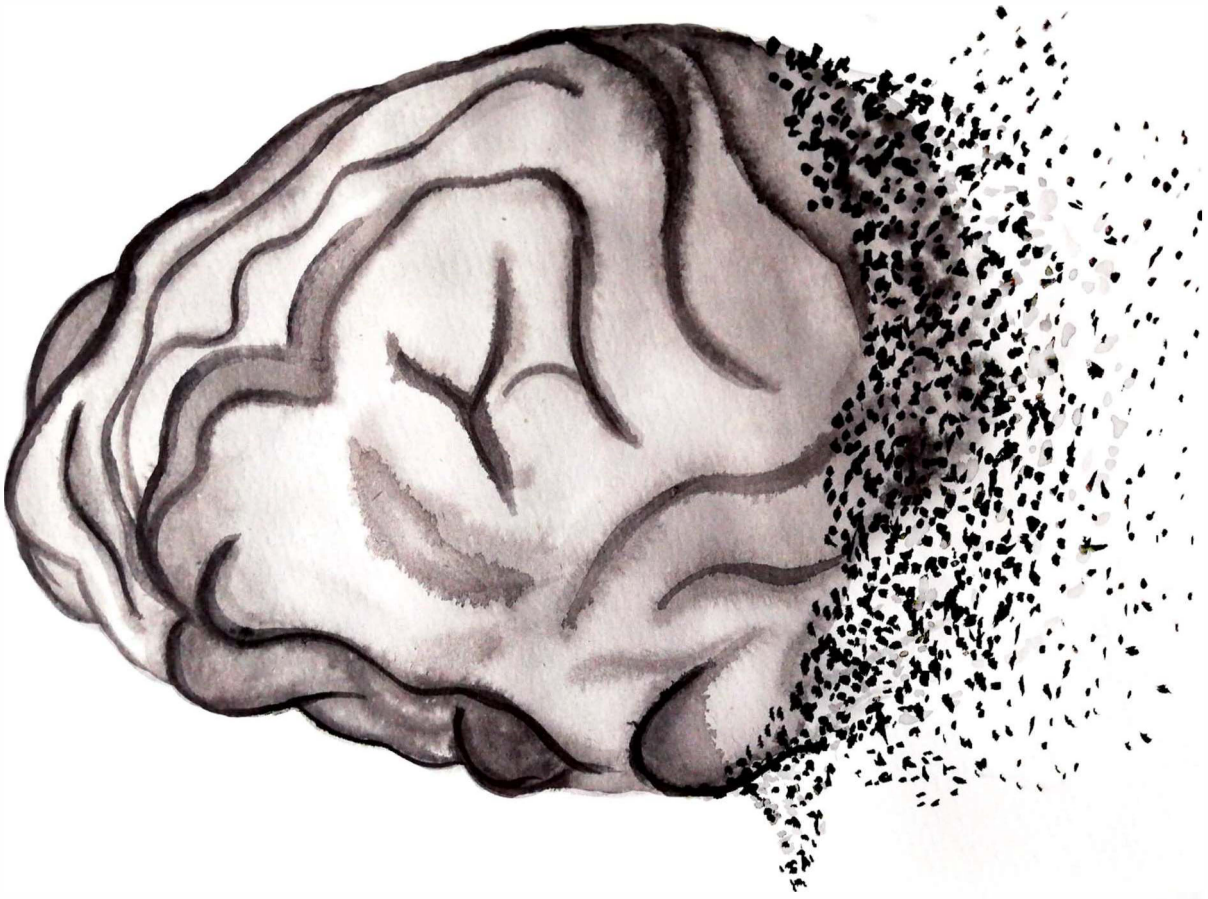
Aging in Myotonic Dystrophy Type 1: Analysis from a neuropsychological and neuroradiological approach

Garazi Labayru Isusquiza

DOCTORAL THESIS



Donostia - San Sebastián, 2020

Biodonostia Health Research Institute, Neuroscience Department

University of the Basque Country, Personality, Assessment and Psychological Treatment Department

Aging in Myotonic Dystrophy Type 1: Analysis from a neuropsychological and neuroradiological approach

Garazi Labayru Isusquiza

2020

Thesis supervisors:

Dra. Andone Sistiaga Berrondo

Dr. Adolfo López de Munain Arregui

Biodonostia Health Research Institute, Neuroscience Department

University of the Basque Country, Personality, Assessment and Psychological Treatment Department

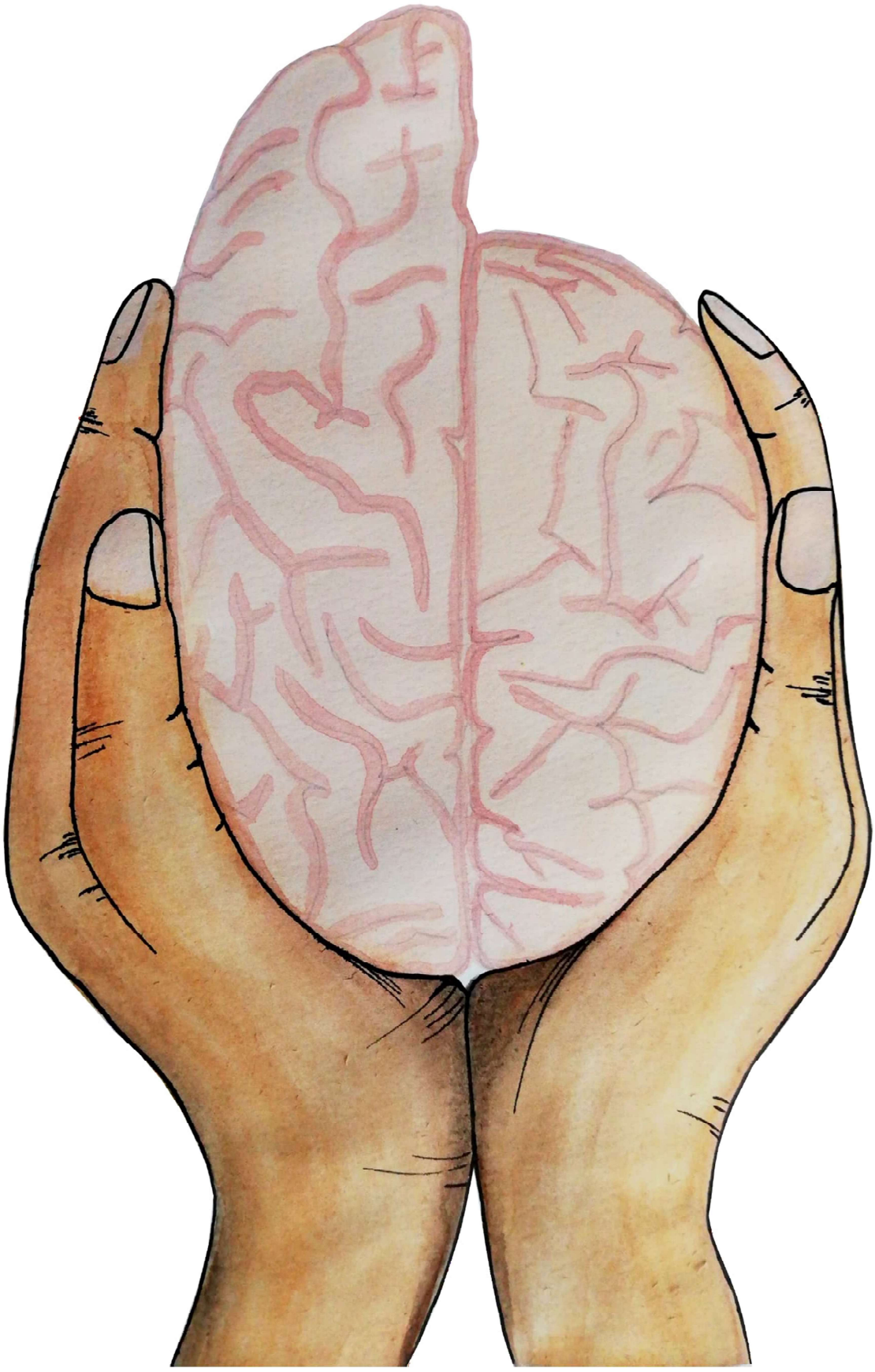
Donostia - San Sebastián, Gipuzkoa, Spain

Pour toi.

– Para saber que sabemos lo que sabemos, y saber que no sabemos lo que no sabemos, hay que tener cierto conocimiento –

Nicolás Kopérniko

CONTENTS



ACRONYMS

$A\beta_{42}$	42-amino-acid form of β-amyloid
AD	Axial diffusivity
ASD	Autism spectrum disorder
BOLD	Blood oxygen level-dependent
CALCAP	California Computerized Assessment Package
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CTG	Cytosine-thymine-guanine
DM1	Myotonic dystrophy type 1
DM2	Myotonic dystrophy type 2
DMN	Default mode network
DMPK	Myotonic dystrophy protein kinase
DNA	Deoxyribose nucleic acid
DTI	Diffusion tensor imaging
FTD	Fronto-temporal dementia
FA	Fractional anisotropy
fMRI	Functional magnetic resonance imaging
GM	Grey matter
LH	Luteinizing hormone
MD	Mean diffusivity
MRI	Magnetic resonance imaging
NAWM	Normal appearing white matter
POFA	Pictures of facial affect

RAVLT	Rey Auditory Verbal Learning Test
RD	Radial diffusivity
ROCF	Rey-Osterrieth Complex Figure
RS	Resting state
RT	Reaction time
SCD	Social-pragmatic communication disorder
SD	Standard deviation
TECA	Cognitive and affective empathy test
TOM	Theory of mind
TSH	Thyrotropin
VBM	Voxel based morphometry
WAIS III	Wechsler Adult Intelligence Scale III
WCST	Wisconsin Card Sorting Test
WM	White matter

ABSTRACT

Myotonic Dystrophy Type 1 (DM1) is the most common form of Myotonic Dystrophy (MD) in adults. It is a genetic-based disorder transmitted in an autosomal dominant manner. The mutation of the 3'- untranslated region of the *dystrophia myotonica* protein kinase (DMPK) gene on chromosome 19q13 is responsible for the expanded and unstable CTG repeat. Diagnosis is confirmed with a CTG length of >50, and the anticipation phenomenon has been described. The inheritance pattern can be maternal or paternal, with the former leading to more severe forms of the disease. DM1 is classified according to the age of onset, which can range from pre-birth up to older age, and up to five disease phenotypes have been described: congenital, childhood, juvenile, adult/classical and late/mild.

DM1 is a multisystemic disease and, to varying degrees, CNS involvement is included among the systems affected by the condition. CNS symptoms include excessive sleepiness, fatigue, affective, mood and behavior disorders, whilst specific personality traits have also been described, along with a wide range of cognitive effects, including social cognition deficits. Whilst cognition has been widely investigated in DM1, the studies have yielded conflicting results, and the exact pattern of cognitive dysfunction is still to be established. The same is true for brain abnormalities, where both gray matter (GM) and white matter (WM) anomalies have been described in studies employing structural magnetic resonance imaging (MRI), and altered connectivity patterns have been found in functional MRI (fMRI) studies. Although still inconclusive, these studies have shown a widespread alteration throughout the brain, along with some associations with genetic, clinical, and neuropsychological features of the disease.

Several data, including muscular, cell and histopathological findings, provide support for the hypothesized involvement of a neurodegeneration process in DM1, which is further encouraged by clinical observations. However, follow-up of CNS features such as cognition and brain abnormalities have only scarcely been conducted in a longitudinal manner, and, from within the scientific community there has been a clear call for clarification of the neurodevelopmental/neurodegenerative basis of CNS abnormalities.

The present thesis aims to address the previously mentioned gaps in the literature through four scientific reports. First, social cognition deficits are explored in order to discover whether these represent a core deficit or can instead be regarded as a secondary deficit of a more general cognitive impairment. Second, GM and WM structural anomalies are examined in order to define the specific pattern of brain involvement. Finally, two longitudinal studies with a timespan of nearly a decade are conducted with the purpose of delineating the profile of

progressive cognitive decline, as well as the profile of natural brain history with a focus on structural neurodegenerative trajectories.

RESUMEN

La Distrofia Miotónica Tipo 1 (DM1) es la forma más común de Distrofia Muscular (DM) en adultos. Se trata de una enfermedad con base genética transmitida de manera autosómica dominante. La mutación en la región 3 no traducida del gen proteinquinasa de la distrofia miotónica (DMPK) en el cromosoma 19q13, es la responsable de la expansión e inestabilidad de la repetición CTG. El diagnóstico se confirma con una longitud CTG >50 y se ha descrito un fenómeno de anticipación. El patrón de herencia puede ser materno o paterno, produciendo el primero formas más severas de la enfermedad. La DM1 se clasifica en base a la edad de inicio de la enfermedad, la cual puede ser desde prenatal hasta la edad adulta tardía; y se han descrito hasta cinco fenotipos: congénito, infantil, juvenil, adulto/clásico y tardío/leve.

La DM1 es una enfermedad multisistémica que incluye afectación del SNC en grado variable. Entre la sintomatología del SNC se han descrito somnolencia elevada, fatiga, trastornos del ánimo y de comportamiento y determinados rasgos de personalidad; así como una variedad de déficits cognitivos, incluyendo dificultades en cognición social. Si bien la cognición ha sido ampliamente estudiada en la DM1, los estudios han arrojado resultados poco consistentes, y el perfil concreto de afectación cognitiva está aún por determinar. Lo mismo ocurre en cuanto a las anomalías cerebrales, dónde se han reportado alteraciones tanto de sustancia gris (SG) como de sustancia blanca (SB) a partir de estudios que emplean resonancia magnética (RM) estructural; así como patrones de conectividad funcional alterados en estudios de resonancia magnética funcional (RMf). Aunque aún de manera no concluyente, estos estudios han mostrado alteraciones de forma diseminada en el cerebro y algunas asociaciones con aspectos genéticos, clínicos y neuropsicológicos de la enfermedad.

Varios datos, incluidos hallazgos musculares, celulares e histopatológicos apoyan una hipótesis neurodegenerativa en DM1, a la que además dan soporte las observaciones clínicas. Sin embargo, apenas se han realizado estudios de seguimiento sobre aspectos del SNC como la cognición o las anomalías cerebrales con un enfoque longitudinal, y existe una clara demanda por parte de la comunidad científica de clarificar la base neurodegenerativa/del neurodesarrollo de las anomalías del SNC.

Esta tesis tiene como objetivo responder a estas lagunas de conocimiento que existen en la literatura científica a través de cuatro estudios. En primer lugar, se explora la cognición social con el propósito de clarificar si representan un déficit central en la enfermedad, o por el

contrario, es secundario a un deterioro cognitivo más global. En segundo lugar, se examinan las anomalías estructurales en SG y SB con el objetivo de definir el patrón específico de afectación cerebral. Finalmente, dos estudios longitudinales tratan de describir el perfil de deterioro cognitivo, así como la historia cerebral natural analizando las trayectorias de neurodegeneración estructural.

SECTION I.

SYNTHESIS

INTRODUCTION

As a first step to introducing this thesis, it is important to make clear to the reader the framework in which this work has been conducted. Despite the fact that DM1 is a rare disease, the geographical area of Gipuzkoa is known for having one of the highest prevalence rates worldwide, with an estimated 300 cases per million inhabitants. This geographical location therefore provides an ideal setting for the study of DM1. Moreover, the work presented in this thesis has been conducted in a research group headed by thesis supervisors that have a long-standing interest and expertise in work related to the clinical, cognitive and neuroradiological characterization of the DM1 population. In particular, Dr. Adolfo López de Munain successfully completed his thesis "Distrofia Miotónica en Guipúzcoa: aspectos clínicos, epidemiológicos y genético." [Myotonic Dystrophy in Guipúzcoa: clinical, epidemiological and genetic aspects] in 1992; and Dr. Andone Sistiaga successfully defended her thesis titled "Alteración cognitiva en la distrofia miotónica tipo 1 (DM1) y la distrofia facioescapulohumeral (FSH): Caracterización neuropsicológica y estructural" [Cognitive impairment in Myotonic Dystrophy Type 1 (DM1) and Facioscapulohumeral Dystrophy (FSH): Neuropsychological and structural characterization] in 2010. Therefore, when beginning this doctoral thesis, the group was able to draw on an extensive dataset, including data collected from DM1 patients and healthy volunteers; whilst a longstanding patient-clinician rapport had already been established.

The present doctoral thesis represents the outcome of a 3-year period of research work. During this period (which formed part of a wider research project), the research work consisted of two main parts. The first part consisted of a retrospective analysis of previously collected and unpublished data by the thesis supervisor Dra. Andone Sistiaga. The second part included the collection and analysis of a new dataset consisting of, for the most part, a longitudinal follow-up of the data retrospectively analyzed. Figure 1 summarizes the design in order to provide the reader with a clear overview of the entire thesis.

This work has yielded three published studies and a manuscript that is currently under review. Two of the published studies correspond to the first and retrospective part of the thesis, whilst the other published study and the manuscript under review are the outcome of the second and prospective part of the thesis.

Published studies resulting from the retrospective analysis:

Study #1:

Labayru, G., Arenzana, I., Aliri, J., Zulaica, M., López de Munain, A. & Sistiaga, A (2018). Social cognition in Myotonic Dystrophy Type 1: Specific or secondary impairment? *PLoS ONE, 13,* 1–11. https://doi.org/10.1371/journal.pone.0204227

Study #2:

Labayru, G., Diez, I., Sepulcre, J., Fernández, E., Zulaica, M., Cortés, J.M., López de Munain, A. & Sistiaga, A. (2019). Regional brain atrophy in gray and white matter is associated with cognitive impairment in Myotonic Dystrophy type 1. *NeuroImage: Clinical, 24*, 102078. https://doi.org/10.1016/j.nicl.2019.102078

Studies resulting from the prospective analysis:

Study #3 (published):

Labayru, G., Aliri, J., Zulaica, M., López de Munain, A., & Sistiaga A. (2019). Age-related cognitive decline in myotonic dystrophy type 1: An 11-year longitudinal follow-up study. *Journal of Neuropsychology*. https://doi.org/10.1111/jnp.12192

Study #4 (under review):

Labayru, G., Jiménez-Marin, A., Fernández, E., Villanua, J., Zulaica, M., Cortés, J.M., Díez, I., Sepulcre, J., López de Munain, A., & Sistiaga, A. (under review). Trajectories of brain degeneration in pediatric and adult/late DM1: A follow-up MRI study over a decade.

The reader should note that the numbers used to denote each study will be employed hereafter and during the whole thesis.

The completion of these studies required a multidisciplinary effort. The close and ongoing collaboration between the PhD candidate and professionals in areas such as biology, engineering, neurology, radiology, nursery and neuropsychology has been a key factor. Additionally, the PhD candidate has had the opportunity to work with other Basque, national and international laboratories, which has undoubtedly enriched the resultant work presented in this thesis. The combined work of the above-mentioned fields of knowledge, has enabled the study of the disease using an interdisciplinary approach encompassing biological, neuropsychological and neuroradiological perspectives.

This work has been supported by a predoctoral grant awarded by the Basque Government to the author of this thesis (PRE_2016_1_0187), CIBERNED (Ref: 609) and by the Institute of Health Carlos III (ISCI-III) co-founded by Fondo Europeo de Desarrollo Regional-FEDER (Ref: PI17/01231 and PI17/01841), and by the Basque Government (S-PE13UN030).

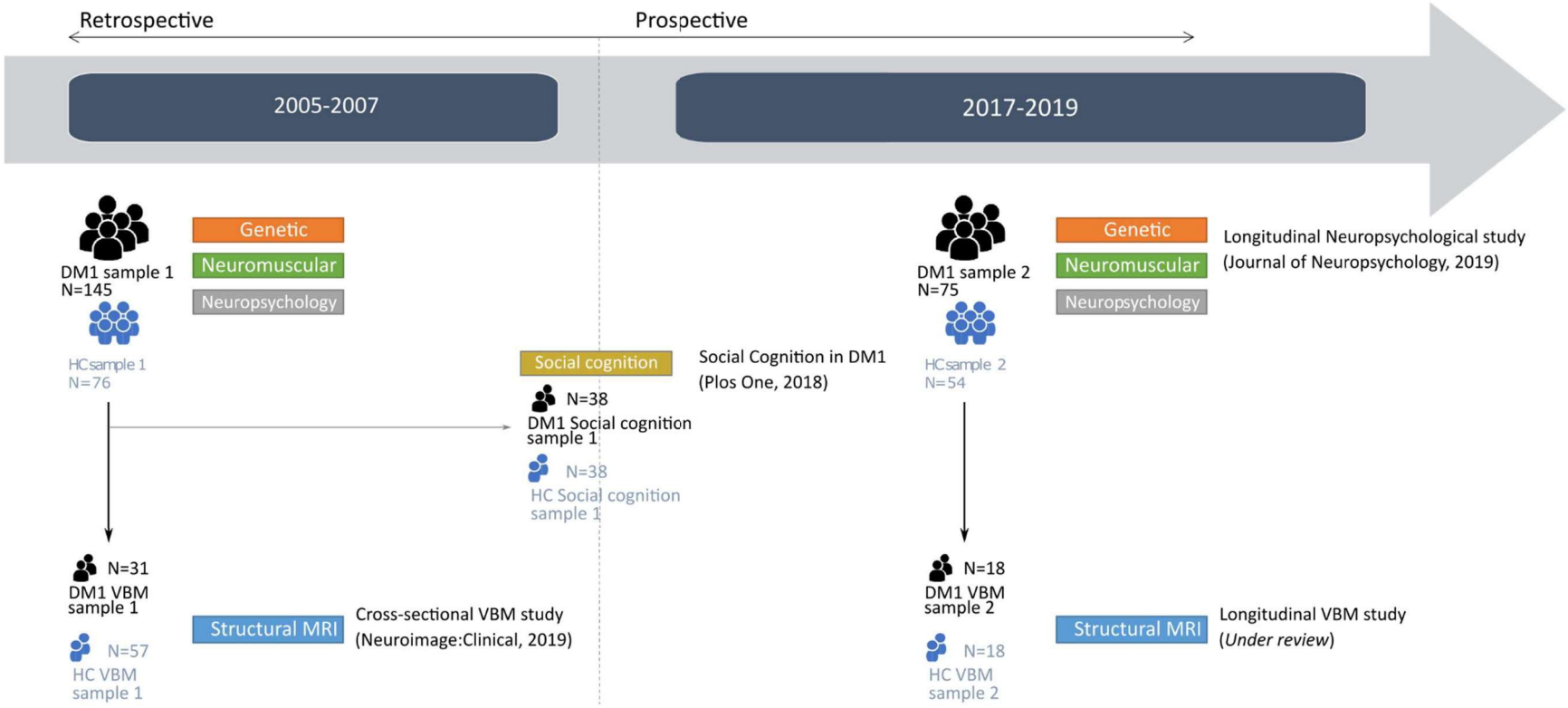


Figure 1. General overview of the work presented in this thesis

1. Muscular Dystrophies: Myotonic Dystrophy Type 1 (DM1)

Muscular dystrophy (MD) is the term employed to refer to a group of muscular dystrophies that includes as many as 9 main diagnostic groups (Figure 2). All share the common feature of progressive muscle weakness and loss of muscle mass, leading to mobility problems. Further, in all of these, genetic defects underlie the altered production of muscle proteins, resulting in the death of muscle cell and tissue. However, each is recognized as an independent entity due to variations in the way in which muscles are affected, differences related to the onset of symptoms (i.e. age of onset and first muscle group affected) along with the severity of the effects, or the specific genetic causes underlying each entity (Huml, 2015).

