores, although three subjects discontinued treatment due to side effects. The recent observations in mouse models suggest effects of methylphenidate on CNS symptoms in DM1 may be further-reaching than EDS symptoms alone, and hence calls for a further large randomised control trial with detailed cognitive phenotyping may be anticipated.

With further regard to EDS symptoms, a recent platform presentation by Dr David Rye at the 11th International Myotonic Dystrophy Consortium meeting in San Francisco, USA (September 2017) drew comparisons between the phenotype of DM1 and idiopathic hypersomnolence (IH). Some individuals affected by IH appear to have naturally occurring GABA receptor agonists, or 'endozapines', present in their CSF, thought to increase signalling by $GABA_A$ receptors, and hence account for the IH phenotype of increased sleep requirements, unrefreshing sleep and 'brain fog' [380]. Blocking of $GABA_A$ receptors by administration of flumazenil has markedly improved symptoms in a proportion of IH patients [381]. Dr Rye has reported similar findings with administration of

flumazenil to patients with DM1 [382], although at the time of writing the findings are not yet published in a peer-reviewed journal. This hypothesis is further supported by the observation that increased sensitivity to GABA signalling has been described in DM1 [383]. If such a mechanism were closely related to dysregulated splicing due to presence of CUG repeat RNA, this could account for our own observation that sleep latency was inversely correlated with repeat length. Given the potential dangers of blocking central GABA signalling however, which might include seizures, worsening of panic disorders and transient hypertension (with associated, though theoretical cardiac risk) [384], further functional studies, work in animal models and, if supported, a randomised control trial in humans under close supervision would be required before treatment with flumazenil could be routinely recommended.

8.9 Final conclusions

Excessive daytime sleepiness, fatigue, cognitive deficits and impaired social functioning are common symptoms in DM1, and appear to arise from CNS involvement. There is continued progress towards targeted therapies for DM1, and so there is a need to identify valid outcome measures for CNS disease. We have demonstrated that issues of mood and insight confound self-reported symptom questionnaires, and primary muscle weakness may affect performance in some standard neuropsychology tests. As such, identification of imaging biomarkers is a desirable goal to improve clinical trial readiness. To this end, we identified specific regional volumetric changes in the DM1 brain compared with controls. This identified promising candidate regions, including volume loss in thalamus as a potential mediator of sleep disturbance and slowing of cognitive processing, and relative changes in volume of accumbens and amygdala as possible drivers of apathy and social avoidance. Further work to explore clinical correlations, and longitudinal studies using a variety of imaging modalities are required to take these observations forward.

Despite the current absence of a pharmacological disease modifying therapy, our data also highlight several readily deliverable strategies that might improve lives of people with DM1. Subjects' self-rating of CNS symptoms was not consistent with objective measures of CNS disease, but was instead closely linked to mood score. This suggests that detection and treatment for low mood, ideally

encompassing psychological interventions such as CBT and exercise therapy, may be effective in limiting the impact of CNS symptoms on quality of life. Furthermore, we identified that SDB was extremely common in our cohort, and was associated with loss of cerebral white matter. Combined with other published data, this should prompt a proactive approach to ascertainment of SDB in DM1 patient cohorts, and implementation of strategies to overcome issues of adherence to therapy.

Finally, the nature of excessive somnolence and sleep disorders in DM1 remains largely enigmatic. Our data suggest an association between excessive somnolence and grey matter loss in anterior cingulate. However, while this observation should undoubtedly be further explored through more detailed imaging analysis, it is unlikely that structural changes in the ascending arousal system alone account for this complex aspect of the DM1 phenotype. Patient registries, biobanks and wearable technology offer the opportunity to explore chronobiology, activity levels and neuroendocrine factors in a large number of DM1 patients, and should be exploited by future work with the aim of better understanding this important symptom.

9 References

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