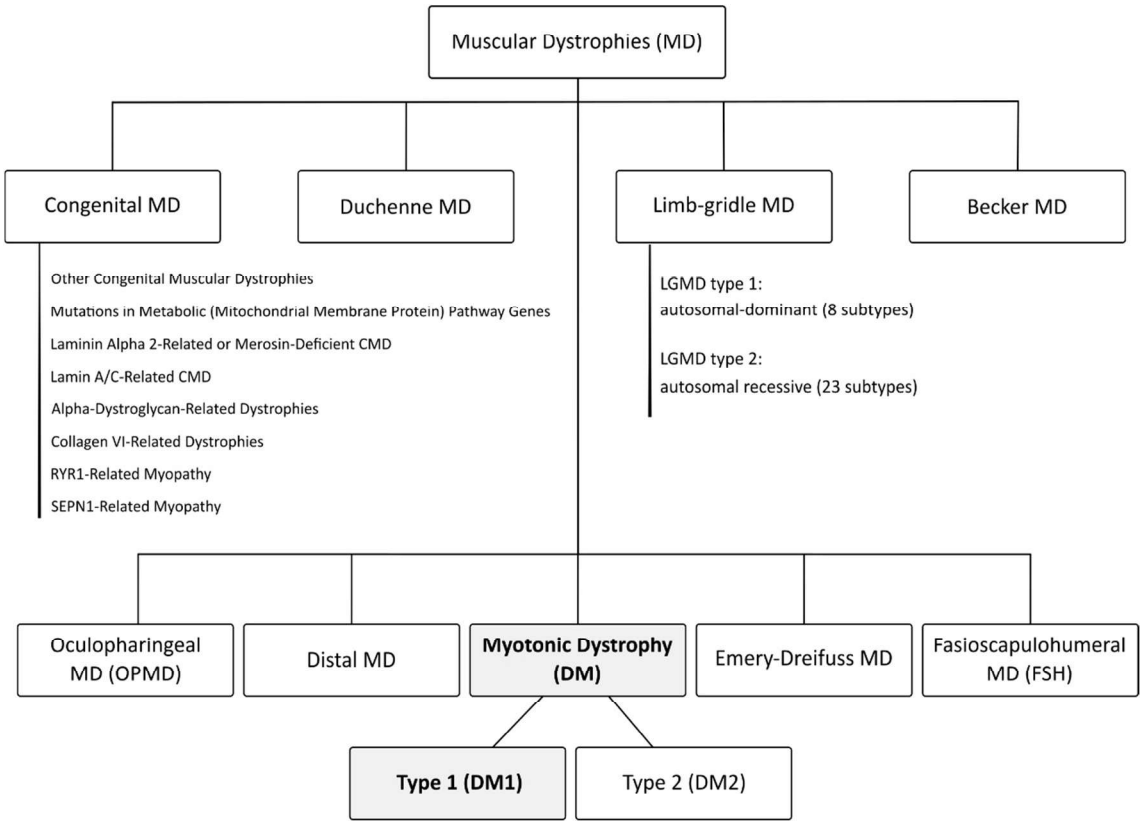


Figure 2. Classification of Muscular Dystrophies (MD)

Myotonic Dystrophy (Distrophia Myotonica, DM) is included in this group and can be distinguished from other forms of MD, not only because this particular myotonia primarily affects distal muscles (in contrast with other MDs that primarily affect proximal muscles), but also on account of its multisystemic nature, implying the involvement of multiple organs in the

body. Two types of DM have been described: Type 1 (DM1) and Type 2 (DM2); each of them representing an independent nosologic entity based on both the affected genes, and distinct clinical manifestations. Reviewing the specific disease features of DM2 is beyond the scope of this work, and therefore only a detailed revision of DM1 will be provided.

1.1. Epidemiology

DM1 is considered to be one of the many rare diseases, assuming the generally accepted definition of rare disease as being that with an estimated prevalence of less than 5 individuals in 10,000 (Auvin et al., 2018). However, DM1 is the most common form of MD in adults, with an estimated worldwide prevalence of 8 cases per 100,000 inhabitants. In a recent systematic literature review (Theadom et al., 2014), results from studies identified as having a lower bias yielded a prevalence that ranged from 0.5 to 18.1 per 100,000 inhabitants. However, prevalence rates vary considerably between different countries and, therefore, efforts should be made in order to assess the specific prevalence in each country or region. The highest occurrence has historically been reported in the Saguenay-Lac-Saint-Jean (SLSJ) region in Northeastern Quebec (Canada), where a prevalence of 189 per 100,000 inhabitants (0.00189%) was estimated (Mathieu et al., 1990). Although this prevalence has declined slightly over the years – 158/100,000 or 0.00158% – due mostly to genetic counselling and predictive testing, this region still remains the one with the highest rate of DM1 (Mathieu & Prévost, 2012). A particularly high prevalence, superior to that registered worldwide, has also been reported in the current geographical area (Gipuzkoa, Spain), where a prevalence of 26.5 per 100,000 inhabitants (0.00026%) has been estimated (López de Munain et al., 1993). Although current prevalence might have been reduced, the high prevalence reported has encouraged an ongoing research trajectory of the disease in this region, which provides the ideal setting for conducting the work described in this thesis.

The relatively few studies that have addressed sex-related differences regarding prevalence indicate a slightly higher rate for males compared with females (M/F ratio 1.18) with further sex-specific variations in age and genotypic distribution. In particular, there is an almost 3-fold prevalence of males among patients younger than 30 years, and a higher prevalence of males within the subgroup of patients with shorter CTG expansion size (50-1000), whereas females are more prevalent within the subgroups of patients with longer CTG expansion size (1000-3500) (Vanacore et al., 2016).

1.2. Genetics and inheritance pattern

As mentioned earlier, all DMs share the common trait of being genetic-based disorders. However, the exact genes involved in each disease and, further, the underlying genetic and molecular mechanisms that are ultimately responsible for the clinical symptoms, are unique to each of the different disorders. Indeed, DM1 and DM2 are based on different gene mutations.

In DM1, the mutation takes place in the 3'- untranslated region of the *dystrophia myotonica* protein kinase (DMPK) gene on chromosome 19q13 (Brook et al., 1992). The mutation produces an unstable replica of CTG (cytosine-thymine-guanine) trinucleotide (Figure 3). The normal variation of the trinucleotide is between 5 and 27. Up to 35 CTG triplets can be considered normal, whilst premutation is indicated when there are between 35 and 45 repeats. When there are more than 50 CTG repetitions, the disease is diagnosed, and overall, longer repeats result in a more severe phenotype.

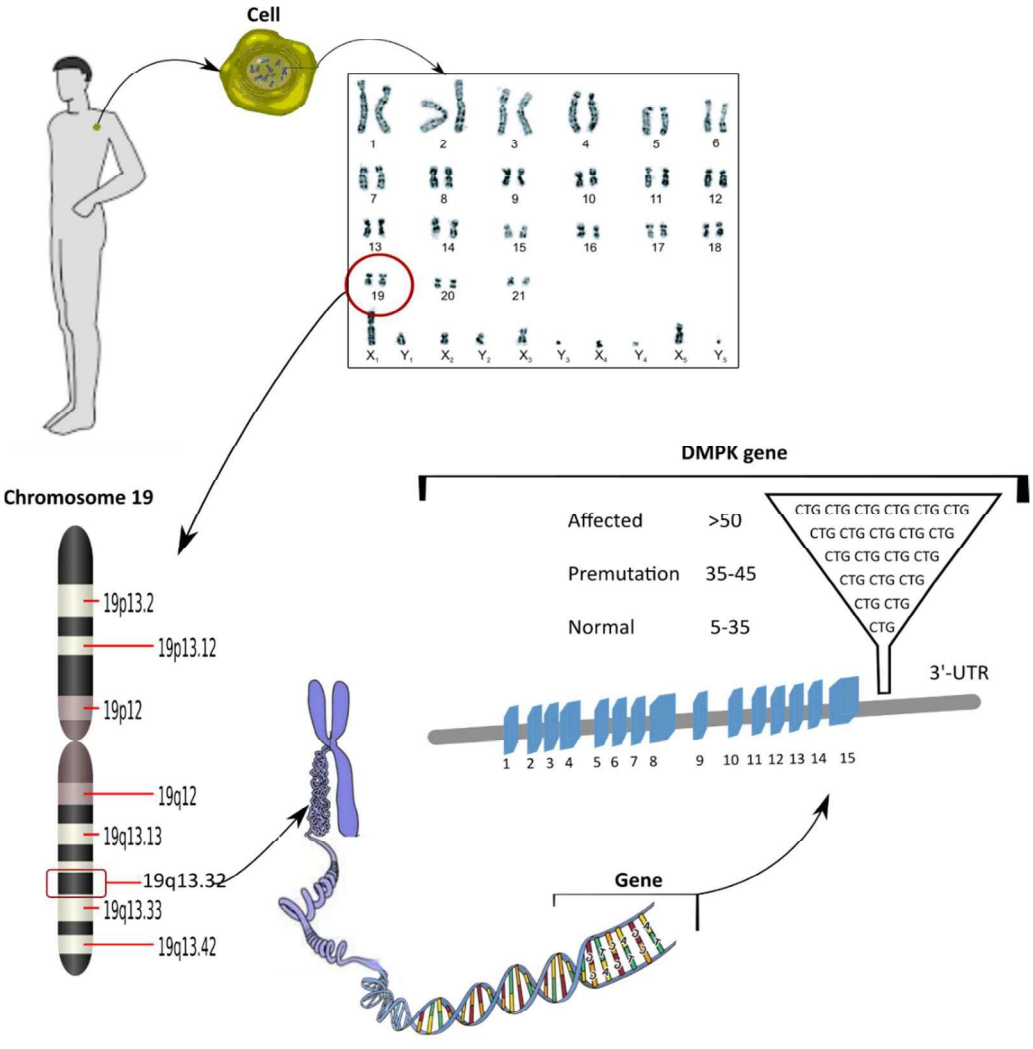


Figure 3. Genetic involvement in DM1: Mutation of the DMPK gene

The disease is transmitted in an autosomal dominant manner. Therefore, the child of an affected parent has a 50% chance of inheriting the disease – the abnormal gene – even if the matching gene from the other parent is unaffected.

The disease can be inherited from the mother (maternal transmission) or the father (paternal transmission). It is more likely that congenital and childhood forms of the disease are inherited by maternal transmission, whilst the opposite is the case for adult and late onset forms. Above 80%, 50%, and almost 40% of congenital, infantile, and juvenile onset patients show maternal inheritance, respectively (Stokes et al., 2019).

It is noteworthy that genetic transmission in DM1 is characterized by the anticipation phenomenon, implying that when the disease is transmitted from a parent to his/her offspring, the latter will develop a more premature and severe form of the disease.

1.3. Disease classification

DM is classified into two major groups (DM1 and DM2), but there are also different DM1 subtypes. DM1 has traditionally been classified according to age of onset. However, as a result of the second international workshop held in San Sebastian, an expert panel of clinicians and researchers working in DM1 argued for the need to set out a series of common classification criteria, described in the report of the second Outcome Measures in Myotonic Dystrophy type 1 (OMMYD-2) (Gagnon et al., 2015). In the subsequent OMMYD-3 meeting, a preliminary consensus was reached on the five-form classification based on the age of onset and most prevalent symptoms: congenital, childhood, juvenile, adult, and late-onset (Gagnon et al., 2018); which has been scientifically validated by De Antonio et al. (2016). More recently, in the last meeting (OMMYD-4), held in Gothenburg (Sweden) during the International Myotonic Dystrophy Consortium Meeting (IDMC-12, June 2019), a new proposal was put forward regarding disease classification. According to this proposal, childhood and juvenile onset patients should be classified as a unique group under the term *pediatric onset*. The other forms of the disease remained unchanged.

Therefore, disease age of onset is currently the main and most recognized feature employed in the classification of DM1. Nonetheless, there is a lack of consensus throughout the scientific literature regarding the exact range of age of onset for each disease form. As an example, Tuikka et al. (1993) classified patients in the congenital group when muscle symptoms began within the first years of life; while Turner & Hilton-Jones (2010) classified patients as having the childhood

form when symptoms began between the age of 1 and 10 years; the classic form (usually referred to as the adult form) is diagnosed when symptoms appear between the ages of 10 and 30 years; and late onset when symptomatology begins between 20 and 70 years. Conversely, Okkersen, Buskes, et al. (2017) classify patients as juvenile onset when symptoms begin before the age of 18 years, and as adult onset when they begin after that age.

Whilst disease age of onset is the most frequently employed classification system, this is not exempt from controversy. When DM1 is diagnosed on the basis of age of onset, the symptoms used to make such a diagnosis are purely muscular. That is, the disease is assumed to start with a muscular defect (usually myotonia and muscle weakness) along with cataracts. However, symptoms reflecting CNS involvement (such as cognitive impairment) are not considered as a "trigger symptom" of the disease. Thus, symptoms that are often overlooked might in fact be present before the onset of muscular symptoms. It is common, for instance, to find pre-existing subtle learning difficulties during school age, which could be the consequence of abnormal neurodevelopment of the CNS. Moreover, establishing the patient's age of onset of the disease is based not only on signs (objective, measurable and/or observable), but also — and usually — on symptoms, which are subjective experiences of disease or malfunction. Indeed, these are reported by patients with approximate accuracy regarding severity, onset, etc., which gives the clinician the task of ultimately determining an approximate age of onset.

This approach directly leads to the potential misclassification of patients, particularly those for whom the disease could have begun earlier, but who are instead classified as having juvenile or adult forms. Indeed, Turner & Hilton-Jones (2010) state that "the diagnosis of childhood DM1 is often missed in affected adolescents or children due to the lack of neurological problems and apparently negative family history". Finally, disease duration, proportional to age of onset, is considered to be one of the most important variables regarding disease severity based on the multiple correlations found with various clinical parameters (Gallais et al., 2017; Salehi et al., 2007). But, again, disease duration is calculated using age of onset, and, therefore, in some cases this could be an inaccurate measure.

Aside from disease age of onset, other grouping methods such as genotype-based classifications have also been employed, clustering patients according to their CTG expansion size. Examples within this classification method can be found in Modoni et al. (2008) (E1 50-150 CTG, E2 150-500 CTG, E3: 500-1000 CTG, E4 >1000 CTG) or Baldanzi et al. (2016) (E1 <150 CTG, E2 150-1000 CTG).

1.4. Clinical features

As a multisystemic disorder, DM1 affects a variety of organs in the body. The impact on the organs can take the following forms (see also Figure 4, for a summary of multisystem involvement in DM1):

- Muscular: Typical muscular symptoms include myotonia (predominantly affecting the fingers – grip myotonia); and muscular weakness and atrophy, which predominantly affect distal muscles. Since atrophy and weakness also affect facial muscles, leading to ptosis and insufficient eyelid closure, DM1 patients show what is known as myopathic face or hatched face. Finally, weakness of oropharyngeal muscles cause nasal and slurred speech, and in more severe cases, difficulties in chewing and swallowing (Wenninger et al., 2018).
- Cardiac: The most frequent cardiac symptoms include arrythmia, atrial fibrillation, left ventricular dysfunction and conduction defects, representing one of the principal causes of early death. The most frequent cause of death due to cardiac complications is sudden cardiac death (Wahbi & Furling, 2020).
- Eyes: Early onset cataracts are a hallmark of DM1 and represent one of the main diagnostic clues for late onset patients.
- Endocrine system: Diabetes, adrenocortical dysfunction, androgen deficiency, increased luteinizing hormone (LH), abnormal thyrotropin (TSH) and secondary hyperparathyroidism are characteristic of endocrine system affectation in DM1 (Dahlqvist et al., 2015; Ørngreen et al., 2012) and some of these may interact with muscle affectation to cause greater impairment and fatigue (Passeri et al., 2013).
- Gastrointestinal symptoms: cholecystitis, gallbladder dysfunction, and elevated liver function tests are common (Hilbert et al., 2017). As a consequence of smooth muscle affectations, patients might present constipation, megacolon, slow gastric emptying, diarrhea, incontinence, and gastrointestinal symptom complexes similar to irritable bowel syndrome (Turner & Hilton-Jones, 2010).
- Cancer: an increased risk of cancer in general has been reported in DM1, with approximately a 2-fold increase in risk (Fernández-Torrón et al., 2016). The cancers with the highest probability of occurrence in DM patients compared with the DM1-free population are cancers of the thyroid, uterus, and cutaneous melanoma (Alsaggaf et al., 2018).
- CNS: Several symptoms have been described in relation to CNS involvement in DM1. Excessive daytime sleepiness, alterations in several cognitive functions, avoidant

behavior, anxiety, apathy, paranoid and aggressive personality traits, are some of the manifestations that patients may show in varying degrees (Thornton, 2014). Some of these symptoms still need to be further examined in order to establish well characterized profiles of affectation and to determine the precise underlying neuroanatomical and neuropathological correlates.

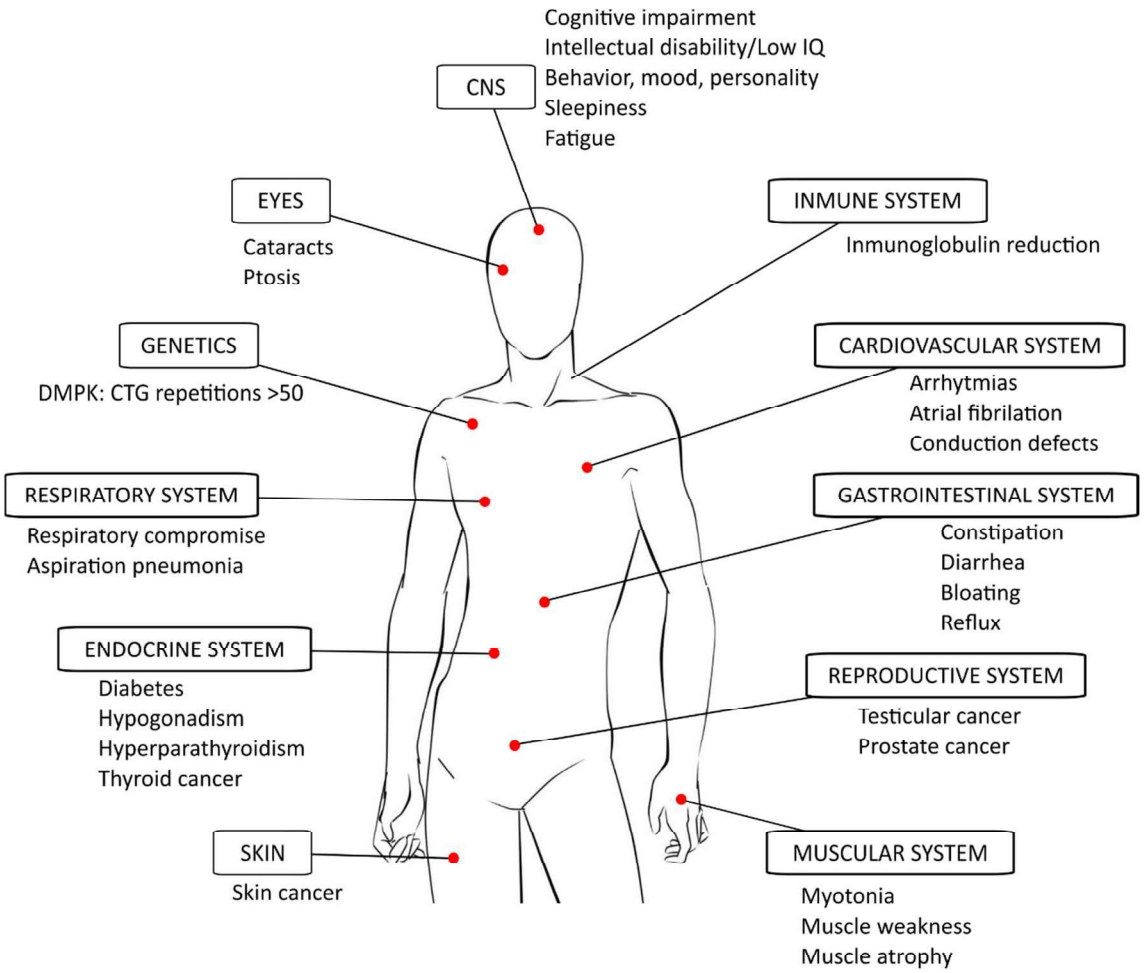


Figure 4. Multisystem involvement in DM1.

Recently, some sex-related differences have been reported with regard to the incidence and severity of certain clinical features in DM1. For example, male patients display more severe endocrine and metabolic symptoms than their female counterparts (Spaziani et al., 2019) and women show higher probability of cancer than males when compared with the general population; with ovarian and endometrial cancers being the most frequent cancers in women, and cancers of the thyroid and brain being more frequent in men (Fernández-Torrón et al., 2016).

The clinical features of DM1 must be described with respect to temporality, the bodily organs affected, and the severity of the symptoms. These parameters are highly dependent on disease age of onset, with earlier onset patients showing more global impairments, whilst more benign or milder forms are usually displayed in patients with adult or late onset of the disease. A summary of the main clinical features of patients with each age of disease onset can be found in Table 1

- Congenital:

Congenital DM1 has been suggested to be a distinct clinical phenotype, and not merely a more severe form of DM1 (Meola & Sansone, 2007; Turner & Hilton-Jones, 2010). It is typically inherited from the mother, although a few cases of paternal inheritance have been reported (Stokes et al., 2019). Prenatal findings include polyhydramnios, talipes, borderline ventriculomegaly and reduced fetal movements (Zaki et al., 2007). At birth, congenital DM1 babies present generalized weakness, hypotonia and respiratory compromise (the latter leading to high mortality) (Turner & Hilton-Jones, 2010). Delay in the acquisition of developmental milestones is a characteristic, and at the cognitive level, intellectual disability is present in 50-90% of these patients (Meola & Sansone, 2007). As a result, learning difficulties and the requirement of special educational needs are common in these patients.

- Childhood:

At the muscular level, patients with childhood form show hand muscle myotonia and facial weakness (less prominent than congenital patients). Developmentally, these patients frequently show delayed motor development, learning difficulties, and low IQ. Indeed, 90% of children and adolescents with childhood onset may show mild intellectual disability (Ho et al., 2019). As they grow up, early conduction defects appear to be a common feature.

- Juvenile:

Rather less is known about the specific disease features of juvenile onset DM1, due to the fact that these patients have frequently been classified or studied along with either childhood or adult onset patients. When juvenile onset has been considered as a separate form, a less severe phenotype has been described in comparison with congenital and childhood forms, although patients with the juvenile form have been reported to have the highest probability of suffering from severe myotonia (De Antonio et al., 2016).

- Adult:

This is the most extensively described phenotype in the scientific literature. Muscular features include distal muscle and facial weakness, leading to ptosis and myotonia. Aside from the muscular symptoms, the multisystemic involvement of adult onset patients includes a variety of clinical conditions: early cataracts (sometimes as the trigger symptom); conduction defects and arrhythmias; cholecystitis and gallbladder function related symptoms; dysphagia and aspiration pneumonia; endocrine abnormalities involving thyroid, pancreas, hypothalamus and gonads; and early frontal balding and epitheliomas (Turner & Hilton-Jones, 2010). Psychological disorders such as anxiety and depression and behavioral symptoms such as apathy are frequent. Moreover, various personality traits such as paranoid and aggressive have been reported (Sistiaga et al., 2010). At the cognitive level, a variety of deficits have been described (see point 2 below) and, even milder than in earlier onset forms, these patients display an IQ below normal range (Jean et al., 2014).

- Late:

Patients diagnosed with late onset DM1 — also known as mild or partial forms — are frequently asymptomatic or show fewer symptoms of milder severity, such as mild weakness and myotonia. The appearance of cataracts after the age of 40 is a typical feature (Thornton, 2014) and represents the most prevalent symptom, occurring in 78.5% of cases. High percentages of cardiac conduction defects (55.8%) and obesity (51.5%) are also reported. Even though the late form is considered the mildest and least aggressive form of the disease, the three features mentioned are significantly more prevalent in late onset forms than in earlier onset forms (De Antonio et al., 2016).

Table 1. Clinical characteristics of distinct disease forms in DM1

Disease form	Age of onset	CTG	Inheritance pattern	Clinical features		Neuropsychological functioning
				Muscular	Others	
Congenital	Birth	750-1400	>80% maternal	Infantile hypotonia	Respiratory failure	Severe Intellectual disability
Childhood	1-10	500-1100	>50% maternal	Facial weakness Myotonia	Conduction defects	Moderate Intellectual disability Learning difficulties
Juvenile	10-20	400-800	40% maternal			Low IQ
Adult	20-40	250-750	70% paternal	Distal and facial muscle weakness Myotonia	Cataracts Conduction defects Insulin resistance Respiratory failure	Low IQ Cognitive impairment
Late	>40	100-600	70% paternal	Mild myotonia	Cataracts	Mild cognitive deficits

Note. CTG: cytosine-thymine-guanine; IQ: Intelligence Quotient

Finally, an important point that needs to be addressed is the fact that mortality in the DM1 population is higher than that of healthy subjects, with a markedly reduced survival rate to the age of 65. Indeed, 18% of DM1 patients (as opposed to 78% of the general population) survives to the age of 65 (de Die-Smulders et al., 1998). The primary causes of death in DM1 are pulmonary (respiratory failure) and cardiac complications (cardiac arrythmia, heart block, sudden death) (de Die-Smulders et al., 1998; Groh et al., 2008) and the higher risk of cancer implies an additional risk (Best et al., 2019). Further, younger age of onset has been reported as a risk factor for early death (Mladenovic et al., 2006).

2. Neuropsychology in DM1

Although cognitive affectation in DM1 has long been an issue of interest in the scientific literature, there is still no consensus regarding the exact pattern of impairment, with some researchers arguing for a more global and unspecific involvement and others describing a profile restricted to a limited number of cognitive skills. In a systematic review and meta-analysis, the results of 40 studies found significantly worse performance in DM1 patients compared with healthy controls (HC) across the following cognitive domains: global cognition, intelligence, executive functioning, language, memory (overall, verbal, and visual), visuoconstruction, visuospatial ability, speed of information processing, attention, and social cognition (Okkersen, Buskes, et al., 2017). These results do not necessarily imply that each DM1 patient will suffer from impairment in all cognitive domains, but instead, they indicate that a single patient could experience difficulties in any of these. Further, the meta-analysis included studies covering the whole spectrum of DM1 (from congenital to late onset patients), and therefore, the possibility that each disease onset has a specific cognitive profile cannot be ruled out.

Under the umbrella of scientific literature examining higher order cognitive functioning in DM1, three main areas deserve individual attention: global intelligence, specific cognitive domains, and social cognition.

2.1. Intelligence

Intelligence — as measured by IQ — has been largely studied in the DM1 population. From normal IQ to severe intellectual disability, a range of possible outcomes can be found among

patients. In general terms, it appears that intellectual level is strongly linked to the severity of the disease. As in the case of global disease severity, IQ is inversely correlated with age of onset of the disease; the more severe the disease form, the lower the IQ (Ekström et al., 2009). Thus, congenital DM1 patients almost invariably suffer from moderate to severe intellectual disability (Ekström et al., 2009). In childhood onset patients, a milder phenotype — but still within the low IQ range — has been described (Douniol et al., 2012), where patient scores can be classified as borderline (IQ: 70-85), mild intellectual disability (IQ: 55-70) or even moderate intellectual disability (IQ< 55) (Angeard et al., 2007, 2011).

When considering the whole DM1 population, conflicting results have been found regarding IQ distribution. Whilst some early studies reported an IQ distribution curve that is skewed towards the lower end (Bird et al., 1983); others have found that IQ scores followed the normal curve in a sample of congenital and adult onset patients. This is mainly due to the fact that the scores of congenital patients were at the lower end (IQ range: 39-69) whilst adult onset patients generally showed scores consistent with normal intelligence (Tuikka et al., 1993).

With regard to adult and late onset patients, the results appear to be less conclusive. Although severe intellectual disability is unfrequently described, a low average IQ in both adult (Sistiaga et al., 2010) and late onset patients (Jean et al., 2014) has been reported. Other studies, however, have reported IQ scores that fall within the normal range, in patients with both disease forms (Rubinsztein et al., 1997; Tanaka et al., 2012; Turnpenny et al., 1994; Winblad et al., 2006; Zalonis et al., 2010).

Given the apparent relationship between disease form and IQ, and the above mentioned greater genetic load of earlier onset forms, a clear hypothesis emerged within the scientific community, that is, the possibility that lower IQ scores are correlated with longer CTG repeat sizes. The results obtained so far appear to be mixed, with most studies providing support for this hypothesis (Douniol et al., 2012; Jean et al., 2014; Marchini et al., 2001; Perini et al., 1999; Sistiaga et al., 2010; Turnpenny et al., 1994); and others that do not (Kuo et al., 2007; Meola et al., 2003).

2.2. Cognitive domains

The DM1 population as a whole is known to suffer from deficits in various cognitive domains. However, the exact pattern of cognitive impairment has been suggested to depend on the age of onset (Peric et al., 2017). In this regard, most studies have focused their attention on the

study of cognitive deficiencies in adult onset patients and, in recent years, a significant body of research has focused on clarifying the specific profile of cognitive impairment. Here, a review of the works published within the past two decades is presented.

In a recent meta-analysis, some of the largest effect sizes were found, among others, in visuospatial perception and visuoconstruction abilities, meaning that either a large number of patients exhibit an impairment in such domains, or that the deficits – albeit in a smaller DM1 subpopulation – are highly severe (Okkersen, Buskes, et al., 2017). In relation to this, work conducted by some authors has pointed to a pattern of cognitive impairment with executive function and visuoconstructive affectation (Meola et al., 2003; Sistiaga et al., 2010), whilst others describe a profile characterized by attentional, language, and memory impairments (Modoni et al., 2004). In a recent study, Peric et al. (2017) identified three main clusters of cognitive impairment in adult onset DM1 patients with a predominance of certain cognitive functions: 1) dominant visuospatial impairment (typically younger patients with shorter duration of disease), 2) dominant language impairment, and 3) a combination of visuospatial and memory impairment.

A number of studies, however, have described a more widespread and non-domain-specific pattern with impaired performance in multiple cognitive functions. For instance, Winblad et al. (2006) reported impaired performance (1 SD below the normative mean) on executive function, speed of information processing, attention and arithmetic, memory, visuoconstructive abilities and vocabulary. Kuo et al. (2007) found borderline impairment in digital repetition, constructional ability, memory recall, calculation, and reasoning. Similarly, (Rakocevic-Stojanovic et al., 2014) found decreased scores on visuospatial/visuoconstructive abilities, executive functions, naming, and visual memory. In more recent studies, a combination of executive (abstract reasoning, working memory), attentional and visuoconstructive deficits has been described (in addition to a slower speed of information processing) (Fujino et al., 2018), and similarly, other studies have reported deficits in a combination of working memory, processing speed, and perceptual reasoning (Langbehn et al., 2020).

Relatively fewer studies have directed their efforts to describing the cognitive profile in patients with the milder form of the disease. Late onset DM1 patients have traditionally been described as less cognitively impaired, presenting cognitive impairment limited only to memory (Rubinsztein et al., 1997). Conversely, although in a small sample of eight patients, Caso et al. (2014) found that late onset patients showed the highest prevalence of memory, attention,

visuospatial and language impairment. Thus, with regard to this phenotype, the existing literature is still scarce, and the results do not allow for drawing any firm conclusions.

Juvenile onset patients have similarly received relatively little attention in the literature on cognitive impairments, although some studies comparing different phenotypes have included juvenile onset patients among the assessed disease forms. When compared with adult onset patients, juvenile onset patients have been found to show less severe impairment of phonemic fluency (Caso et al., 2014) and lower scores on verbal IQ and memory tests (Woo et al., 2019). Further, in comparison with normative data, juvenile onset patients have been shown to achieve low scores on performance IQ tests (Woo et al., 2019) and a high rate of patients (above the 16% expected in the normative data population) score below 1SD from the mean in reasoning and problem solving, verbal and visual memory, visuospatial abilities, executive functions and language (Caso et al., 2014). Other authors have reported that executive/attentional deficits and visuospatial impairment define the cognitive profile of juvenile onset patients, irrespective of sociodemographic and clinical parameters (Peric et al., 2017) Finally, congenital and childhood onset DM1 patients have been characterized as those most cognitively affected. In both congenital (Ekström et al., 2009) and childhood onset patients (Angeard et al., 2007, 2011; Ekström et al., 2009) verbal abilities appear to be more preserved compared with non-verbal or performance skills, as measured by Verbal IQ and Performance IQ.

In light of these results, it can be concluded that cognitive alterations are undoubtedly present in the DM1 population. Nevertheless, although several studies have attempted to describe the exact pattern of impairment, no consensus has yet been reached. There are a variety of possible reasons for these conflicting results. For instance, the above-mentioned disparities regarding disease classification and the different definitions of the disease forms makes cross-study comparisons difficult. Further, whilst is true that certain neuropsychological tests are frequently used in different studies, in general assessment batteries differ considerably between studies, making it impossible to make direct comparisons. It is also worth noting that most cognitive tests are not pure (i.e. do not measure a unique and isolated function) and not all researchers describe certain cognitive tests as measuring the same cognitive function. Thus, standardized assessment protocols for the DM1 population are clearly needed for both research and clinical settings, whilst efforts should be made to homogenize study samples.

Finally, associations between CTG length and cognitive performance have been investigated and, as in the case of IQ scores, both negative correlations (longer CTG - poorer cognitive performance) (Winblad et al., 2006) and an absence of correlations (Antonini et al., 2006; Kuo

et al., 2007; Meola et al., 2003; Modoni et al., 2004) have been reported. Somatic mosaicism of CTG across body tissues could underlie these conflicting results. Most studies reporting such correlations measured CTG repeat expansion in blood. Nevertheless, CTG expansion in brain tissue has been found to be non-correlated with that measured in blood lymphocytes and, further, CTG mosaicism also appears among distinct brain regions (Sergeant et al., 2001). Thus, the pathological mechanism underlying correlations between genetic load and cognitive functioning should be clarified. This will help researchers to choose the most reliable body tissue for quantifying CTG when studying correlations with cognitive abilities and will also facilitate the interpretation of such correlates.

2.2.1. Social cognition in DM1

Clinicians working with DM1 population and relatives of these patients have pointed out certain difficulties in social participation. This, along with the presence of autistic-like traits and the comorbidity with Autism Spectrum Disorder (ASD), has led to an increase in research into social cognitive functions in DM1. Although still relatively low in number, in recent decades some studies have addressed the issue of social cognition in the DM1 population. In general, most research has focused on facial emotion recognition, finding in all cases greater difficulties in DM1 patients compared with HC (or with normative data) (Kobayakawa et al., 2010; Takeda et al., 2009; Winblad, Hellström, et al., 2006). Patients appear to have particular difficulties with the recognition of certain emotions such as fear, anger and disgust (all with a negative undertone) (Takeda et al., 2009; Winblad, Hellström, et al., 2006) but not happiness; a finding that has been suggested to rely on a diminished sensitivity to facial expressions related to limbic system impairment (Takeda et al., 2009). Relatively fewer studies can be found on other social cognition abilities in DM1, but so far, the results point to an impaired ability to perform Theory of Mind (ToM) tasks (Kobayakawa et al., 2012; Serra, Cercignani, et al., 2016), social abilities, moral judgments and emotion attribution (through brief verbal stories rather than visual stimuli) (Serra et al., 2020). Similar to previous studies assessing facial emotion recognition, Serra et al. (2020) confirmed the difficulty of processing negative emotion recognition (sadness, embarrassment and anger), although these authors also found that the patients had difficulties with recognition of happiness.

It should also be noted that measuring social cognition is a challenging issue, since there are relatively few standardized procedures available. Moreover, this issue is not exempt from controversy. Indeed, social cognition tasks could rely on the correct functioning of other higher

order cognitive abilities such as attention, language comprehension, memory, and reasoning and, thus it is challenging to isolate and provide a pure measure of social cognition. Methodological control of such higher order cognitive functions could be a possible way of increasing the accuracy of social cognition assessment.

2.3. Affect, mood, behavior, and personality

The DM1 population is at higher risk of developing a psychiatric disorder (Bertrand et al., 2015). In particular, a number of studies have described avoidant personality traits (Meola et al., 2003; Winblad et al., 2005), aggressive and paranoid traits (Serra et al., 2014; Sistiaga et al., 2010) and schizotypal personality traits (Serra et al., 2014).

Some reports have indicated a higher incidence of depression and anxiety than that expected for the normal population. For example, mild depression rates can be found in 50% of patients, and significant depression in 20%; and marked anxiety has been reported in 16-40% of patients (Antonini et al., 2006; Rakocevic-Stojanovic et al., 2014). A recent systematic meta-analysis showed that the prevalence of depression is 19%, anxiety 17%, and apathy 55% (pooled prevalence) (van der Velden et al., 2019), with a gender bias of higher rates in women than in men (Castellano-Guerrero et al., 2018).

3. The brain in DM1

3.1. Neuropathology

Pathological findings in DM1 include neuronal loss (Mizukami et al., 1999; Ono, Takahashi, Jinnai, Kanda, Fukuoka, Kurisaki, Mitake, Inagaki, Yamano, & Nagao, 1998; Ono, Takahashi, Jinnai, Kanda, Fukuoka, Kurisaki, Mitake, Inagaki, Yamano, Shimizu, et al., 1998) and the presence of neurofibrillary tangles (NFT), Lewy bodies, Marinesco bodies (Itoh et al., 2010), and RNA nuclear foci across several brain regions and cells (reviewed in Gourdon & Meola, 2017). Brain involvement biomarkers, including levels of cerebrospinal fluid (CSF) total tau, phosphorylated tau, and 42-amino-acid form of β-amyloid ($A\beta_{42}$) have been investigated in DM1. Some studies have reported a significant decrease of $A\beta_{42}$ and increased total tau (but normal phosphorylated tau) in non-congenital DM1 patients (Winblad et al., 2008), whilst others have

found a significant decrease of $A\beta_{42}$ in juvenile onset patients and an increased (non-significant) total and phosphorylated tau in adult onset patients (Peric et al., 2014).

3.2. Brain structure

From a visual assessment of brain alterations to the most precise and fully automated methods, the DM1 brain has been explored through a wide range of parameters. Table 2 displays the number of published studies employing each brain measure parameter, as reviewed by Okkersen et al. (2017). Within the quantitative and fully automated methods, those most employed to assess both gray matter (GM) and white matter (WM) brain anomalies through structural MRI are Voxel Based Morphometry (VBM) and Diffusion Tensor Imaging (DTI), respectively. A summary review of the scientific literature focused on structural brain imaging published during the last decade, is collected in Table 3. Although some of the manuscripts reported here contain semi-quantitative techniques, all include at least one of the following methods: VBM or DTI.

Table 2. Number of published studies in relation to the brain measure used in the DM1 population

Parameter	Number of studies[¶]
Whole brain volume	
Unknown method of assessment	7
Subjective visual assessment	9
Ventricle brain-ratio or comparable measurements	13
Semi-automated segmentation methods	8
Gray matter	
Semiautomated segmentation methods	2
VBM	9
Cortical thickness analysis	1
White matter. Hyperintensities	
Prevalence of white matter hyperintensities (WMH)	49
Total lesion load or total lesion area of WMH	8
Fazekas scale	2
ARWMC/Wahlund scale	6
White matter. Normal-appearing white matter evaluation	
T2-relaxometry	1

Magnetization transfer	2
DTI-ROI approach	6
DTI-TBSS	5

Note. ARWMC: Age-related white matter change scale; DTI: Diffusion tensor imaging; ROI: Region of interest; TBSS: Tract-based spatial statistics

[¶] Parameters will be counted separately if a particular study employed two or more of the brain analysis techniques.

3.2.1. Voxel Based Morphometry (VBM)

The use of VBM has only recently been documented in the DM1 population, with the work of Antonini et al. (2004) being the first reported use of this approach. In their study, Antonini et al. (2004) conducted a quantitative analysis of both global and regional brain volume, finding a statistically significant global and regional (both GM and WM) atrophy in DM1 patients compared with sex-age matched healthy participants. The location of the regional GM volume loss was widespread, involving bilateral frontal, parietal and temporal cortex, left occipital and left caudate. Further, unlike CTG expansion of muscular impairment, age significantly correlated with a decrease in GM volume, and this correlation was greater for patients than for HC.

Later, several studies emerged employing this technique in DM1. In general, the findings indicate the widespread nature of brain atrophy in DM1, involving both cortical and subcortical regions. On the cortical surface, regions from all lobes have been reported as atrophied, and with regard to subcortical structures, almost all studies have found decreased volumes in various deep gray structures such as the thalamus, caudate, putamen, or nucleus accumbens (see Table 3). In view of all the results reported, the specific pattern of GM atrophy – if there is such a pattern – is still to be determined. For this purpose, certain methodological concerns would need to be overcome, such as homogenization of samples regarding clinical and demographic factors or the creation of standardized scanning protocols to be employed in multicenter imaging studies.

Relatively few studies have examined voxel-level WM atrophy in the DM1 population. Whilst some authors have reported WM atrophy that is restricted to corpus callosum (genu, anterior, medioanterior, central and mediodorsal regions) (Cabada et al., 2017; Ota et al., 2006), others have revealed a more widespread reduction in WM volume involving all cerebral lobes, brainstem, cerebellum, and corpus callosum (Minnerop et al., 2011) or reductions in the thalamus nuclei, left pre and postcentral gyrus adjacent WM, and posterior corpus callosum (Schneider-Gold et al., 2015).

Overall, VBM studies have not systematically assessed the relationship between brain findings and demographic, genetic, clinical and neuropsychological outcomes, and, when they have, the findings are somewhat mixed, meaning that at present, no firm conclusions can be drawn on this issue (Table 3).

3.2.2. Diffusion Tensor Imaging (DTI)

Since its introduction in the mid-'90s, DTI has become one of the most widely employed techniques to assess WM both in pathological processes and in the healthy population. Several measures can be obtained from DTI analyses that are indicative of WM disintegration, including reduced fractional anisotropy (FA), increased Mean diffusivity (MD), increased Radial diffusivity (RD) and increased Axial diffusivity (AD).

With regard to VBM, DTI was not applied in DM1 until very recently. Most of the published studies have focused on specific WM tracts or regions. For example, Fukuda et al. (2005) employed a ROI-based DTI approach and found significantly reduced FA and increased MD in DM1 compared with controls across all ROIs considered, including frontal, parietal, temporal and occipital white matter, splenium of the corpus callosum, and the posterior limb of the internal capsule. Other researchers have analyzed more restricted areas such as those of the corpus callosum, finding significant differences in FA and MD in all areas but one (isthmus), which was suggested to be the result of Wallerian degeneration linked to atrophy at the frontal, temporal, and occipital cortex, as well as subcortical regions (thalamus and striatum), but not the parietal cortex (Ota et al., 2006). Following these first two studies, in the last decade studies employing DTI have become more frequent and have, overall, provided a more comprehensive assessment of WM tissue. For instance, Minnerop et al. (2011) found WM impairment (reduced FA and increased RD, MD and AD) in all association fibers, all regions of the corpus callosum (except for the splenium) and several projection fibers. Wozniak et al. (2014) reported reduced FA and increased MD in all seven tracts evaluated: corticospinal tracts, inferior longitudinal fasciculus, uncinate fasciculus, cingulum bundle, superior longitudinal fasciculus, forceps major and forceps minor. Caso et al. (2014) reported a distributed and symmetric reduction in FA and an increased MD and RD (but not AD) in the corpus callosum, the majority of association and projection tracts, and the brain stem; whilst Baldanzi et al. (2016) described more severe impairment in bilateral association and projection tracts. Finally, Zanigni et al. (2016) and Cabada et al. (2017) reported diffusely distributed lower FA and higher MD, RD, and AD.

Although relatively fewer studies have been conducted in children and adolescents with DM1, the results so far indicate similar widespread compromise of WM tracts (Franc et al., 2012; Wozniak et al., 2013). In sum, diffuse WM microstructural alteration appears to be a hallmark of DM1, and has been suggested to play a role of disconnection in the disease (Caso et al., 2014; Serra et al., 2015).

Correlations between DTI measures and demographic, genetic, clinical and neuropsychological outcomes have not been reported in every study, and, when they have, they have yielded inconclusive results. Whilst some studies have found no correlation between mean FA or MD values in NAWM with patient age, age at onset, duration of illness (Fukuda et al., 2005; Ota et al., 2006; van Dorst et al., 2019), CTG repeat expansion size (Caso et al., 2014; Ota et al., 2006; van Dorst et al., 2019) or neuropsychological outcomes (Minnerop et al., 2011); others have found greater WM abnormalities, correlating with older age and longer disease duration (Minnerop et al., 2011); greater muscular impairment (Minnerop et al., 2011; Serra et al., 2015; Wozniak et al., 2014) and larger CTG repeat expansion sizes (Minnerop et al., 2011; Serra et al., 2015; Wozniak et al., 2014; Zanigni et al., 2016); and neuropsychological outcomes such as working memory in children and adolescents (Wozniak et al., 2013); working memory and processing speed in adults (Wozniak et al., 2014); MMSE (Serra et al., 2015; Zanigni et al., 2016); attention and orientation (Caso et al., 2014); and attention, memory, and visuospatial domains (Baldanzi et al., 2016; van Dorst et al., 2019).

3.3. Brain function

fMRI measures *in vivo* brain activity through blood oxygenation parameters, known as the BOLD (blood oxygen level-dependent) signal. fMRI studies are still in the preliminary stages in terms of studying DM1. During performance of a motor task consisting of self-paced sequential finger-to-thumb opposition, functional alterations have been shown in the central motor system, including bilateral sensorimotor regions, inferior parietal lobules, basal ganglia, thalami, premotor area, insula, and the supplementary motor area (SMA) (Caramia et al., 2010). The authors hypothesized that the observed functional changes may be the result of compensatory mechanisms of an accelerated aging process causing neurodegenerative and neurochemical changes in DM1. More recently, cortical involvement of high-order motor control areas (SMA and dorsal anterior cingulate cortex) during myotonia has been demonstrated, and interpreted as secondary, and possibly compensational, cortical activation in myotonia (Toth et al., 2015).

The remaining studies employing fMRI have focused on brain connectivity during resting. This method is known as resting-state fMRI and permits the exploration of brain connectivity patterns when a specific task is not being performed. This provides information on the overall organization of global and local brain networks based on synchronic frequency fluctuations between several brain regions. Studies employing this technique in DM1 have been led by neuropsychologists investigating the interaction between functional connectivity and personality traits, personality disorders, and social cognition. The results indicate an increased functional connectivity of the default mode network (DMN) (a network with high sensitivity to brain dysfunction-related disorders) in association with schizotypal-paranoid traits (Serra et al., 2014). Later, the same research group investigated the ToM network in relation to resting-state and, despite observing similar global topological properties of the ToM network in healthy subjects, various differences were found regarding local properties, along with an association between ToM deficits and specific patterns of abnormal connectivity (Serra, Cercignani, et al., 2016). Finally, employing graph theoretical methods along with resting-state fMRI, the authors found that the reduced connectivity in the frontoparietal network correlated with visuospatial affectation (Serra, Mancini, et al., 2016).

Taken together, the results from fMRI studies provide evidence in favor of a widespread alteration in functional connectivity in DM1, and show promising results for a better understanding of the neurological basis of the observed clinical features of the disease, particularly motor and neuropsychological manifestations.

Table 3. Summary of studies employing neuroimaging in DM1, published in the last decade (2010-2020).

(Authors, year)	Sample	Method	Results			Conclusion
			GM	WM	Associations	
(van Dorst et al., 2019)	28 DM1 patients: - 1 childhood - 12 juvenile onset - 12 adult onset - 2 late onset 3 excluded from MRI (forms not reported) HC: 16	• **GM:** - • **WM:** - WMH - DTI - TBSS	-	- Increased WMH in periventricular and deep WM - Widespread decrease in FA, increased MD, AD and RD in projection, association and commissural fibers	• **CTG**: No correlation • **Muscular Impairment:** - • **Age:** - • **Neuropsychology:** Whole brain FA, MD, global and local network efficiency correlated with d2 Test, Purdue Pegboard Test, and ROCF • **Others:** Age of onset: no correlation	DM1 as a complex network disorder with WM network alterations related to neuropsychological outcomes
(Cabada et al., 2017)	40 adult onset HC: 42	• **GM:** Volumetric • **WM:** - Volumetric - WMH - DTI	- Enlargement of the ventricular system, reduction in total and widespread regional cortical volume - Subcortical atrophy in thalami, putamen, nucleus accumbens, and ventral diencephalon	- Increased frequency of WMH (77.5% vs 23.8% in HC) - Increased total WMH, frontal, parietooccipital and temporal WMH - More prominent WMH in frontal and temporal regions - Decreased total FA and increased MD, AD and RD in association fibers, CC, and projection fibers	• **CTG**: - • **Muscular Impairment:** - • **Age:** - • **Neuropsychology:** - Decreased FA and increased MD in the posterior CC correlated with visuospatial impairment - Ventricle enlargement, volume loss in central and anteromedial CC, bilateral cingulated isthmus, right lateral occipital and right pericalcarine cortex correlated with visuospatial impairment • **Others:** -	DM1 shows widespread GM and WM involvement (cortical and subcortical) that correlates with progressive cognitive impairment
(Sugiyama et al., 2017)	28 DM1 patients: - 4 childhood - 10 juvenile onset - 14 adult onset	• **GM:** - VBM - Graph Theoretical analysis • **WM: -**	- Almost symmetric GM atrophy including cortical and subcortical areas: orbitofrontal, medial frontal, precentral, anterior insular, posterior cingulate, superior temporal, hippocampal, parahippocampal, fusiform, lingual	-	• **CTG**: No correlation • **Muscular Impairment:** No correlation • **Age:** - • **Neuropsychology: -** • **Others:** - Age of onset: no correlation	Widespread GM atrophy but preserved global network measures consistent with preserved general cognitive function.

	HC: 28		areas, thalamus, caudate and putamen. - No differences in global network measures, but differences in betweenness centrality in the left fusiform gyrus, superior temporal and frontal gyrus, right precuneus (increased) and right caudate and putamen (decreased)		- Disease duration: no correlation	Altered connectivity in left fusiform gyrus and right striatum, which could be related to face perception and ToM impairment
(Baldanzi et al., 2016)	30 adult onset DM1 HC: 30 and 21¶	• **GM:** - BPF - VBM • **WM:** - WMH - DTI Network tractography (TBSS)	- VBM diffuse atrophy: perirolandic, orbitofrontal, dorsolateral frontal, insula, temporo occipital, parieto-mesial, anterior and posterior cingulate	% of patients with WMH according to grades: - Grade 0: 16.7 - Grade 1: 36.7 - Grade 2: 33.3 - Grade 3: 13.3 - Decreased FA and increased MD, RD and AD in association and projection tracts - Decreased FA and increased MD in brainstem and cerebellum - Increased AD in brainstem	• **CTG**: - • **Muscular Impairment:** - • **Age:** - • **Neuropsychology:** - BPF correlated with visuospatial and executive performance - Left postcentral, left middle and inferior temporal gyri and left supramarginal gyrus correlated with RAVLT - No correlation with WMH - RD correlated with ROCF, RAVLT and CBT - AD correlated with RAVLT **Others:** -	Widespread GM atrophy and areas of altered DTI measures, with several correlates between brain abnormalities and cognitive functions, including left temporal atrophy and verbal memory; RD and mnesic and visuospatial impairment, and AD and verbal memory.
(Zanigni et al., 2016)	24 DM1 patients - 7 congenital (E3) - 13 adult onset (E2) - 4 mild (E1) HC:25	• **GM:** - VBM - Cortical thickness • **WM:** - WMH - DTI - TBSS	- Bilateral cortical and subcortical atrophy: thalamus, hippocampus, putamen, caudate, cingulate, frontal, parietal, occipital, insular and temporal cortices - Reduced cortical thickness in lateral-occipital cortices, right precentral and left superior-parietal, superior-temporal and fusiform cortices.	- Widespread decreased FA and increased MD, RD and AD.	• **CTG**: - Correlation with DTI measures diffusely in all tracts# - No correlation with VBM or cortical thickness analyses • **Muscular Impairment:** - Correlation with all DTI measures# - No correlation with VBM or cortical thickness analyses • **Age:** Correlated with WML • **Neuropsychology:** - Negative correlation of WMH with MMSE - DTI measures correlated with MMSE in CC, thalamic radiations and internal capsule# - **Others:** -	GM is less involved than WM, with the latter being correlated with both clinical and genetic features

(Schneider-Gold et al., 2015)	12 juvenile/adult onset HC: 33	• **GM:** VBM • **WM:** WMH	- Significant total atrophy - Areas of cortical atrophy: lingual gyrus, cuneus, insula, middle and superior temporal gyrus, precentral gyrus, medial frontal gyrus - Areas of subcortical atrophy: bilateral putamen and caudate head - Significant widening of the ventricles (lateral, 3rd, 4th)	- Considerable WMH in patients aged >40 years, located primarily in frontal, parietal, and occipital regions - Significant total atrophy - Areas of atrophy: posterior CC, bilateral mediodorsal and pulvinar nuclei of the thalamus and left pre and postcentral adjacent WM	• **CTG**: - • **Muscular Impairment:** Inverse correlation with supratentorial volume • **Age:** - • **Neuropsychology:** Inverse correlation between GM volume in medio-parietal cortex (secondary visual cortex) and cognitive flexibility • **Others:** No gender differences	Widespread GM and WM atrophy, with cortical and subcortical GM loss more pronounced than WM loss; and visual cortex plays a role in executive impairment in DM1
(Wozniak et al., 2014)	45 juvenile/adult onset HC: 44	• **GM:** - • **WM:** DTI	-	- Bilateral affectation in all 7 WM tracts studied: CST, ILF, uncinate fasciculus, cingulum, SLF, forceps major and forceps minor	• **CTG**: Moderate correlation with WM impairment (MD) in CST and cingulum • **Muscular Impairment:** Strong correlation with WM impairment in all tracts except forceps major • **Age:** - • **Neuropsychology:** Strong correlation between WM impairment all tracts but forceps major with working memory and in CST, SLF, cingulate and uncinate with processing speed • **Others:** -	Widespread WM impairment in both early and adult onset patients. The various correlations suggest DTI metrics are a useful index of disease burden in the CNS
(Caso et al., 2014)	51 DM1 patients: - 17 juvenile onset - 34 adult onset HC: 34	• **GM:** VBM • **WM:** - WMH - DTI	- Global and almost symmetrical GM atrophy, including cortical and subcortical regions. - Areas of cortical atrophy: bilateral pre-postcentral, SMA, orbitofrontal, medial and dorsolateral frontal, insular, anterior and posterior cingulate, lateral and medial parietal, lateral temporal, hippocampal and occipital cortex. - Areas of subcortical atrophy: bilateral thalamus, caudate, putamen.	- Significantly greater WMH load - Areas in order of most frequent involvement: frontal, temporal, parieto-occipital and posterior and anterior periventricular regions. - Decreased FA and increased MD and RD in CC, the majority of association tracts (SLF, ILF, inferior fronto-occipital and uncinate fasciculi, fornixes, and cingulum), projection fibers (internal and external capsule), and brainstem	• **CTG**: No correlation • **Muscular Impairment:** No correlation • **Age:** No correlation • **Neuropsychology:** - No correlation with GM VBM - WMH correlated with ACE-R memory and Raven's test scores - MD correlated with ACE-R attention, in corona radiata, internal and external capsule and frontotemporal WM regions belonging to the inferior fronto-occipital/uncinate fasciculi, ILF and SLF. • **Others:** Disease duration: correlated with WMH	DM1 is characterized by severe brain involvement. The correlations between WM impairment and cognitive deficits suggest a disconnection of GM structures due to damage of WM tracts

			- When juveniles assessed separately: less distributed atrophy			
(Wozniak et al., 2013)	16 childhood onset DM1 HC: 15	• **GM:** - • **WM:** DTI	-	- Decreased total FA and increased total MD - Regional WM alterations in all four lobes, with largest effect sizes in frontal and temporal lobes. Increased MD in CC - WM alterations in all tracts examined: CST, ILF, SLF, uncinate	• **CTG**: - • **Muscular Impairment:** - • **Age:** - • **Neuropsychology:** - WM alterations correlated with working memory • **Others:** -	Widespread WM microstructural damage playing only a partial role in the observed cognitive impairment
(Minnerop et al., 2011)	22 juvenile/adult onset DM1 HC: 22	• **GM:** VBM • **WM:** - WMH - VBM - DTI - TBSS	- Bilateral cortical atrophy mainly in frontal and parietal regions. - Subcortical atrophy in right posterior thalamus and anterior right putamen	- WMH more prevalent, with frontal WM being the most prominently impaired - Extensive WM atrophy in all cortical lobes, brainstem and CC - Widespread decreased FA and increased MD and RD in association fibers, CC, projection fibers, CST and brainstem	• **CTG**: correlated with decreased FA • **Muscular Impairment:** correlated with decreased FA • **Age:** correlated with decreased FA • **Neuropsychology:** - Marginal and inconsistent associations • **Others:** - Disease duration: correlated with decreased FA	DM1 is predominantly a WM disease in which WM affectation might be progressive over time
(Wozniak et al., 2011)	8 DM1 patients: - 3 congenital - 5 juvenile onset HC: 8	• **GM:** - • **WM:** - DTI	-	- Decreased FA and increased MD, RD and AD in all four ROIs: inferior frontal, superior frontal, occipital and supracallosal regions	• **CTG**: - • **Muscular Impairment:** - • **Age:** - • **Neuropsychology:** - Lower IQ correlated with decreased FA • **Others:** -	Young patients with DM1 suffer from significant WM alterations, which are implicated in cognitive functioning
(Weber et al., 2010)	20 DM1 patients (not specified, except for the exclusion of congenital forms) HC: 13 and 20 *	• **GM:** - BPF - VBM • **WM:** WMH	- Global and widespread atrophy - Subcortical atrophy: bilateral thalami	- WMH mainly located in subcortical frontal areas and centrum semiovale, less pronounced in temporal and parietal lobes	• **CTG**: - • **Muscular Impairment:** - • **Age:** Correlated with the extent of WMH • **Neuropsychology:** - The extent of WMH correlated with TMT - Nonverbal memory deficits correlated with hippocampal atrophy • **Others:** - Disease duration: Correlated with the extent of WMH and BPF - Clinical score[$]: Correlated with the extent of WMH and GM atrophy in medial and	DM1 shows widespread brain abnormalities, with some cognitive deficits linked to specific structural changes

posterior regions of the bilateral hippocampus

Note. DM1: Myotonic Dystrophy Type 1; JCR: Journal Citations Reports; CH: Healthy controls; GM: Gray matter; WM: White matter; WMH: White matter hyperintensities; TBSS: Tract Based Spatial Statistics; FA: Fractional anisotropy; MD: Mean diffusivity; AD: Axial diffusivity; RD: Radial diffusivity; ROCF: Rey-Osterrieth Complex Figure; CC: Corpus callosum; ToM: Theory of Mind; BPF: Brain parenchymal fraction; RAVLT: Rey Auditory Verbal Learning Test; CBT: Corsi block-tapping Test; MMSE: Mini Mental State; CST: corticospinal tracts; ILF: inferior longitudinal fasciculus; SLF: superior longitudinal fasciculus; ACE-R: Addenbrooke's Cognitive Examination-Revised.

Only manuscripts published in the English language and indexed in Journal Citation Reports (JCR) are included, in an effort to display those studies with the greatest scientific rigor.

[¶] 30 HC were employed for VBM analyses, and 21 for DTI analyses

* 13 (MRI+Neuropsychology), 20 (MRI), 18 (PET)

[#] No correlation if congenital patients were excluded

[$] Clinical score was calculated to represent general clinical status from current age, CTG length, and disease duration

4. Aging in DM1

4.1. DM1 as a progeroid disease

The muscular impairment observed in DM1 is known to be of progressive nature. However, other clinical symptoms — that are a consequence of the multisystemic affectation of the disease — also seem to reflect an accelerated aging process. Examples of these symptoms are the early presence of cataracts, baldness, diabetes, or cardiac arrhythmia. Thus, it has recently been suggested that DM1 is a progeroid syndrome (Meinke et al., 2018), on the basis of studies conducted from different perspectives. Indeed, DM1 has been proposed to resemble a model of premature aging, considering the process of muscle wasting (Mateos-Aierdi et al., 2015) or the skin abnormalities suffered by these patients (Campione et al., 2017). Further, at the cellular level, several mechanisms implicated in aging also occur in DM1, including genomic instability, mitochondrial dysfunction, insulin resistance, and satellite cell senescence (Mateos-Aierdi et al., 2015).

With regard to CNS involvement, several processes have been found to occur in DM1 that are closely linked to neurodegeneration. In fact, DM1 has been described as the first pathology in which tauopathy, spliceopathy and RNAopathy are combined (Caillet-Boudin et al., 2014; Fernandez-Gomez et al., 2019). These studies provide support for proposals of a possible degenerative brain process, which, in turn, constitute the rationale for longitudinal studies aimed at determining a possible profile of progressive cognitive and brain deterioration.

4.2. Cognitive decline and longitudinal studies in cognition

As mentioned in the previous section, CNS findings have been reported that suggest neurodegeneration. Based on these data, and on observations by clinicians, a progressive cognitive decline has been hypothesized, far beyond that expected in normal aging people.

While scientific literature regarding the neuropsychological profile of the DM1 population still needs to be extended, this gap in the literature is even more noticeable among studies examining longitudinal changes in cognitive abilities. There are a number of possible reasons for this lack of studies. Research on DM1 has mainly focused on other manifestations of the disease. Moreover, longitudinal studies are challenging for research teams, not only because of the greater costs and amount of time involved, but also for methodological reasons; for example, it

is often difficult to recruit equal numbers of participants at baseline and at follow-up (particularly control participants), and almost invariably, there will be a certain rate of experimental death. Finally, as already mentioned, there is a higher mortality rate in the DM1 population, particularly in those patients with an earlier onset of the disease. These factors make it somewhat challenging to conduct follow-up studies in DM1 — and even more to ensure representativeness of the population — all of which could explain the reduced number of longitudinal neuropsychological studies of this disease. Indeed, only five longitudinal studies, aside from Study #3 in this thesis, have been published so far.

Before formal longitudinal studies were published, some reports could be found that suggest a cognitive degeneration process in DM1. As early as 1937, Maas and Paterson (1937) described a series of cases presenting cognitive deterioration after school age; and, later in 1960, Vanier (1960) documented a progressive fall in the verbal IQ of a childhood onset patient. Bird et al. (1983) reevaluated five patients in their sample 11-19 years following the first assessment, and found no significant decline, except for one patient who comorbidly suffered from depression and a stroke. Finally, a case study was reported in 1999, which described the progressive cognitive decline (over an 11-year period) of a juvenile onset DM1 patient (Wilson et al., 1999) and later, another published case study reported progressive major deterioration, with particular affectation of executive functioning and episodic and semantic memory (Macniven et al., 2005). In both case studies, the observed alterations could be attributed to a comorbid diagnosis of dementia.

A summary of the longitudinal studies (as defined in its strictest sense) published so far can be found in Table 4. The first study was conducted by Tuikka et al. (1993), who assessed 15 adult onset and 1 congenital patient and found some progressive decline in memory and visuoconstruction tests, but no overall global and severe cognitive decline over a period of 12 years. Around 15 years later, two more studies emerged. The first of these reported a progressive decline that was specifically restricted to attentional functions (Sansone et al., 2007), whilst the second (Modoni et al., 2008) described a selective progressive cognitive decline of linguistic and executive functions. Both of these studies provide support for frontal involvement in the cognitive impairment.

During the last five years, three new studies have yielded results that shed light on the cognitive decline hypothesis (Gallais et al., 2017; Lindeblad et al., 2019; Winblad et al., 2016). Whilst Winblad et al. (2016), found a progression of cognitive deterioration in executive, arithmetic, attention, visuospatial construction and vocabulary functions in adult onset DM1 patients;

Gallais et al. (2017), found a decline in verbal memory, attention, and psychomotor speed. The most recent longitudinal study was conducted on congenital and childhood onset patients at school age or young adulthood (< 30 years old). Therefore, whilst no conclusions could be drawn regarding cognitive decline (as defined in its strictest sense), the results revealed a developmental trajectory that was characterized by a slower pace of development and a progressively wider gap between the patients and their peers, particularly in those with the congenital form of the disease.

Only some of these studies have examined the correlation between clinical or demographic factors and a potential cognitive decline, and the results were inconclusive, with some authors finding that the correlations depend on age (Gallais et al., 2017; Lindeblad et al., 2019; A. Modoni et al., 2008) age at onset, and disease duration (Gallais et al., 2017; Winblad et al., 2016). All studies, however, indicate the non-involvement (poor or no correlation) of genetic load (CTG expansion size) (Gallais et al., 2017; Modoni et al., 2008; Winblad et al., 2016) or MIRS score (Gallais et al., 2017; Winblad et al., 2016).

In sum, longitudinal studies on cognition in the DM1 population are still relatively scarce, and the progressive decline hypothesis needs further clarification. In particular, an agreement is needed on whether or not deterioration is an established fact, and if so, the exact pattern of cognitive decline is still to be defined. Moreover, the scope of this phenomenon needs to be determined, that is, does it affect all disease forms equally?, and what are the protective and risk factors?

None of the longitudinal studies on cognition described thus far included a broad spectrum of DM1 patients with disease forms from childhood to late onset. Moreover, none of the existing literature reviewed in this thesis has included a control group of healthy participants that were followed up longitudinally alongside the DM1 group, and only one study has made use of a decade-long observation period. In contrast, the current research group had access to a well characterized (clinically and socio-demographically) and extensive sample of patients with all disease forms assessed more than 10 years ago through a comprehensive neuropsychological battery. This provided the ideal setting for initiating a prospective longitudinal follow-up study.

Table 4. Summary of longitudinal studies on cognition in the DM1 population.

				Population							
Study	**Authors**	**Year**	**Country**	**DM1**	**HC**	**Duration**	**Age at baseline**	**Age at follow-up**	**Neuropsychological assessment**	**Results**	**Conclusion**
1	Tuikka et al.	1993	Finland	- 1 congenital - 15 adult onset	-	$\bar{x}$=12y	- DM1: 35.4y - HC: n/a	- DM1: 47.3y - HC: n/a	- WAIS (FSIQ, VIQ, PIQ) - WMS (MQ) - ROCF - Benton Visual Retention Test - Copy of Drawings Test - Finger-Tapping Test	- The IQ of 10 patients deteriorated by more than 10 points - 1 patient moderately demented - Significant decrease: Two WMS subtests (Mental control and Logical memory) and ROCF	No severe global cognitive impairment over time
2	Sansone et al.	2007	Italy	- 14 adult onset	-	$\bar{x}$=7.3y (SD= 2.7y)	Unk.	Unk.	- MMSE - RPM- colored - Token test - Verbal fluency - Digit span - Spatial span - Story recall - ROCF - TMT - TLT - TEA	- Significant worsening in alertness, TMT B, TMT B-A, Token test - Significant improvement on Story recall	Selective progressive frontal cognitive (attentional) impairment
3	Modoni et al.	2008	Italy	34 patients: - 32 juvenile/ adult onset • 2 E1 • 12 E2 • 18 E3 - 2 congenital • 2 E4	-	Per group: - E1: $\bar{x}$=23mo. (SD= 2.1mo.) - E2: $\bar{x}$=36mo. (SD= 10.3mo.) - E3: $\bar{x}$=31mo. (SD= 11.8mo.) - E4: $\bar{x}$=25.5mo. (SD= 0.7mo.)	Unk	Unk	- MMSE - RAVLT - ROCF - Digit span - Verbal fluency - Naming - Stroop color-word test - TMT	- Significant worsening in linguistic and executive function abilities: semantic verbal fluency and naming of nouns and verbs - Significant improvement in visual memory (ROCF recall) and verbal memory (RAVLT immediate recall)	Selective cognitive decline of linguistic and executive functions (fronto-temporal areas), similar to that observed in FTD

									- Multiple Features Target Cancellation test - RPM-colored		
4	Winblad et al.	2016	Sweden	- 37 adult onset	-	5y	DM1: $\bar{x}$=40y (SD= 10y) HC: n/a	DM1: $\bar{x}$=45y (SD= 10y) HC: n/a	- WAIS: Vocabulary, Arithmetic, Block design, Picture completion, digit span - Verbal fluency - ROCF - RAVLT - TMT - Stroop color-word - Spatial span - WCST	- Significant worsening in raw scores: RACLT, TMT A, TMT B, Spatial span, Block design and Picture completion - Significant worsening in standardized T scores: RAVLT, TMT A, TMT B, Spatial span, Block design and Vocabulary	Selective cognitive decline in adult onset DM1 is characterized by worsening in memory, attention, verbal ability and visuoconstruction
5	Gallais et al.	2017	Canada	115 patients: - 90 adult onset - 25 late onset	-	$\bar{x}$=8.9y (SD= 0.31y)	DM1 (total group): $\bar{x}$=43.6y (SD= 10.3y) Adult onset DM1: $\bar{x}$=41.0y (SD= 7.7y) Late onset DM1: $\bar{x}$=53.1y (SD= 13.0y) HC: n/a	DM1 (total group): $\bar{x}$=52.3y (SD= 10.3y) Adult onset DM1: $\bar{x}$=49.7y (SD= 7.7y) Late onset DM1: $\bar{x}$=61.6y (SD= 13.0y) HC: n/a	- CVLT - ROCF - Verbal fluency - BNT - Stroop color-word - Ruff 2 & 7 Selective Attention Test - WAIS-R (FSIQ, VIQ, PIQ)	- Significant worsening in verbal memory, visual memory and processing speed - Significant improvement in verbal fluency and global intelligence (FSIQ) - Significant increase in % of patients with an impaired score (<1 SD) from baseline to follow-up - Higher rate of decline in late onset patients than adult onset patients	A global cognitive alteration occurs in patients aged 50 years and older. The impairment is present earlier in life for executive, language and visual memory functions, and extends later to verbal memory, visual attention, and processing speed
6	Lindebald et al.	2019	Sweden	51 patients: - 16 severe congenital¶ - 17 mild congenital	-	$\bar{x}$=7y 8mo. (range: 6y 8mo. – 9y 4mo.)	Severe congenital: $\bar{x}$=9y 11mo. (range: 2y 6mo.- 21y 4mo.)	Severe congenital: $\bar{x}$=18y (range: 11y-28y 10mo.)	- WPPSI-R - WISC-R - WAIS-R - VABS	- No significant worsening in intellectual level (Wechsler scales) in any of the groups - Significant worsening in severe congenital DM1	No cognitive decline over time in congenital and childhood onset patients, although a slowed rhythm of development. Wider

	- 18 childhood onset	Mild congenital: $\bar{x}$=11y 1mo. (range: 3y 3mo.- 18y 8mo.) Childhood onset: $\bar{x}$=13y 8mo. (range: 8y 1mo.- 21y) HC: n/a	Mild congenital: $\bar{x}$=18y 9mo. (range: 10y 10mo.- 26y 5mo.) Childhood onset: $\bar{x}$=21y 5mo. (range: 16y 5mo.- 28y 11mo.) HC: n/a	for all adaptive behavior domains - Significant worsening in mild congenital DM1 for communication, socialization and daily living domains - Significant worsening in childhood onset DM1 for communication domain	development gap between DM1 patients (particularly congenital) and their peers, which can be expected to increase over time.

Note. DM1: Myotonic Dystrophy Type 1; HC: Healthy Controls; y: years; mo.: months; SD: Standard Deviation; n/a: non applicable; Unk.: Unknown; WAIS: Wechsler Adult Intelligence Scale; WAIS-R: Wechsler Adult Intelligence Scale- Revised; FSIQ: Full Scale Intelligence Quotient; VIQ: Verbal Intelligence Quotient; PIQ; Performance Intelligence Quotient; ROCF: Rey-Osterrieth Complex Figure test; MMSE: Mini-Mental State Examination; RPM-colored: Raven's Pogressive Colored Matrices; TMT: Trail Making Test; TLT: Tower of London Test; TEA: Test of Everyday Attention; RAVLT: Rey Auditory Verbal Learning Test; WCST: Wisconsin Card Sorting Test; CVLT: California Verbal Learning Test; BNT: Boston Naming Test; WPPSI-R: Wechsler Preschool and Primary Scale of Intelligence- Revised; WISC-R: Wechsler Intelligence Scale for Children- Revised; VABS: Vineland Adaptive Behavior Scales FTD: Fronto Temporal Dementia
Only manuscripts with samples larger than 10 patients, published in the English language and indexed in Journal Citation Reports (JCR) are included in an effort to display those studies with the greatest scientific rigor.
¶Severe congenital implies a life-threatening condition at birth, requiring the need for resuscitation and/or respiratory assistance.

4.3. Brain decline and longitudinal neuroimaging studies

The cross-sectional design of most neuroimaging studies in DM1 do not allow for determining whether the observed alterations are the consequence of a neurodevelopmental process of brain aberrant maturation, progressive neurodegeneration, or both. The greatest gap in the scientific literature regarding the topic of the current thesis undoubtedly concerns longitudinal studies examining brain changes in DM1. So far, only two MRI longitudinal studies have been published in this population with the aim of testing the hypothesized progressive impairment in brain structures.

The first attempt was made by Conforti et al. (2016), who conducted a follow-up ranging from 7 up to 20 years employing semi-quantitative methods to assess WM lesions and ventricular/brain ratio. These authors found a progression in WM lesions located at frontal, temporal, and periventricular regions, along with an increase in brain atrophy, as measured by the ventricular/brain ratio. More recently, Gliem et al. (2019) conducted a 5-year follow-up study employing VBM and DTI and did not find a greater volume loss over time in DM1, for either gray or white matter tissue.

In the absence of any other longitudinal study on MRI, there are still several neuroimaging findings that encourage follow-up studies, such as the correlations reported between age or disease duration and various MRI results such as brain parenchymal fraction (BPF) or WM lesion load (Baldanzi et al., 2016; Cabada et al., 2017; Caso et al., 2014; Hamilton et al., 2018). These correlations are often taken to indicate an ongoing process of brain degeneration over time. However, as noted, no other attempts have been made, aside from the studies cited above, to describe the natural history of brain outcomes in this population. Therefore, given that the current research group had obtained a neuroimaging dataset recorded between 2008-2009, not only in DM1 patients but also in healthy participants, this provided the ideal framework for studying the potential progression of brain anomalies in patients, compared with a healthy sample.

HYPOTHESES AND OBJECTIVES

Hypotheses-related objectives are presented in Table 5 with a cross-reference to the specific study in which they are addressed.

Table 5. Hypotheses and objectives addressed in each study

	HYPOTHESES		OBJECTIVES	STUDY
	Global		Global	
	DM1 patients will show a greater impairment in both cognition and brain structure, which will additionally suffer an accelerated aging process.		To study the characterization of CNS involvement in DM1 regarding cognitive and structural brain findings — both transversally and longitudinally — and to examine the association with clinical and molecular data.	Study #1-4
	Specific hypotheses		General and specific objectives	
1.	DM1 patients without global cognitive impairment will not perform worse on social cognition tasks than healthy individuals with normal IQ.	1.	To study the existence of social cognition impairment in DM1, regardless of global cognitive functioning.	Study #1
		1.1.	To determine whether social cognition impairment is a primary or secondary deficit in DM1.	
2.	DM1 patients without global cognitive impairment will not show differences in social cognition tasks associated with clinical and molecular variables	1.2.	To examine whether clinical and molecular features are associated with social cognition outcomes.	
3.	DM1 patients will show a greater global GM and WM atrophy and global white matter integrity impairment compared with healthy individuals.	2.	To analyze the structural pattern of brain organization through multimodal neuroimaging techniques in DM1 patients.	Study #2
4.	DM1 patients will show a specific pattern of regional GM and WM structural impairment and white matter integrity impairment compared with healthy individuals.	2.1.	To examine the structural brain GM impairment through VBM in DM1 patients.	
		2.2.	To examine the structural WM connectivity impairment through DTI in DM1 patients.	
5.	The extent of brain structural impairment (global regional GM volume and white matter integrity) will positively correlate with greater clinical, molecular and cognitive affectation.	2.3.	To search for potential associations between clinical, demographic, and cognitive outcomes and structural brain findings in DM1	

6.	DM1 patients will show areas of reduced GM volume and white matter integrity correlated with age, in comparison with healthy individuals.			
7.	DM1 patients will show a specific pattern of cognitive decline in comparison with healthy individuals.	3.	To determine the presence and pattern of longitudinal cognitive decline in DM1 patients.	Study #3
		3.1.	To study the specific pattern of cognitive decline in DM1 patients.	
8.	The cognitive decline from baseline to follow-up in DM1 will be associated with potential demographic and clinical predictors.	3.2.	To search for potential clinical and demographic associations with cognitive decline in DM1 patients.	
9.	DM1 patients will suffer a greater cerebral GM and WM loss over time in comparison with healthy individuals.	4.	To determine the presence and pattern of longitudinal structural brain deterioration in DM1 patients.	Study #4
		4.1.	To study the specific pattern of progressive structural brain deterioration in DM1 patients	
10.	Pediatric onset DM1 patients will differ from adult/late onset DM1 patients in cross-sectional brain structural impairment and in the trajectory of impairment progression over time.	4.2.	To separately examine pediatric onset and adult/late onset DM1 regarding structural brain deterioration.	
11.	GM and WM cerebral loss over time in DM1 patients will correlate positively with clinical, molecular, and cognitive affectation in DM1 patients.	4.3.	To search for potential associations between clinical, demographic, and cognitive outcomes and structural brain changes in DM1.	

Note. DM1: Myotonic Dystrophy Type 1; VBM: Voxel Based Morphometry; DTI: Diffusion Tensor Imaging

METHODS

As outlined in Figure 1 (see Introduction), the methodology employed in the work presented in this thesis uses both a retrospective and longitudinal design. A detailed description of the specific procedure, study sample, and applied instruments can be found in each study (see Section II). Nonetheless — and given that all of the studies share a global objective within this thesis — a general procedure has been used throughout the course of this work, which is described next.

1. Participants

The participants in the four studies of this thesis are DM1 patients attending the outpatient service of the Neurology Department at Donostia University Hospital (Gipuzkoa, Spain), and HC subjects recruited from both non-affected relatives of patients and healthy volunteers.

Inclusion criteria for DM1 patients were being aged above 16 years and having a molecular confirmation of the clinical diagnosis. Patients with the congenital form of the disease were not included in any study as this subtype was considered to be a qualitatively distinct phenotype. Patients were excluded if they presented a history of major psychiatric disorder, acquired brain damage, alcohol or drug abuse, or had any condition that could impede the application of any of the medical tests or planned neuropsychological assessments. The same inclusion criteria were applied to the HC participants, except for the molecular confirmation of the clinical diagnosis. Whenever possible, non-affected relatives were included in an effort to obtain the most similar control sample in terms of socioeconomic background.

For follow-up studies, only participants that had previously participated were eligible, and, consequently, statistical analyses were carried out using only the same participants at baseline and follow-up (i.e. complete case analysis).

Table 6 summarizes the final sample sizes included for analysis in each of the studies of this thesis. The sample in Study #4 was reclassified according to the most recent proposal of disease classification put forward by the OMMYD-4 group during the IDMC-12, thus, dividing the sample into two groups: pediatric onset (childhood + juvenile) and adult/late onset (adult + late). The specific sample recruiting process (including clinical and sociodemographic data and dropout rates at follow-up), is detailed in each study (see Section II).

Table 6. DM1 and healthy control samples employed in each study.

	Study #1		Study #2		Study #3		Study #4	
	N (%)	**Mean age (range)**	**N (%)**	**Mean age (range)**	**N (%)**	**Mean age* (range)**	**N (%)**	**Mean age* (range)**
DM1 patients	38	44.84 (27-65)	31	43.94 (22-61)	75	38.65 (19-67)	21	39.05 (22-60)
Childhood onset					7 (9.3)		3 (14.3)	
Juvenile onset	12 (31.6)		9 (29.03)		20 (26.7)		6 (28.6)	
Adult onset	21 (55.3)		22 (70.97)		35 (46.7)		8 (38.1)	
Late onset	5 (13.2)				13 (17.3)		4 (19)	
Healthy controls	38	45.47 (25-62)	57	45.14 (18-70)	54	42 (16-71)	18	39.3 (18-58)
Total	76		88		129		39	

Note. DM1: Myotonic Dystrophy Type 1
*Mean age at baseline is reported

2. Assessment instruments

Three main types of assessment can be distinguished throughout the four studies: clinical, neuropsychological, and neuroradiological.

2.1. Clinical and molecular assessment

Clinical and molecular assessment was conducted only in DM1 patients and initially helped to identify those patients who met all inclusion criteria and those who needed to be excluded.

The assessment comprised the collection of two types of data. First, a muscular impairment assessment was conducted by the neurologist through the Muscular Impairment Rating Scale (MIRS) (Mathieu et al., 2001). The MIRS scale is one of the most widely employed indices in the assessment of muscular impairment in DM1 both at a clinical and research level. It consists of a five-point scale ranging from No muscular impairment (Grade 1) to Severe proximal weakness (Grade 5) (Table 7).

Table 7. Muscular Impairment Rating Scale (MIRS)

Grade	Description
1	***No muscular impairment***
2	***Minimal signs***
	Myotonia, jaw and temporal wasting, facial weakness, neck flexor weakness, ptosis, nasal speech, no distal weakness except for isolated digit flexor weakness
3	***Distal weakness***
	No proximal weakness except for isolated elbow extensor weakness
4	***Mild to moderate proximal weakness***
5	***Severe proximal weakness***

Note. Adapted from Mathieu et al. (2001)

Second, patient blood samples were requested in order to obtain an updated CTG expansion size. The instability of CTG over time has been described in DM1, with increases of CTG repeat length for an individual patient across small time periods (Martorell, 1998). This was only done in case patients had not had a recent CTG estimation within the last 5 years. The CTG expansion size was obtained through genetic assessment of the DMPK gene isolated from circulating

leucocyte DNA. PCR was used to measure repeat length in DMPK alleles up to approximately 100 CTG repeats and Southern blot analysis was used for larger expansions.

2.2. Neuropsychological assessment

Two neuropsychological assessment batteries were designed for the purpose of the studies in this thesis. The first corresponds to Study #1 and is mainly aimed at evaluating social cognition abilities. The second corresponds to Studies #2, #3 and #4, and represents a comprehensive cognitive assessment battery, the purpose of which is to describe global cognitive functioning and to specifically evaluate various cognitive domains. In some cases, as can be seen in each study, short forms of the original tests were selected in order to avoid excessive tiredness in patients, since fatigue and daytime sleepiness are well-documented characteristics of the disease (Laberge et al., 2020). For each test, when considered appropriate, standardized T values were calculated from participants' raw scores according to Spanish population-based norms taking into account the participant's age and, where applicable, sex and years of education.

Table 8 summarizes the specific tests employed in each study.

Table 8. Summary of the neuropsychological tests employed in each study.

	Study #1	Study #2	Study #3	Study #4
K-BIT	●			
POFA	●			
Faux Pas Test	●			
TECA	●			
WAIS III				
Block design		●	●	●
Digit span	●	●	●	●
Vocabulary		●	●	●
Object assembly		●		●
Arithmetic		●		●
Similarities		●		●
IQ estimate		●	●	●
RAVLT		●	●	●
Raven's SPM		●	●	●

ROCF	●	●	●
Verbal fluency	●	●	●
Stroop color and word test	●	●	●
CALCAP	●	●	●
Benton's Judgement of Line Orientation	●		●
WCST	●		●

Note. K-BIT: Kaufman Brief Intelligence Test; POFA: Pictures of Facial Affect; TECA: the Test of Cognitive and Affective Empathy; WAIS III: Wechsler Adult Intelligence Scale III; RAVLT: Rey Auditory Verbal Learning Test; Raven's SPM: Raven's Standard Progressive Matrices; ROCF: Rey-Osterrieth Complex Figure; CALCAP: California Computerized Assessment Package; WCST: Wisconsin Card Sorting Test

2.2.1. Neuropsychological assessment tools for Study #1

The neuropsychological assessment in Study #1 comprised a global cognitive functioning assessment and a specific social cognitive functioning assessment. The former included an IQ estimation through the Kaufman Brief Intelligence Test (K-BIT) (Kaufman, 1997), including measures of verbal (vocabulary subtest) and nonverbal (matrix subtest) intelligence; and a measure of attention performance through the digit span subtest from the Wechsler Adult Intelligence Scale (WAIS-III) (Wechsler, 1999). The latter included three measures of social cognitive functioning. First, facial emotion recognition was assessed through the Pictures of Facial Affect (POFA) (Ekman & Friesen, 1976). Second, the Faux Pas test was employed to assess a specific ToM ability – the ability to adequately interpret socially awkward situations in which someone mistakenly said something they should not have said – ; and the Cognitive and Affective Empathy Test (TECA in its Spanish acronym) (López-Pérez et al., 2008) to assess self-reported cognitive and affective aspects of empathy. The overall assessment session lasted approximately one hour.

2.2.2. Neuropsychological assessment tools for Studies #2, #3 and #4

The second neuropsychological battery was partially or completely employed in the analyses of Studies #2, #3 and #4, and included the following tests:

- Wechsler Adult Intelligence Scale (WAIS-III) (Wechsler, 1999). This is one of the most widely employed instruments to assess global cognitive functioning in the adult

population. It is often administered to obtain an IQ score for the subject, but its use is extended to the examination of specific cognitive functions. The following subtests were administered throughout the studies: block design, digit span, vocabulary, object assembly, arithmetic and similarities. Only block design, digit span and vocabulary subtests were re-administered at the follow-up assessment. The reason for doing so was that since the first administration, the research group had decided to revise the neuropsychological assessment protocol for DM1 patients in order to include some new tests. This decision was made in order to follow the recommendations provided by expert clinicians and researchers working on DM1 regarding the best outcome measures (OM) to be used based on metrological properties and experience [Outcome Measures in Myotonic Dystrophy Type 1-1 OMMYD-1 (Gagnon et al., 2013) and OMMYD-2 (Gagnon et al., 2015)].

- Rey Auditory Verbal Learning Test (RAVLT) (Lezak et al., 2004). This test measures verbal learning and memory capacity through 5 learning trials of a 15-word list, followed by a delayed recall trial (30 to 45 minutes later).
- Raven's Standard Progressive Matrices (SPM). This test measures nonverbal reasoning ability and it is widely employed as an intelligence estimate. High correlations of this measure with WAIS-based IQ have been reported.
- Rey-Osterrieth Complex Figure test (ROCF) (Rey, 2009). In this test, participants are asked to reproduce a complex figure drawing, first by copying, and then by drawing it from memory 30 to 45 minutes later. The purpose of this test is to measure perceptual organization, visuoconstruction skills, and visual memory.
- Verbal fluency: Semantic (animals) and phonemic (P) (Casals-Coll et al., 2013; Pena-Casanova et al., 2009). In one minute, participants were asked to produce as many words as possible within the given category or beginning with the given letter. This test measures both verbal ability (lexical knowledge and lexical retrieval ability) and executive functioning.
- Stroop color and word test (Golden, 2001). This test is employed to measure both information processing speed and inhibition of the cognitive interference resulting from the processing of one stimulus over (or impeding) another, known as the Stroop Effect. The test consists of three sheets that the subject is required to read as fast as possible. The first two sheets contain congruent information (names of colors printed in black ink and non-verbal stimuli – XXXXX – written in color ink). The third sheet, known as the color-word condition, contains incongruent stimuli – names of colors printed in a different color ink than that stated by the word.

- California Computerized Assessment Package (CALCAP) (Miller, 1990). This computer-based test assesses information processing speed through measures of reaction time (RT). The participant has to react (press the spacebar) or inhibit her/his response when presented with target stimuli according to the instructions given. Here, the brief version consisting of four tasks was administered, with an approximate duration of 10 minutes. Each task produces an output measure: simple RT, election RT, sequential 1 RT and sequential 2 RT.
- Benton's Judgement of Line Orientation (Benton et al., 1994). This test evaluates visuospatial ability through 30 items where the participant has to judge the angle and orientation of a line in a blank space. The participant has to choose which of the 11 lines displayed in a semicircle matches the target item. This test was only administered at baseline evaluation.
- Wisconsin Card Sorting Test (WCST) (Heaton et al., 2001). As a standard measure of executive functioning, this test assesses cognitive flexibility and categorization or abstract reasoning. The participant is presented with four stimulus cards and asked to match 128 cards to these (similar to the stimulus cards, with varying forms, numbers and colors) following one of three categories: form, number and color. Each time the participant achieves 10 consecutive correct answers in a row – a complete category – the examiner changes the classification criteria. The examiner, without revealing how to match, gives the participant feedback of whether their response is correct or incorrect. From the variety of possible scores, only "number of categories completed" was included in the studies. This test was only administered at baseline evaluation.

2.3. Neuroradiological assessment

DM1 patients and HC underwent a neuroradiological assessment in Studies #2 and #4. In both studies, this neuroradiological assessment was identical; it was conducted in the same MRI scanner and under the same scanning protocol. A detailed description of the MRI scanning material and protocol can be found in Study #2 and Study #4.

To study voxel-based GM volume loss in DM1 patients and its association with various clinical and neuropsychological outcomes, FSL (version 6.01) Voxel Based Morphometry (VBM) was used (Douaud et al., 2007), which is an optimized VBM protocol (Good et al., 2001) carried out with FSL tools (Smith et al., 2004). VBM is mainly employed to assess global and regional GM volume, although it also enables the assessment of WM volume. VBM allows for a thorough examination of voxel-level concentration of brain tissue by comparing multiple local volume

changes in brain anatomy using a parametric mapping statistical approach. This is an automated technique, and as such, allows for the analysis of multiple subjects at the same time, avoiding the time-consuming traditional approach of manual measurements. The VBM procedure segments T1-weighted images into the three main brain tissues: GM, WM and CSF, allowing for separate analyses of each tissue. The resulting segmented images are then co-registered into standard anatomical templates (such as the Montreal National Institute [MNI 152] standard space), GM modulated for correcting local expansion, and finally smoothed. This procedure allows for comparing the same voxels in multiple subjects, as well as detecting correlations between continuous outcomes (i.e. neuropsychological performance, clinical variables, etc.) and voxel-level changes (Ashburner & Friston, 2000). A pipeline depicting the T1-voxel morphometry computation analyses is displayed in Figure 5.

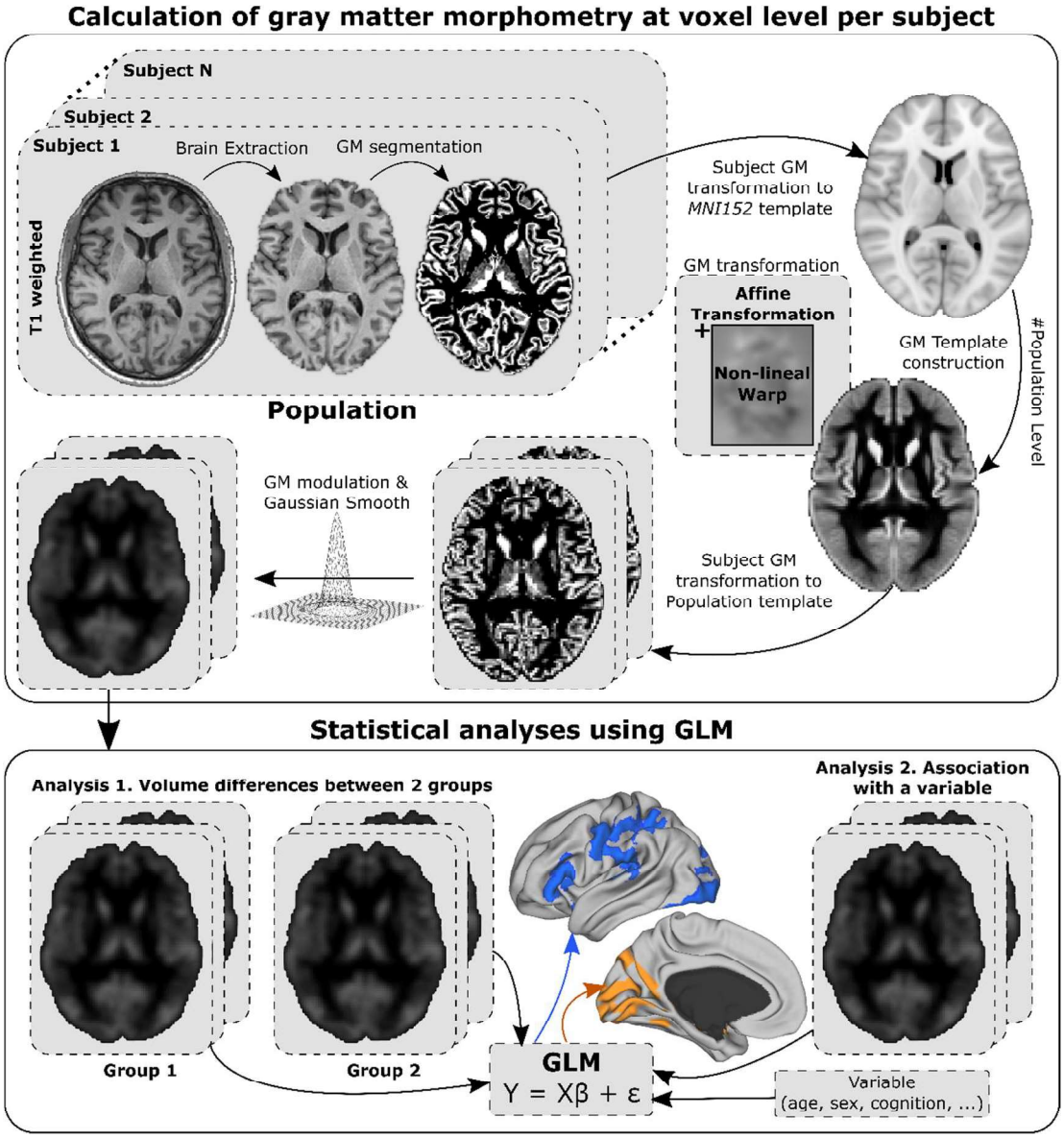


Figure 5. T1-voxel morphometry computation pipeline.

To estimate global brain tissue volume, normalized for the participant's head size, the SIENAX tool was used (Smith et al., 2002).

DTI was employed to study WM microstructural damage in cortical and subcortical regions. This technique examines WM integrity based on the rate and direction of the diffusion of water molecules within fibers. The DTI technique is built on the principle that due to the structural restrictions of WM tracts, the diffusion of water molecules is anisotropic (straightforward). When the permeability of these tracts is disrupted as a consequence of microstructural damage, water anisotropic diffusion is altered. Thus, DTI allows for the inspection of WM microstructural impairment with greater sensitivity in comparison with traditional MRI and even allows for detecting lesions in the so-called normal NAWM. In the DTI procedure used here, the brain was divided into 86 regions (68 cortical and 18 subcortical, as defined by Freesurfer Desikan parcellation and Freesurfer segmentation, respectively). After DTI preprocessing, probabilistic tractography was applied to obtain a density map defining the number of fibers passing through each voxel from a given seed. The thresholded density maps of each region were used to compute the weighted FA values and GLM was applied to conduct intergroup comparisons of weighted FA of the 86 regions and associations of targeted variables.

3. Procedure

Patients and HCs were recruited either during medical appointments by the neurologist specialized in DM1 at the Neurology Department of the hospital (and the usual neurologist of all the patients) or via phone calls in case the patient or HC had participated in a previous study conducted by any member of the group. If the participant agreed to take part, he/she was informed about the main objectives and implications of his/her participation and was given an information sheet containing all the relevant details, including treatment of personal information and rights of the participant. Finally, the participant was asked to provide written informed consent for each of the tests to be conducted (i.e. blood sample, MRI, and neuropsychological assessment).

Once written informed consent had been provided, the various assessments were scheduled in one or two separate appointments. Clinical and genetic assessments were conducted by the neurologists and staff nurses at the Neurology Department of the Donostia University Hospital. All neuropsychological assessments were conducted by an experienced neuropsychologist, and under the same conditions (time, location) at the same hospital. Neuroradiological assessments were carried out in the usual facility within the hospital (Osatek, S.L.).

Each of the studies reported in this thesis has received the approval of the Ethics Committee of the Donostia University Hospital.

RESULTS AND DISCUSSION

This section provides a summary of the results obtained from the four studies described in this thesis, along with a brief discussion in each case. For a more specific and thorough description of the results and discussion, the reader is referred to Section II (Published manuscripts and manuscripts under review). For the sake of clarity, the following description of the results is arranged according to the study hypotheses.

The first set of hypotheses referred to the performance shown by DM1 patients without global cognitive impairment on social cognition tasks (Hypotheses 1 and 2). The results obtained in Study #1 indicated that DM1 patients show significantly worse performance than age- and gender- paired HC on facial emotion recognition (POFA) ($U = 460.0$, $p < .006$, $r = 0.31$), with anger and disgust being the two emotions for which the patients had the greatest difficulties. These results are in line with other published studies reporting deficits in facial emotion recognition, particularly negative emotions, in patients (Kobayakawa et al., 2010; Takeda et al., 2009; Winblad, Hellström, et al., 2006), although it has also been reported that in some isolated cases, patients have shown difficulty with positive emotions (i.e. happiness (Serra et al., 2020). In contrast, DM1 patients in Study #1 showed similar performance to that of HC participants on other measures of social cognition, that is, the ability to identify socially awkward situations (The Faux Pas test) and self-reported cognitive and affective empathy (TECA). Other studies have reported difficulties in correctly performing a variety of social cognition tasks (Kobayakawa et al., 2012; Serra, Cercignani, et al., 2016; Serra et al., 2020). The discrepancies between the results of Study #1 and those of these other works could be due to methodological differences. In particular, in the present study, global cognitive impairment was controlled by limiting inclusion to those DM1 patients with IQ in the normal range and adequate attentional capacity. Thus, the results are interpreted as reflecting a core deficit in facial emotion recognition in DM1, while other social cognition tasks demanding higher order cognitive processes appear to be secondary to global cognitive functioning.

With regard to the association between social cognition deficits and clinical and demographic features, POFA total score was negatively correlated with age (Spearman's rho = -.35; $p = .03$), whereas no correlation was found between POFA and CTG length (Spearman's rho = .146, $p = .380$). Interestingly, the correlation between POFA and age was only found in DM1 patients, but not in HC, (Spearman's rho = .15; $p = .37$), indicating that facial emotion recognition deficits in

DM1 are age-related and could reflect an accelerated cognitive decline process, as already suggested.

So far, the results of Study #1 do not rule out the existence of a social cognition impairment in DM1. Indeed, there is evidence of comorbidity with Autism Spectrum Disorder (ASD) in childhood and particularly congenital phenotypes (Ekström et al., 2008), a disorder that includes the presence of social cognition deficits. More recently, while still considering the ASD-comorbidity issue as an open debate, a proposal on considering the Social-pragmatic Communication Disorder (SCD) has instead been put forward, suggesting that this could provide a better fit with some of the social cognition deficits shown by childhood onset DM1 patients (Angeard et al., 2018). In any case, social cognition assessment is highly controversial for several reasons. First, as outlined in the background, social cognition abilities could be highly dependent on global cognitive functioning, a possibility that has been specifically addressed in Study #1. Second, most social cognition tasks employed rely on verbal materials (i.e. texts describing situations) or visual materials (i.e. pictures of facial expressions), whereas in real life, correctly functioning social cognition could depend on the integration of multiple sources of information (movement, speech tone, past and present contextual information). Therefore, there is a need to develop more ecologically valid tasks for use in clinical and research fields. Finally, social interactions are highly dependent on the specific cultural contexts of a particular population; a given behavior might be considered socially correct in one culture but not in another. However, the currently available social cognition tasks have been developed in a certain country (and therefore based on a certain culture) and may not be completely accurate to measure the intended construct in other countries. An effort should be made to culturally adapt and validate the existing social cognition assessment tools to each target population, in order to ensure that examiners correctly grasp the essence of what they wish to measure.

In order to overcome the above-mentioned limitations, future studies might aim at designing and testing experimental paradigms of social cognition in order to clarify whether socially-related information processing is hindered in the disease and to confirm the observed difficulties in social communication, emotional recognition, or the assessment of others' mental states.

Regarding the following set of hypotheses (Hypotheses 4-7) addressed through the cross-sectional assessment of DM1 patients from a neuroradiological approach (Study #2), the obtained results confirmed the global brain involvement in DM1 with clinical, demographic, and neuropsychological correlates. Total GM and WM volume, as well as WM integrity (measured as FA) were significantly reduced in comparison with HC (p= .000; p= .011 and p= .001, respectively). These results confirm those of previous reports and support the suggestion of the

core involvement of CNS in the disease. DM1 has been proposed to be a disease with predominantly white matter affectation, given the more pronounced decrease of WM than GM (Minnerop et al., 2011). The results of Study #2, however, point to the possibility of a slightly greater impairment of GM, which, in any case, appeared to be closely linked to microstructural impairment of WM tracts. A similar trend was observed with regard to the specific location of structural anomalies. Widespread bilateral decrease in GM, including cortical and subcortical structures, has been reported (Okkersen, Monckton, et al., 2017) and confirmed in our results with a pattern of greater fronto-parietal and subcortical atrophy. However, the precise localization of the decreased regions requires replication in further studies and an effort should be made to select homogeneous samples with equivalent disease phenotypes and comparable (if not identical) scanning protocols.

In relation to brain correlates with the variables of interest, several vulnerability predictors were identified. Older patients with larger CTG repeats and greater muscular impairment (as measured by MIRS) showed greater total GM atrophy (age: r= -.56, p= .001; CTG: r= -.66, p= .000; MIRS: r= -.58, p= .003) and regional GM decrease. Muscular impairment was further associated with decreased total and regional FA (r= -.5, p= .016) and CTG length with regional FA. Neuropsychological performance, particularly on executive functioning and visuoconstruction tasks, emerged as a strong predictor of both total and regional brain abnormalities (Block design: r= .49, p= .006; Raven's progressive matrices: r= .52, p= .006; ROCF copy: r= .55, p= .001). Nevertheless, the non-specificity of such an anatomo-functional relationship suggests a rather network-wise organization of cognitive functions in DM1, along with a functional reorganization of such cognitive functions due to global brain atrophy. Taken together, these results encourage the continued exploration of these target variables as potential markers of brain impairment, whilst age-related vulnerability provides support for the potential neurodegenerative process previously hypothesized.

With regard to this latter idea, researchers have explicitly claimed that longitudinal studies would allow for clarifying the debate surrounding progressive neurodegeneration of the CNS in DM1 patients. This claim formed the basis for the final cluster of hypotheses (Hypotheses 7-11), which are concerned with both cognitive decline and brain progressive degeneration. Overall, the results of Study #3 and Study #4 provide support for the neurodegenerative hypothesis revealing disease-specific worsening trajectories that could clearly be distinguished from those that would be naturally expected in HC.

In particular — and regarding the hypothesis predicting that DM1 patients would show a specific cognitive decline pattern compared with healthy individuals — the results of Study #3 confirmed this prediction. Patients did not show a global deterioration over time but did show a greater cognitive worsening on tasks measuring visuospatial/visuoconstructive ability and visual memory when compared with HC. Interestingly, the visuoconstruction domain had previously been suggested as a strong predictor of brain atrophy (Study #2) and in a recent meta-analysis, this domain was identified as the one with the largest effect sizes (Okkersen, Buskes, et al., 2017). Undoubtedly, this domain deserves special attention in DM1 and, as proposed for social cognition, an effort should be made to analyze in more depth the basic cognitive processing deficits that could potentially underlie these outcomes. Indeed, visuoconstruction tasks (such as block design of ROCF) are thought to require the considerable involvement of executive organization and control. Therefore, deficits in various executive functions (already well-documented in DM1), could account for some of the difficulties shown in visuoconstruction tasks. A subsequent analysis sought to identify the significant predictive factors of cognitive decline in these tasks. Interestingly, age emerged as one such predictor, and a cut-off point at 31 years of age was detected, meaning that, from the thirties onwards, there is a more rapid decline in visuoconstruction abilities.

So far, relatively few studies have addressed the cognitive decline in DM1 through a longitudinal approach. Overall, a great variability exists between these studies regarding the phenotypes included, neuropsychological batteries administered, or statistical analyses conducted; all of which hinders comparability and the possibility of reaching common conclusions. So far —and on the basis of the present results and several reports of the cognitive profile in DM1 and brain-cognition correlates from MRI studies, visuoconstruction tasks such as block design and ROCF, emerge as essential tools for the neuropsychological evaluation of DM1 population. Such instruments should therefore be included in assessment protocols in future studies. Further, the lack of control groups and small DM1 samples are some of the methodological concerns that need to be overcome. Additionally, taking into account the inherent difficulties of longitudinal studies in rare diseases with such high heterogeneity regarding the age of patients, an effort should be made to recruit homogeneous samples assessed throughout the lifespan in order to identify clear cut-off points of cognitive decline in each cognitive domain.

Finally, an accelerated process of GM and WM loss over time was hypothesized in DM1. The MRI follow-up conducted in a cohort of patients and HC revealed three main interesting findings regarding natural brain history in the disease. First, and contrary to our expectations, the decrease in total GM or WM volume was not significantly greater in DM1 patients compared

with HC. However, the effect sizes were large for GM. While pediatric DM1 lost 3.86% of GM volume, HC-pediatric lost 0.63% (F= 3.497, p= .078, *Hedges' g*= 0.94); and whereas adult/late DM1 lost 2.71%, HC-adult/late lost 0.85% (F= 1.721, p= .203, *Hedges' g* =0.57). Statistical power was lacking, possibly as a consequence of the small sample sizes, but the large effect size could imply that, regardless of the small samples employed, the difference exists. These results are of particular interest since they imply that even in young adulthood (the mean age of pediatric patients at baseline was 30) or middle adulthood (the mean age of adult/late patients at baseline was 45.83), a neurodegeneration process may be emerging. Further, the early brain differences between DM1 patients and HC suggest that brain abnormalities might have a neurodevelopmental origin, an idea that needs further clarification through neuroimaging studies exploring brain findings during neurodevelopment, or at least, as soon as the diagnosis is made.

Second, the analysis of regional volume loss at each timepoint and over time, revealed distinct patterns of cross-sectional brain atrophy as well as a trajectory of brain progressive damage. While adult/late onset patients showed greater brain atrophy that was restricted to subcortical areas and further enlarged at follow-up (but still restricted to deep GM); the brain atrophy of pediatric onset patients was located primarily at subcortical areas at baseline and progressed to mainly frontal areas at follow-up. Although these results require replication in larger samples, they can be interpreted as a sign of a distinct CNS involvement pattern in each phenotype and could be at the basis of the different cognitive affectation profiles displayed by patients with early or adult onset of the disease.

Finally, whereas genetic features (CTG length and inheritance pattern), muscular impairment, and years of education at baseline showed no capacity to predict a decrease in volume over time or total brain atrophy at follow-up, baseline neuropsychological assessment did show such predictive capacity. The poorer performance on certain neuropsychological tasks (CALCAP and ROCF) correlated with greater WM loss over time; and several cognitive tests were associated with regional decrease of GM at follow-up. Again, the lack of topological specificity of such correlates supports the notion of a network-wise organization, and the association between neuropsychological outcomes and several areas of reduced volume corresponding to the multimodal integration network might be at the basis of higher order cognitive dysfunction in DM1.

In summary, the results from our longitudinal studies suggest a domain-specific cognitive decline of visuospatial/visuoconstructive abilities and the potential existence of an early

neurodegenerative process that plays a role in a neurodevelopmentally compromised CNS in DM1 patients. These results provide additional (and important) information regarding the natural brain history in DM1 and encourage continued research in this area. Future works combining cognitive and neuroimaging data from a longitudinal perspective should be enhanced in order to gain knowledge on the neurodevelopmental/neurodegenerative hypothesis of CNS involvement. These studies should include HC samples and representative samples of the various disease forms, which would allow for clarifying the distinct profiles in each phenotype. Further, more than two timepoints should be included, starting from as soon as the first symptoms appear. This would potentially help to delineate natural cognitive and brain trajectories of the disease from its onset, and could help to detect cognitive, clinical, and biomarkers of disease progression, which will in turn provide crucial information for clinical trials.

CONCLUSIONS

Cognition in DM1:

- Deficits in social cognition tasks demanding higher order cognitive processes such as ToM, might be secondary to the global cognitive impairment frequently associated with DM1.

- In contrast, affective aspects of social cognition, such as facial emotion recognition, emerge as a primary deficit independent of global cognitive functioning and genetic load. Further, such deficits could be a marker of age-related decline in DM1.

- In contrast with normal ageing, DM1 patients present their own trajectory of cognitive decline, which is domain-specific, rather than global, with visuospatial/visuoconstructive abilities showing the greatest vulnerability.

Structural brain involvement in DM1:

- DM1 is characterized by global GM and WM atrophy, as well as global WM integrity impairment. The regional GM loss depicts a pattern of greater fronto-temporo-parietal and subcortical impairment.

- The extension of GM damage, which exceeds WM integrity impairment, questions the previously proposed predominance of white matter impairment in DM1. So far, DM1 could be understood as a disease with both GM and WM involvement; and CNS involvement in DM1 could be linked to a complex neuronal network disruption.

- The involvement of a multimodal network is noteworthy and constitutes a potential basis of the impairment observed in higher order cognitive functions.

- Longitudinal brain changes suggest that CNS involvement in DM1 is of a developmental nature. Additionally, a potential neurodegenerative process could be expected.

- Pediatric and adult/late onset DM1 patients show distinctive patterns of brain damage and progressive deterioration, supporting the recently proposed classification of DM1.

- The impairment in pediatric onset DM1 patients is far more severe. In these patients, GM atrophy starts in subcortical regions and progresses into frontal areas, while in adult/late onset DM1 patients, atrophy remains in subcortical regions.

- The potential neurodegenerative process could start earlier in life for pediatric DM1 patients (from the thirties onwards) and later in life for adult/late DM1 patients (not before the age of 55).

Clinical and neuropsychological markers of brain damage in DM1:

- Age, CTG expansion size, muscular impairment and neuropsychological performance are vulnerability predictors of the severity of cross-sectional CNS involvement.

Neuropsychological assessment in DM1

- Neuropsychological assessment tools emerge as strong but unspecific predictors of GM damage in DM1. In particular, performance on visuoconstructive and executive function tests, along with IQ estimates, need to be considered as potential indicators of brain abnormalities in DM1.

- As a translational contribution from research to the field of clinical neuropsychology, the inclusion of block design and ROCF tests in cognitive assessment protocols is highly recommended, not only when depicting the cognitive profile of patients at a given time-point, but also when assessing the possibility of a cognitive decline.

REFERENCES

Alsaggaf, R., St. George, D. M. M., Zhan, M., Pfeiffer, R. M., Wang, Y., Wagner, K. R., Greene, M. H., Amr, S., & Gadalla, S. M. (2018). Cancer Risk in Myotonic Dystrophy Type I: Evidence of a Role for Disease Severity. *JNCI Cancer Spectrum*, *2*(4), pky052. https://doi.org/10.1093/jncics/pky052

Angeard, N., Huerta, E., Jacquette, A., Cohen, D., Xavier, J., Gargiulo, M., Servais, L., Eymard, B., & Héron, D. (2018). Childhood-onset form of myotonic dystrophy type 1 and autism spectrum disorder: Is there comorbidity? *Neuromuscular Disorders*, *28*(3), 216-221. https://doi.org/10.1016/j.nmd.2017.12.006

Angeard, N., Gargiulo, M., Jacquette, A., Radvanyi, H., Eymard, B., & Héron, D. (2007). Cognitive profile in childhood myotonic dystrophy type 1: Is there a global impairment? *Neuromuscular Disorders*, *17*(6), 451-458. https://doi.org/10.1016/j.nmd.2007.02.012

Angeard, N., Jacquette, A., Gargiulo, M., Radvanyi, H., Moutier, S., Eymard, B., & Héron, D. (2011). A new window on neurocognitive dysfunction in the childhood form of myotonic dystrophy type 1 (DM1). *Neuromuscular Disorders*, *21*(7), 468-476. https://doi.org/10.1016/j.nmd.2011.04.009

Antonini, G, Mainero, C., Romano, A., Giubilei, F., Ceschin, V., Gragnani, F., Morino, S., Fiorelli, M., Soscia, F., Di Pasquale, A., & Caramia, F. (2004). Cerebral atrophy in myotonic dystrophy: A voxel based morphometric study. *Journal of Neurology, Neurosurgery & Psychiatry*, *75*(11), 1611-1613. https://doi.org/10.1136/jnnp.2003.032417

Antonini, G., Soscia, F., Giubilei, F., De Carolis, A., Gragnani, F., Morino, S., Ruberto, A., & Tatarelli, R. (2006). Health-related quality of life in myotonic dystrophy type 1 and its relationship with cognitive and emotional functioning. *Journal of Rehabilitation Medicine*, *38*(3), 181-185. https://doi.org/10.1080/16501970500477967

Ashburner, J., & Friston, K. J. (2000). Voxel-Based Morphometry—The Methods. *NeuroImage*, *11*(6), 805-821. https://doi.org/10.1006/nimg.2000.0582

Auvin, S., Irwin, J., Abi-Aad, P., & Battersby, A. (2018). The Problem of Rarity: Estimation of Prevalence in Rare Disease. *Value in Health*, *21*(5), 501-507. https://doi.org/10.1016/j.jval.2018.03.002

Baldanzi, S., Cecchi, P., Fabbri, S., Pesaresi, I., Simoncini, C., Angelini, C., Bonuccelli, U., Cosottini, M., & Siciliano, G. (2016). Relationship between neuropsychological impairment and grey and white matter changes in adult-onset myotonic dystrophy type 1. *NeuroImage. Clinical*, *12*, 190-197. https://doi.org/10.1016/j.nicl.2016.06.011

Benton, A., Sivan, A., Hamsher, K., Varney, N. R., & Spreen, O. (1994). *Contributions to neuropsychological assessment: A clinical manual*. Oxford University Press.

Bertrand, J. A., Jean, S., Laberge, L., Gagnon, C., Mathieu, J., Gagnon, J. F., & Richer, L. (2015). Psychological characteristics of patients with myotonic dystrophy type 1. *Acta Neurologica Scandinavica*, *132*(1), 49-58. https://doi.org/10.1111/ane.12356

Best, A. F., Hilbert, J. E., Wood, L., Martens, W. B., Nikolenko, N., Marini-Bettolo, C., Lochmüller, H., Rosenberg, P. S., Moxley, R. T., Greene, M. H., & Gadalla, S. M. (2019). Survival patterns and cancer determinants in families with myotonic dystrophy type 1. *European Journal of Neurology*, *26*(1), 58-65. https://doi.org/10.1111/ene.13763

Bird, T. D., Follett, C., & Griep, E. (1983). Cognitive and personality function in myotonic muscular dystrophy. *Journal of Neurology, Neurosurgery & Psychiatry*, *46*(11), 971-980. https://doi.org/10.1136/jnnp.46.11.971

Brook, J. D., McCurrach, M. E., Harley, H. G., Buckler, A. J., Church, D., Aburatani, H., Hunter, K., Stanton, V. P., Thirion, J.-P., Hudson, T., Sohn, R., Zemelman, B., Snell, R. G., Rundle, S. A., Crow, S., Davies, J., Shelbourne, P., Buxton, J., Jones, C., … Housman, D. E. (1992). Molecular basis of myotonic dystrophy: Expansion of a trinucleotide (CTG) repeat at

the 3' end of a transcript encoding a protein kinase family member. *Cell*, *68*(4), 799-808. https://doi.org/10.1016/0092-8674(92)90154-5

Cabada, T., Iridoy, M., Jericó, I., Lecumberri, P., Seijas, R., Gargallo, A., & Gomez, M. (2017). Brain Involvement in Myotonic Dystrophy Type 1: A Morphometric and Diffusion Tensor Imaging Study with Neuropsychological Correlation. *Archives of Clinical Neuropsychology*, *32*(4), 401-412. https://doi.org/10.1093/arclin/acx008

Caillet-Boudin, M.-L., Fernandez-Gomez, F.-J., Tran, H., Dhaenens, C.-M., Buee, L., & Sergeant, N. (2014). Brain pathology in myotonic dystrophy: When tauopathy meets spliceopathy and RNAopathy. *Frontiers in Molecular Neuroscience*, *6*. https://doi.org/10.3389/fnmol.2013.00057

Campione, E., Botta, A., Di Prete, M., Rastelli, E., Gibellini, M., Petrucci, A., Bernardini, S., Novelli, G., Bianchi, L., Orlandi, A., Massa, R., & Terracciano, C. (2017). Cutaneous features of myotonic dystrophy types 1 and 2: Implication of premature aging and vitamin D homeostasis. *Neuromuscular Disorders*, *27*(2), 163-169. https://doi.org/10.1016/j.nmd.2016.11.004

Caramia, F., Mainero, C., Gragnani, F., Tinelli, E., Fiorelli, M., Ceschin, V., Pantano, P., Bucci, E., Barra, V., Bozzao, L., & Antonini, G. (2010). Functional MRI changes in the central motor system in myotonic dystrophy type 1. *Magnetic Resonance Imaging*, *28*(2), 226-234. https://doi.org/10.1016/j.mri.2009.07.006

Casals-Coll, M., Sánchez-Benavides, G., Quintana, M., Manero, R. M., Rognoni, T., Calvo, L., Palomo, R., Aranciva, F., Tamayo, F., & Peña-Casanova, J. (2013). Estudios normativos españoles en población adulta joven (proyecto NEURONORMA jóvenes): Normas para los test de fluencia verbal. *Neurología*, *28*(1), 33-40. https://doi.org/10.1016/j.nrl.2012.02.010

Caso, F., Agosta, F., Peric, S., Rakočević-Stojanović, V., Copetti, M., Kostic, V. S., & Filippi, M. (2014). Cognitive impairment in myotonic dystrophy type 1 is associated with white matter damage. *PLoS ONE*, *9*(8), 1-8. https://doi.org/10.1371/journal.pone.0104697

Castellano-Guerrero, A. M., Guerrero, R., Relimpio, F., Losada, F., Mangas, M. A., Pumar, A., & Martínez-Brocca, M. A. (2018). Prevalence and predictors of depression and anxiety in adult patients with type 1 diabetes in tertiary care setting. *Acta Diabetologica*, *55*(9), 943-953. https://doi.org/10.1007/s00592-018-1172-5

Conforti, R., de Cristofaro, M., Cristofano, A., Brogna, B., Sardaro, A., Tedeschi, G., Cirillo, S., & Di Costanzo, A. (2016). Brain MRI abnormalities in the adult form of myotonic dystrophy type 1: A longitudinal case series study. *The Neuroradiology Journal*, *29*(1), 36-45. https://doi.org/10.1177/1971400915621325

Dahlqvist, J. R., Ørngreen, M. C., Witting, N., & Vissing, J. (2015). Endocrine function over time in patients with myotonic dystrophy type 1. *European Journal of Neurology*, *22*(1), 116-122. https://doi.org/10.1111/ene.12542

De Antonio, M., Dogan, C., Hamroun, D., Mati, M., Zerrouki, S., Eymard, B., Katsahian, S., & Bassez, G. (2016). Unravelling the myotonic dystrophy type 1 clinical spectrum: A systematic registry-based study with implications for disease classification. *Revue Neurologique*, *172*(10), 572-580. https://doi.org/10.1016/j.neurol.2016.08.003

de Die-Smulders, C., Höweler, C. J., Thijs, C., Mirandolle, J. F., Anten, H. B., Smeets, H. J., Chandler, K. E., & Geraedts, J. P. (1998). Age and causes of death in adult-onset myotonic dystrophy. *Brain*, *121*(8), 1557-1563. https://doi.org/10.1093/brain/121.8.1557

Douaud, G., Smith, S., Jenkinson, M., Behrens, T., Johansen-Berg, H., Vickers, J., James, S., Voets, N., Watkins, K., Matthews, P. M., & James, A. (2007). Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain*, *130*(9), 2375-2386. https://doi.org/10.1093/brain/awm184

Douniol, M., Jacquette, A., Cohen, D., Bodeau, N., Rachidi, L., Angeard, N., Cuisset, J.-M., Vallée, L., Eymard, B., Plaza, M., Héron, D., & Guilé, J.-M. (2012). Psychiatric and cognitive phenotype of childhood myotonic dystrophy type 1: Psychiatric and Cognitive Phenotype of Childhood Myotonic Dystrophy. *Developmental Medicine & Child Neurology*, *54*(10), 905-911. https://doi.org/10.1111/j.1469-8749.2012.04379.x

Ekman, P., & Friesen, W. V. (1976). *Pictures of Facial Affect*. Consulting Psychologists Press.

Ekström, A.-B., Hakenäs-Plate, L., Samuelsson, L., Tulinius, M., & Wentz, E. (2008). Autism spectrum conditons in myotonic dystrophy type 1: A study on 57 individuals with congenital and childhood forms. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *147B*(6), 918-926. https://doi.org/10.1002/ajmg.b.30698

Ekström, A.-B., Hakenäs-Plate, L., Tulinius, M., & Wentz, E. (2009). Cognition and adaptive skills in myotonic dystrophy type 1: A study of 55 individuals with congenital and childhood forms. *Developmental Medicine & Child Neurology*, *51*(12), 982-990. https://doi.org/10.1111/j.1469-8749.2009.03300.x

Fernandez-Gomez, F., Tran, H., Dhaenens, C.-M., Caillet-Boudin, M.-L., Schraen-Maschke, S., Blum, D., Sablonnière, B., Buée-Scherrer, V., Buee, L., & Sergeant, N. (2019). Myotonic Dystrophy: An RNA Toxic Gain of Function Tauopathy? En A. Takashima, B. Wolozin, & L. Buee (Eds.), *Tau Biology* (Vol. 1184, pp. 207-216). Springer Singapore. https://doi.org/10.1007/978-981-32-9358-8_17

Fernández-Torrón, R., García-Puga, M., Emparanza, J.-I., Maneiro, M., Cobo, A.-M., Poza, J.-J., Espinal, J.-B., Zulaica, M., Ruiz, I., Martorell, L., Otaegui, D., Matheu, A., & López de Munain, A. (2016). Cancer risk in DM1 is sex-related and linked to miRNA-200/141 downregulation. *Neurology*, *87*(12), 1250-1257. https://doi.org/10.1212/WNL.0000000000003124

Franc, D. T., Muetzel, R. L., Robinson, P. R., Rodriguez, C. P., Dalton, J. C., Naughton, C. E., Mueller, B. A., Wozniak, J. R., Lim, K. O., & Day, J. W. (2012). Cerebral and muscle MRI

abnormalities in myotonic dystrophy. *Neuromuscular Disorders*, *22*(6), 483-491. https://doi.org/10.1016/j.nmd.2012.01.003

Fujino, H., Shingaki, H., Suwazono, S., Ueda, Y., Wada, C., Nakayama, T., Takahashi, M. P., Imura, O., & Matsumura, T. (2018). Cognitive impairment and quality of life in patients with myotonic dystrophy type 1: Cognition & QoL in DM1. *Muscle & Nerve*, *57*(5), 742-748. https://doi.org/10.1002/mus.26022

Fukuda, H., Horiguchi, J., Ono, C., Ohshita, T., Takaba, J., & Ito, K. (2005). Diffusion tensor imaging of cerebral white matter in patients with myotonic dystrophy. *Acta Radiologica*, *46*(1), 104-109. https://doi.org/10.1080/02841850510015974

Gagnon, C., Heatwole, C., Hébert, L. J., Hogrel, J.-Y., Laberge, L., Leone, M., Meola, G., Richer, L., Sansone, V., & Kierkegaard, M. (2018). Report of the third outcome measures in myotonic dystrophy type 1 (OMMYD-3) international workshop Paris, France, June 8, 2015. *Journal of Neuromuscular Diseases*, *5*(4), 523-537. https://doi.org/10.3233/JND-180329

Gagnon, C., Meola, G., Hébert, L. J., Laberge, L., Leone, M., & Heatwole, C. (2015). Report of the second Outcome Measures in Myotonic Dystrophy type 1 (OMMYD-2) international workshop San Sebastian, Spain, October 16, 2013. *Neuromuscular Disorders*, *25*(7), 603-616. https://doi.org/10.1016/j.nmd.2015.01.008

Gagnon, C., Meola, G., Hébert, L. J., Puymirat, J., Laberge, L., & Leone, M. (2013). Report of the first Outcome Measures in Myotonic Dystrophy type 1 (OMMYD-1) international workshop. *Neuromuscular Disorders*, *23*(12), 1056-1068. https://doi.org/10.1016/j.nmd.2013.07.004

Gallais, B., Gagnon, C., Mathieu, J., & Richer, L. (2017). Cognitive decline over time in adults with myotonic dystrophy type 1: A 9-year longitudinal study. *Neuromuscular Disorders*, *27*(1), 61-72. https://doi.org/10.1016/j.nmd.2016.10.003

Gliem, C., Minnerop, M., Roeske, S., Gärtner, H., Schoene-Bake, J.-C., Adler, S., Witt, J.-A., Hoffstaedter, F., Schneider-Gold, C., Betz, R. C., Helmstaedter, C., Tittgemeyer, M., Amunts, K., Klockgether, T., Weber, B., & Kornblum, C. (2019). Tracking the brain in myotonic dystrophies: A 5-year longitudinal follow-up study. *PLOS ONE*, *14*(3), e0213381. https://doi.org/10.1371/journal.pone.0213381

Golden, C. J. (2001). *STROOP. Color and word test* (3rd ed.). TEA Ediciones.

Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N. A., Friston, K. J., & Frackowiak, R. S. J. (2001). A voxel-based morphometric study of ageing in 465 normal adult human brains. *NeuroImage*, *14*(1 I), 21-36. https://doi.org/10.1006/nimg.2001.0786

Gourdon, G., & Meola, G. (2017). Myotonic Dystrophies: State of the Art of New Therapeutic Developments for the CNS. *Frontiers in Cellular Neuroscience*, *11*, 101. https://doi.org/10.3389/fncel.2017.00101

Groh, W. J., Groh, M. R., Saha, C., Kincaid, J. C., Simmons, Z., Ciafaloni, E., Pourmand, R., Otten, R. F., Bhakta, D., Nair, G. V., Marashdeh, M. M., Zipes, D. P., & Pascuzzi, R. M. (2008). Electrocardiographic Abnormalities and Sudden Death in Myotonic Dystrophy Type 1. *New England Journal of Medicine*, *358*(25), 2688-2697. https://doi.org/10.1056/NEJMoa062800

Hamilton, M. J., Mclean, J., Cumming, S., Ballantyne, B., Mcghie, J., Jampana, R., Longman, C., Evans, J. J., Monckton, D. G., & Farrugia, M. E. (2018). Outcome Measures for Central Nervous System Evaluation in Myotonic Dystrophy Type 1 May Be Confounded by Deficits in Motor Function or Insight. *Frontiers in Neurology*, *9*, 1-15. https://doi.org/10.3389/fneur.2018.00780

Heaton, R. K., Chelune, G. J., Talley, J. L., Kay, G. G., & Curtiss, G. (2001). *Test de clasificación de tarjetas de Wisconsin*. TEA Ediciones.

Hilbert, J. E., Barohn, R. J., Clemens, P. R., Luebbe, E. A., Martens, W. B., McDermott, M. P., Parkhill, A. L., Tawil, R., Thornton, C. A., & Moxley, R. T. (2017). High frequency of

gastrointestinal manifestations in myotonic dystrophy type 1 and type 2. *Neurology*, *89*(13), 1348-1354. https://doi.org/10.1212/WNL.0000000000004420

Ho, G., Carey, K. A., Cardamone, M., & Farrar, M. A. (2019). Myotonic dystrophy type 1: Clinical manifestations in children and adolescents. *Archives of Disease in Childhood*, *104*(1), 48-52. https://doi.org/10.1136/archdischild-2018-314837

Huml, R. A. (Ed.). (2015). *Muscular Dystrophy*. Springer International Publishing. https://doi.org/10.1007/978-3-319-17362-7

Itoh, K., Mitani, M., Kawamoto, K., Futamura, N., Funakawa, I., Jinnai, K., & Fushiki, S. (2010). Neuropathology does not Correlate with Regional Differences in the Extent of Expansion of CTG Repeats in the Brain with Myotonic Dystrophy Type 1. *Acta Histochemica et Cytochemica*, *43*(6), 149-156. https://doi.org/10.1267/ahc.10019

Jean, S., Richer, L., Laberge, L., & Mathieu, J. (2014). Comparisons of intellectual capacities between mild and classic adult-onset phenotypes of myotonic dystrophy type 1 (DM1). *Orphanet Journal of Rare Diseases*, *9*(1), 186. https://doi.org/10.1186/s13023-014-0186-5

Kaufman, A. S. (1997). *K-BIT: Test Breve deinteligenciade Kaufman*. TEA Ediciones.

Kobayakawa, M., Tsuruya, N., & Kawamura, M. (2012). Theory of mind impairment in adult-onset myotonic dystrophy type 1. *Neuroscience Research*, *72*(4), 341-346. https://doi.org/10.1016/j.neures.2012.01.005

Kobayakawa, M., Tsuruya, N., Takeda, A., Suzuki, A., & Kawamura, M. (2010). Facial emotion recognition and cerebral white matter lesions in myotonic dystrophy type 1. *Journal of the Neurological Sciences*, *290*(1-2), 48-51. https://doi.org/10.1016/j.jns.2009.11.011

Kuo, H.-C., Hsieh, Y.-C., Wang, H.-M., Chuang, W.-L., & Huang, C.-C. (2007). Correlation among subcortical white matter lesions, intelligence and CTG repeat expansion in classic myotonic dystrophy type 1. *Acta Neurologica Scandinavica*, *0*(0), 070902114930001-??? https://doi.org/10.1111/j.1600-0404.2007.00911.x

Laberge, L., Gallais, B., Auclair, J., Dauvilliers, Y., Mathieu, J., & Gagnon, C. (2020). Predicting daytime sleepiness and fatigue: A 9-year prospective study in myotonic dystrophy type 1. *Journal of Neurology*, *267*(2), 461-468. https://doi.org/10.1007/s00415-019-09592-7

Langbehn, K. E., Plas, E., Moser, D. J., Long, J. D., Gutmann, L., & Nopoulos, P. C. (2020). Cognitive function and its relationship with brain structure in myotonic dystrophy type 1. *Journal of Neuroscience Research*, jnr.24595. https://doi.org/10.1002/jnr.24595

Lezak, M., Howieson, H., & Loring, D. (2004). *Neuropsychological assessment* (4th ed.). Oxford University Press.

Lindeblad, G., Kroksmark, A., & Ekström, A. (2019). Cognitive and adaptive functioning in congenital and childhood forms of myotonic dystrophy type 1: A longitudinal study. *Developmental Medicine & Child Neurology*, *61*(10), 1214-1220. https://doi.org/10.1111/dmcn.14161

López de Munain, A. L., Blanco, A., Emparanza, J. I., Poza, J. J., Masso, J. F. M., Cobo, A., Martorell, L., Baiget, M., & Lage, J. M. M. (1993). Prevalence of myotonic dystrophy in Guipuzcoa (Basque Country, Spain). *Neurology*, *43*(8), 1573-1573. https://doi.org/10.1212/WNL.43.8.1573

López-Pérez, B., Fernández-Pinto, I., & Abad, F. (2008). *TECA: Test de empatía cognitiva y afectiva.* TEA Ediciones.

Maas, O., & Paterson, A. S. (1937). Mental changes in families affected by dystrophia myotonica. *The Lancet*, *229*(5914), 21-23. https://doi.org/10.1016/S0140-6736(00)86562-9

Macniven, J. A. B., Graham, N. L., Davies, R. R., & Wilson, B. A. (2005). A 5-year follow-up study of an atypical case of myotonic dystrophy. *Brain Injury*, *19*(14), 1213-1221. https://doi.org/10.1080/02699050500283509

Marchini, C., Lonigro, R., Verriello, L., Pellizzari, L., Bergonzi, P., & Damante, G. (2001). Correlations between individual clinical manifestations and CTG repeat amplification in

myotonic dystrophy: Genotype-phenotype correlation in myotonic dystrophy. *Clinical Genetics*, *57*(1), 74-82. https://doi.org/10.1034/j.1399-0004.2000.570112.x

Martorell, L. (1998). Progression of somatic CTG repeat length heterogeneity in the blood cells of myotonic dystrophy patients. *Human Molecular Genetics*, *7*(2), 307-312. https://doi.org/10.1093/hmg/7.2.307

Mateos-Aierdi, A. J., Goicoechea, M., Aiastui, A., Fernández-Torrón, R., Garcia-Puga, M., Matheu, A., & de Munain, A. L. (2015). Muscle wasting in myotonic dystrophies: A model of premature aging. *Frontiers in Aging Neuroscience*, *7*. https://doi.org/10.3389/fnagi.2015.00125

Mathieu, J., Boivin, H., Meunier, D., Gaudreault, M., & Begin, P. (2001). Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. *Neurology*, *56*(3), 336-340. https://doi.org/10.1212/WNL.56.3.336

Mathieu, J., De Braekeleer, M., & Prevost, C. (1990). Genealogical reconstruction of myotonic dystrophy in the Saguenay-Lac-Saint- Jean area (Quebec, Canada). *Neurology*, *40*(5), 839-839. https://doi.org/10.1212/WNL.40.5.839

Mathieu, J., & Prévost, C. (2012). Epidemiological surveillance of myotonic dystrophy type 1: A 25-year population-based study. *Neuromuscular Disorders*, *22*(11), 974-979. https://doi.org/10.1016/j.nmd.2012.05.017

Meinke, P., Hintze, S., Limmer, S., & Schoser, B. (2018). Myotonic Dystrophy—A Progeroid Disease? *Frontiers in Neurology*, *9*, 601. https://doi.org/10.3389/fneur.2018.00601

Meola, G, Sansone, V., Perani, D., Scarone, S., Cappa, S., Dragoni, C., Cattaneo, E., Cotelli, M., Gobbo, C., Fazio, F., Siciliano, G., Mancuso, M., Vitelli, E., Zhang, S., Krahe, R., & Moxley, R. T. (2003). Executive dysfunction and avoidant personality trait in myotonic dystrophy type 1 (DM-1) and in proximal myotonic myopathy (PROMM/DM-2). *Neuromuscular Disorders*, *13*(10), 813-821. https://doi.org/10.1016/S0960-8966(03)00137-8

Meola, G., & Sansone, V. (2007). Cerebral involvement in myotonic dystrophies. *Muscle & Nerve*, *36*(3), 294-306. https://doi.org/10.1002/mus.20800

Miller, E. N. (1990). *CalCAP: California Computerized Assessment Package*. Norland Software.

Minnerop, M., Weber, B., Schoene-Bake, J.-C., Roeske, S., Mirbach, S., Anspach, C., Schneider-Gold, C., Betz, R. C., Helmstaedter, C., Tittgemeyer, M., Klockgether, T., & Kornblum, C. (2011). The brain in myotonic dystrophy 1 and 2: Evidence for a predominant white matter disease. *Brain*, *134*(12), 3530-3546. https://doi.org/10.1093/brain/awr299

Mizukami, K., Sasaki, M., Baba, A., Suzuki, T., & Shiraishi, H. (1999). An autopsy case of myotonic dystrophy with mental disorders and various neuropathologic features. *Psychiatry and Clinical Neurosciences*, *53*(1), 51-55. https://doi.org/10.1046/j.1440-1819.1999.00470.x

Mladenovic, J., Pekmezovic, T., Todorovic, S., Rakocevic-Stojanovic, V., Savic, D., Romac, S., & Apostolski, S. (2006). Survival and mortality of myotonic dystrophy type 1 (Steinert's disease) in the population of Belgrade. *European Journal of Neurology*, *13*(5), 451-454. https://doi.org/10.1111/j.1468-1331.2006.01261.x

Modoni, A., Silvestri, G., Vita, M. G., Quaranta, D., Tonali, P. A., & Marra, C. (2008). Cognitive impairment in myotonic dystrophy type 1 (DM1): A longitudinal follow-up study. *Journal of Neurology*, *255*(11), 1737-1742. https://doi.org/10.1007/s00415-008-0017-5

Modoni, A., Silvestri, G., Grazia Pomponi, M., Mangiola, F., Tonali, P. A., & Marra, C. (2004). Characterization of the Pattern of Cognitive Impairment in Myotonic Dystrophy Type 1. *Archives of Neurology*, *61*(12), 1943-1947. https://doi.org/10.1001/archneur.61.12.1943

Okkersen, K., Buskes, M., Groenewoud, J., Kessels, R. P. C., Knoop, H., van Engelen, B., & Raaphorst, J. (2017). The cognitive profile of myotonic dystrophy type 1: A systematic review and meta-analysis. *Cortex*, *95*, 143-155. https://doi.org/10.1016/j.cortex.2017.08.008

Okkersen, K., Monckton, D. G., Le, N., Tuladhar, A. M., Raaphorst, J., & van Engelen, B. G. M. (2017). Brain imaging in myotonic dystrophy type 1: A systematic review. *Neurology*, *89*(9), 960-969. https://doi.org/10.1212/WNL.0000000000004300

Ono, S., Takahashi, K., Jinnai, K., Kanda, F., Fukuoka, Y., Kurisaki, H., Mitake, S., Inagaki, T., Yamano, T., & Nagao, K. (1998). Loss of serotonin-containing neurons in the raphe of patients with myotonic dystrophy: A quantitative immunohistochemical study and relation to hypersomnia. *Neurology*, *50*(2), 535-538. https://doi.org/10.1212/WNL.50.2.535

Ono, S., Takahashi, K., Jinnai, K., Kanda, F., Fukuoka, Y., Kurisaki, H., Mitake, S., Inagaki, T., Yamano, T., Shimizu, N., & Nagao, K. (1998). Loss of catecholaminergic neurons in the medullary reticular formation in myotonic dystrophy. *Neurology*, *51*(4), 1121-1124. https://doi.org/10.1212/WNL.51.4.1121

Ørngreen, M. C., Arlien-Søborg, P., Duno, M., Hertz, J. M., & Vissing, J. (2012). Endocrine function in 97 patients with myotonic dystrophy type 1. *Journal of Neurology*, *259*(5), 912-920. https://doi.org/10.1007/s00415-011-6277-5

Ota, M., Sato, N., Ohya, Y., Aoki, Y., Mizukami, K., Mori, T., & Asada, T. (2006). Relationship between diffusion tensor imaging and brain morphology in patients with myotonic dystrophy. *Neuroscience Letters*, *407*(3), 234-239. https://doi.org/10.1016/j.neulet.2006.08.077

Passeri, E., Bugiardini, E., Sansone, V. A., Valaperta, R., Costa, E., Ambrosi, B., Meola, G., & Corbetta, S. (2013). Vitamin D, parathyroid hormone and muscle impairment in myotonic dystrophies. *Journal of the Neurological Sciences*, *331*(1-2), 132-135. https://doi.org/10.1016/j.jns.2013.06.008

Pena-Casanova, J., Quinones-Ubeda, S., Gramunt-Fombuena, N., Quintana-Aparicio, M., Aguilar, M., Badenes, D., Cerulla, N., Molinuevo, J. L., Ruiz, E., Robles, A., Barquero, M. S., Antunez, C., Martinez-Parra, C., Frank-Garcia, A., Fernandez, M., Alfonso, V., Sol, J.

M., Blesa, R., & for the NEURONORMA Study Team. (2009). Spanish Multicenter Normative Studies (NEURONORMA Project): Norms for Verbal Fluency Tests. *Archives of Clinical Neuropsychology*, *24*(4), 395-411. https://doi.org/10.1093/arclin/acp042

Peric, S., Mandic-Stojmenovic, G., Markovic, I., Stefanova, E., Ilic, V., Parojcic, A., Misirlic-Dencic, S., Ostojic, M., Rakocevic-Stojanovic, V., & Kostic, V. (2014). Cerebrospinal fluid biomarkers of neurodegeneration in patients with juvenile and classic myotonic dystrophy type 1. *European Journal of Neurology*, *21*(2), 231-237. https://doi.org/10.1111/ene.12237

Peric, S., Rakocevic Stojanovic, V., Mandic Stojmenovic, G., Ilic, V., Kovacevic, M., Parojcic, A., Pesovic, J., Mijajlovic, M., Savic-Pavicevic, D., & Meola, G. (2017). Clusters of cognitive impairment among different phenotypes of myotonic dystrophy type 1 and type 2. *Neurological Sciences*, *38*(3), 415-423. https://doi.org/10.1007/s10072-016-2778-4

Perini, G. I., Menegazzo, E., Ermani, M., Zara, M., Gemma, A., Ferruzza, E., Gennarelli, M., & Angelini, C. (1999). Cognitive impairment and (CTG)n expansion in myotonic dystrophy patients. *Biological Psychiatry*, *46*(3), 425-431. https://doi.org/10.1016/S0006-3223(99)00016-5

Rakocevic-Stojanovic, V., Peric, S., Madzarevic, R., Dobricic, V., Ralic, V., Ilic, V., Basta, I., Nikolic, A., & Stefanova, E. (2014). Significant impact of behavioral and cognitive impairment on quality of life in patients with myotonic dystrophy type 1. *Clinical Neurology and Neurosurgery*, *126*, 76-81. https://doi.org/10.1016/j.clineuro.2014.08.021

Rey, A. (2009). *Copy and memory reproduction test of complex geometric figures*. TEA Ediciones.

Rubinsztein, J. S., Rubinsztein, D. C., McKenna, P. J., Goodburn, S., & Holland, A. J. (1997). Mild myotonic dystrophy is associated with memory impairment in the context of normal

general intelligence. *Journal of Medical Genetics*, *34*(3), 229-233. https://doi.org/10.1136/jmg.34.3.229

Salehi, L. B., Bonifazi, E., Stasio, E. D., Gennarelli, M., Botta, A., Vallo, L., Iraci, R., Massa, R., Antonini, G., Angelini, C., & Novelli, G. (2007). Risk Prediction for Clinical Phenotype in Myotonic Dystrophy Type 1: Data from 2,650 Patients. *Genetic Testing*, *11*(1), 84-90. https://doi.org/10.1089/gte.2006.0511

Sansone, V., Gandossini, S., Cotelli, M., Calabria, M., Zanetti, O., & Meola, G. (2007). Cognitive impairment in adult myotonic dystrophies: A longitudinal study. *Neurological Sciences*, *28*(1), 9-15. https://doi.org/10.1007/s10072-007-0742-z

Schneider-Gold, C., Bellenberg, B., Prehn, C., Krogias, C., Schneider, R., Klein, J., Gold, R., & Lukas, C. (2015). Cortical and Subcortical Grey and White Matter Atrophy in Myotonic Dystrophies Type 1 and 2 Is Associated with Cognitive Impairment, Depression and Daytime Sleepiness. *PLOS ONE*, *10*(6), e0130352. https://doi.org/10.1371/journal.pone.0130352

Sergeant, N., Sablonnière, B., Schraen-Maschke, S., Ghestem, A., Maurage, C. A., Wattez, A., Vermersch, P., & Delacourte, A. (2001). Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1. *Human Molecular Genetics*, *10*(19), 2143-2155. https://doi.org/10.1093/hmg/10.19.2143

Serra, L., Bianchi, G., Bruschini, M., Giulietti, G., Domenico, C. D., Bonarota, S., Petrucci, A., Silvestri, G., Perna, A., Meola, G., Caltagirone, C., & Bozzali, M. (2020). Abnormal Cortical Thickness Is Associated With Deficits in Social Cognition in Patients With Myotonic Dystrophy Type 1. *Frontiers in Neurology*, *11*, 113. https://doi.org/10.3389/fneur.2020.00113

Serra, L., Cercignani, M., Bruschini, M., Cipolotti, L., Mancini, M., Silvestri, G., Petrucci, A., Bucci, E., Antonini, G., Licchelli, L., Spanò, B., Giacanelli, M., Caltagirone, C., Meola, G., & Bozzali, M. (2016). “I Know that You Know that I Know”: Neural Substrates

Associated with Social Cognition Deficits in DM1 Patients. *PLOS ONE*, *11*(6), e0156901. https://doi.org/10.1371/journal.pone.0156901

Serra, L., Mancini, M., Silvestri, G., Petrucci, A., Masciullo, M., Spanò, B., Torso, M., Mastropasqua, C., Giacanelli, M., Caltagirone, C., Cercignani, M., Meola, G., & Bozzali, M. (2016). Brain Connectomics' Modification to Clarify Motor and Nonmotor Features of Myotonic Dystrophy Type 1. *Neural Plasticity*, *2016*, 1-10. https://doi.org/10.1155/2016/2696085

Serra, L., Petrucci, A., Spanò, B., Torso, M., Olivito, G., Lispi, L., Costanzi-Porrini, S., Giulietti, G., Koch, G., Giacanelli, M., Caltagirone, C., Cercignani, M., & Bozzali, M. (2015). How genetics affects the brain to produce higher-level dysfunctions in myotonic dystrophy type 1. *Functional Neurology*, *30*(1), 21-31.

Serra, L., Silvestri, G., Petrucci, A., Basile, B., Masciullo, M., Makovac, E., Torso, M., Spanò, B., Mastropasqua, C., Harrison, N. A., Bianchi, M. L. E., Giacanelli, M., Caltagirone, C., Cercignani, M., & Bozzali, M. (2014). Abnormal Functional Brain Connectivity and Personality Traits in Myotonic Dystrophy Type 1. *JAMA Neurology*, *71*(5), 603. https://doi.org/10.1001/jamaneurol.2014.130

Sistiaga, A., Urreta, I., Jodar, M., Cobo, A. M., Emparanza, J., Otaegui, D., Poza, J. J., Merino, J. J., Imaz, H., Martí-Massó, J. F., & López de Munain, A. (2010). Cognitive/personality pattern and triplet expansion size in adult myotonic dystrophy type 1 (DM1): CTG repeats, cognition and personality in DM1. *Psychological Medicine*, *40*(3), 487-495. https://doi.org/10.1017/S0033291709990602

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL.

NeuroImage, *23*(SUPPL. 1), 208-219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Smith, S. M., Zhang, Y., Jenkinson, M., Chen, J., Matthews, P. M., Federico, A., & De Stefano, N. (2002). Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *NeuroImage*, *17*(1), 479-489. https://doi.org/10.1006/nimg.2002.1040

Spaziani, M., Semeraro, A., Bucci, E., Rossi, F., Garibaldi, M., Papassifachis, M. A., Pozza, C., Anzuini, A., Lenzi, A., Antonini, G., & Radicioni, A. F. (2019). Hormonal and metabolic gender differences in a cohort of myotonic dystrophy type 1 subjects: A retrospective, case–control study. *Journal of Endocrinological Investigation*, *43*, 663-675. https://doi.org/10.1007/s40618-019-01156-w

Stokes, M., Varughese, N., Iannaccone, S., & Castro, D. (2019). Clinical and genetic characteristics of childhood-onset myotonic dystrophy. *Muscle & Nerve*, *60*(6), 732-738. https://doi.org/10.1002/mus.26716

Sugiyama, A., Sone, D., Sato, N., Kimura, Y., Ota, M., Maikusa, N., Maekawa, T., Enokizono, M., Mori-Yoshimura, M., Ohya, Y., Kuwabara, S., & Matsuda, H. (2017). Brain gray matter structural network in myotonic dystrophy type 1. *PLOS ONE*, *12*(11), e0187343. https://doi.org/10.1371/journal.pone.0187343

Takeda, A., Kobayakawa, M., Suzuki, A., Tsuruya, N., & Kawamura, M. (2009). Lowered sensitivity to facial emotions in myotonic dystrophy type 1. *Journal of the Neurological Sciences*, *280*(1-2), 35-39. https://doi.org/10.1016/j.jns.2009.01.014

Tanaka, H., Arai, M., Harada, M., Hozumi, A., & Hirata, K. (2012). Cognition and event-related potentials in adult-onset non-demented myotonic dystrophy type 1. *Clinical Neurophysiology*, *123*(2), 261-269. https://doi.org/10.1016/j.clinph.2011.06.012

Theadom, A., Rodrigues, M., Roxburgh, R., Balalla, S., Higgins, C., Bhattacharjee, R., Jones, K., Krishnamurthi, R., & Feigin, V. (2014). Prevalence of Muscular Dystrophies: A

Systematic Literature Review. *Neuroepidemiology*, *43*(3-4), 259-268. https://doi.org/10.1159/000369343

Thornton, C. A. (2014). Myotonic Dystrophy. *Neurologic Clinics*, *32*(3), 705-719. https://doi.org/10.1016/j.ncl.2014.04.011

Toth, A., Lovadi, E., Komoly, S., Schwarcz, A., Orsi, G., Perlaki, G., Bogner, P., Sebok, A., Kovacs, N., Pal, E., & Janszky, J. (2015). Cortical involvement during myotonia in myotonic dystrophy: An fMRI study. *Acta Neurologica Scandinavica*, *132*(1), 65-72. https://doi.org/10.1111/ane.12360

Tuikka, R. A., Laaksonen, R. K., & Somer, H. V. K. (1993). Cognitive Function in Myotonic Dystrophy: A Follow-Up Study. *European Neurology*, *33*(6), 436-441. https://doi.org/10.1159/000116989

Turner, C., & Hilton-Jones, D. (2010). The myotonic dystrophies: Diagnosis and management. *Journal of Neurology, Neurosurgery & Psychiatry*, *81*(4), 358-367. https://doi.org/10.1136/jnnp.2008.158261

Turnpenny, P., Clark, C., & Kelly, K. (1994). Intelligence quotient profile in myotonic dystrophy, intergenerational deficit, and correlation with CTG amplification. *Journal of Medical Genetics*, *31*(4), 300-305. https://doi.org/10.1136/jmg.31.4.300

van der Velden, B. G., Okkersen, K., Kessels, R. P., Groenewoud, J., van Engelen, B., Knoop, H., & Raaphorst, J. (2019). Affective symptoms and apathy in myotonic dystrophy type 1 a systematic review and meta-analysis. *Journal of Affective Disorders*, *250*, 260-269. https://doi.org/10.1016/j.jad.2019.03.036

van Dorst, M., Okkersen, K., Kessels, R. P. C., Meijer, F. J. A., Monckton, D. G., van Engelen, B. G. M., Tuladhar, A. M., & Raaphorst, J. (2019). Structural white matter networks in myotonic dystrophy type 1. *NeuroImage: Clinical*, *21*, 101615. https://doi.org/10.1016/j.nicl.2018.101615

Vanacore, N., Rastelli, E., Antonini, G., Bianchi, M. L. E., Botta, A., Bucci, E., Casali, C., Costanzi-Porrini, S., Giacanelli, M., Gibellini, M., Modoni, A., Novelli, G., Pennisi, E. M., Petrucci, A., Piantadosi, C., Silvestri, G., Terracciano, C., & Massa, R. (2016). An Age-Standardized Prevalence Estimate and a Sex and Age Distribution of Myotonic Dystrophy Types 1 and 2 in the Rome Province, Italy. *Neuroepidemiology*, *46*(3), 191-197. https://doi.org/10.1159/000444018

Vanier, T. M. (1960). Dystrophia Myotonica in Childhood. *BMJ*, *2*(5208), 1284-1288. https://doi.org/10.1136/bmj.2.5208.1284

Wahbi, K., & Furling, D. (2020). Cardiovascular manifestations of myotonic dystrophy. *Trends in Cardiovascular Medicine*, *30*(4), 232-238. https://doi.org/10.1016/j.tcm.2019.06.001

Weber, Y. G., Roebling, R., Kassubek, J., Hoffmann, S., Rosenbohm, A., Wolf, M., Steinbach, P., Jurkat-Rott, K., Walter, H., Reske, S. N., Lehmann-Horn, F., Mottaghy, F. M., & Lerche, H. (2010). Comparative analysis of brain structure, metabolism, and cognition in myotonic dystrophy 1 and 2. *Neurology*, *74*(14), 1108-1117. https://doi.org/10.1212/WNL.0b013e3181d8c35f

Wechsler, D. (1999). *WAIS-III: Escala de Inteligencia de Wechsler para Adultos III*. TEA Ediciones.

Wenninger, S., Montagnese, F., & Schoser, B. (2018). Core Clinical Phenotypes in Myotonic Dystrophies. *Frontiers in Neurology*, *9*, 303. https://doi.org/10.3389/fneur.2018.00303

Wilson, B. A., Balleny, H., Patterson, K., & Hodges, J. R. (1999). Myotonic Dystrophy and Progressive Cognitive Decline: A Common Condition or Two Separate Problems? *Cortex*, *35*(1), 113-121. https://doi.org/10.1016/S0010-9452(08)70789-5

Winblad, S., Hellström, P., Lindberg, S. H., & Hansen, S. (2006). Facial emotion recognition in myotonic dystrophy type 1 correlates with CTG repeat expansion. *Journal of Neurology, Neurosurgery & Psychiatry*, *77*(2), 219-223. https://doi.org/10.1136/jnnp.2005.070763

Winblad, S., Lindberg, C., & Hansen, S. (2005). Temperament and character in patients with classical myotonic dystrophy type 1 (DM-1). *Neuromuscular Disorders*, *15*(4), 287-292. https://doi.org/10.1016/j.nmd.2004.12.003

Winblad, S., Lindberg, C., & Hansen, S. (2006). Cognitive deficits and CTG repeat expansion size in classical myotonic dystrophy type 1 (DM1). *Behavioral and Brain Functions*, *2*(1), 16. https://doi.org/10.1186/1744-9081-2-16

Winblad, S., Månsson, J. E., Blennow, K., Jensen, C., Samuelsson, L., & Lindberg, C. (2008). Cerebrospinal fluid tau and amyloid β42 protein in patients with myotonic dystrophy type 1. *European Journal of Neurology*, *15*(9), 947-952. https://doi.org/10.1111/j.1468-1331.2008.02217.x

Winblad, S., Samuelsson, L., Lindberg, C., & Meola, G. (2016). Cognition in myotonic dystrophy type 1: A 5-year follow-up study. *European Journal of Neurology*, *23*(9), 1471-1476. https://doi.org/10.1111/ene.13062

Woo, J., Lee, H.-W., & Park, J.-S. (2019). Differences in the pattern of cognitive impairments between juvenile and adult onset myotonic dystrophy type 1. *Journal of Clinical Neuroscience*, *68*, 92-96. https://doi.org/10.1016/j.jocn.2019.07.029

Wozniak, J. R., Mueller, B. A., Bell, C. J., Muetzel, R. L., Lim, K. O., & Day, J. W. (2013). Diffusion tensor imaging reveals widespread white matter abnormalities in children and adolescents with myotonic dystrophy type 1. *Journal of Neurology*, *260*(4), 1122-1131. https://doi.org/10.1007/s00415-012-6771-4

Wozniak, J. R., Mueller, B. A., Lim, K. O., Hemmy, L. S., & Day, J. W. (2014). Tractography reveals diffuse white matter abnormalities in Myotonic Dystrophy Type 1. *Journal of the Neurological Sciences*, *341*(1-2), 73-78. https://doi.org/10.1016/j.jns.2014.04.005

Wozniak, J. R., Mueller, B. A., Ward, E. E., Lim, K. O., & Day, J. W. (2011). White matter abnormalities and neurocognitive correlates in children and adolescents with

myotonic dystrophy type 1: A diffusion tensor imaging study. *Neuromuscular Disorders*, *21*(2), 89-96. https://doi.org/10.1016/j.nmd.2010.11.013

Zaki, M., Boyd, P. A., Impey, L., Roberts, A., & Chamberlain, P. (2007). Congenital myotonic dystrophy: Prenatal ultrasound findings and pregnancy outcome. *Ultrasound in Obstetrics and Gynecology*, *29*(3), 284-288. https://doi.org/10.1002/uog.3859

Zalonis, I., Bonakis, A., Christidi, F., Vagiakis, E., Papageorgiou, S. G., Kalfakis, N., Manta, P., & Vassilopoulos, D. (2010). Toward Understanding Cognitive Impairment in Patients with Myotonic Dystrophy Type 1. *Archives of Clinical Neuropsychology*, *25*(4), 303-313. https://doi.org/10.1093/arclin/acq016

Zanigni, S., Evangelisti, S., Giannoccaro, M. P., Oppi, F., Poda, R., Giorgio, A., Testa, C., Manners, D. N., Avoni, P., Gramegna, L. L., De Stefano, N., Lodi, R., Tonon, C., & Liguori, R. (2016). Relationship of white and gray matter abnormalities to clinical and genetic features in myotonic dystrophy type 1. *NeuroImage: Clinical*, *11*, 678-685. https://doi.org/10.1016/j.nicl.2016.04.012

SECTION II

ANNEXES

SUMMARY

This section presents the studies that emerged from the current thesis. Specifically, there are four scientific manuscripts, three of which have been published in JCR-indexed journals during the course of the work presented here. The quality indicators are provided for each piece of work:

Published studies resulting from the retrospective analysis:

Study #1:

Labayru, G., Arenzana, I., Aliri, J., Zulaica, M., López de Munain, A. & Sistiaga, A (2018). Social cognition in Myotonic Dystrophy Type 1: Specific or secondary impairment? *PLoS ONE, 13,* 1–11. https://doi.org/10.1371/journal.pone.0204227

Quality indicators 2018:

- Impact factor: 2.776
- Category: Multidisciplinary Sciences
    - Quartile: Q2
    - Rank: 24/69

Study #2:

Labayru, G., Diez, I., Sepulcre, J., Fernández, E., Zulaica, M., Cortés, J.M., López de Munain, A. & Sistiaga, A. (2019). Regional brain atrophy in gray and white matter is associated with cognitive impairment in Myotonic Dystrophy type 1. *NeuroImage: Clinical, 24*, 102078. https://doi.org/10.1016/j.nicl.2019.102078

Quality indicators 2018:

- Impact factor: 3.943
- Category: Neuroimaging
    - Quartile: Q1
    - Rank: 3/14

Studies resulting from the prospective analysis:

Study #3 (published):

Labayru, G., Aliri, J., Zulaica, M., López de Munain, A., & Sistiaga A. (2019). Age-related cognitive decline in myotonic dystrophy type 1: An 11-year longitudinal follow-up study. *Journal of Neuropsychology*. https://doi.org/10.1111/jnp.12192

Quality indicators 2019:

- Impact factor: 2.468
- Category: Psychology
    - Quartile: Q2
    - Rank: 31/77

Study #4 (under review):

Labayru, G., Jiménez-Marin, A., Fernández, E., Villanua, J., Zulaica, M., Cortés, J.M., Díez, I., Sepulcre, J., López de Munain, A., & Sistiaga, A. (under review). Trajectories of brain degeneration in pediatric and adult/late DM1: A follow-up MRI study over a decade.

Journal: Annals of Clinical and Translational Neurology

Quality indicators 2018:

- Impact factor: 4.656
- Category: Clinical Neurology
    - Quartile: Q1
    - Rank: 29/199
- Category: Neurosciences
    - Quartile: Q1
    - Rank: 55/267

PUBLISHED MANUSCRIPTS

Annex 1

PLOS ONE

RESEARCH ARTICLE

Social cognition in myotonic dystrophy type 1: Specific or secondary impairment?

Garazi Labayru[1,2☯], Irati Arenzana[3☯], Jone Aliri[4], Miren Zulaica[1], Adolfo López de Munain[1,5], Andone Sistiaga A.[1,2*]

1 Neuroscience Area, Biodonostia Research Institute, Donostia-San Sebastian, Gipuzkoa, Spain, **2** Personality, Assessment and Psychological Treatment Department, Psychology Faculty, University of the Basque Country (UPV/EHU), Donostia-San Sebastian, Gipuzkoa, Spain, **3** Master's in Child and Adult Neuropsychology, Autonoma University of Barcelona, Spain, **4** Department of Social Psychology and Methodology of Behavioral Sciences, University of the Basque Country (UPV/EHU), Donostia-San Sebastian, Gipuzkoa, Spain, **5** Neurology Department, Donostia University Hospital, Donostia-San Sebastian, Gipuzkoa, Spain

☯ These authors contributed equally to this work.
* andone.sistiaga@ehu.eus

OPEN ACCESS

Citation: Labayru G, Arenzana I, Aliri J, Zulaica M, López de Munain A, Sistiaga A. A (2018) Social cognition in myotonic dystrophy type 1: Specific or secondary impairment? PLoS ONE 13(9): e0204227. https://doi.org/10.1371/journal.pone.0204227

Editor: Mel McKendrick, Heriot-Watt University School of Social Sciences, UNITED KINGDOM

Received: December 5, 2017

Accepted: September 5, 2018

Published: September 24, 2018

Data Availability Statement: All excel files are available from the DANS (Data Archiving and Networked Services) database (DOI: http://dx.doi.org/10.17026/dans-xwd-xyza).

Funding: The present study has been supported by grants of the Instituto de Salud Carlos III co-founded by Fondo Europeo de Desarrollo Regional - FEDER (Ref: PI17/01231).

Competing interests: The authors have declared that no competing interests exist.

Abstract

Aims

The cognitive profile of Myotonic Dystrophy type 1 (DM1) has been described in recent decades. Moreover, DM1 patients show lowered social engagement and difficulties in social-cognitive functions. The aim of the present study is to explore whether social cognition impairment is present in DM1 taking into account the overall cognitive condition.

Method

38 patients and a control group paired in age and gender participated in the study. All the participants had an IQ within the normal range. Subjects were administered an abbreviated neuropsychological battery which comprised a facial emotion recognition test (POFA) and Faux Pas Test, as well as a self-report questionnaire on cognitive and affective empathy (TECA).

Results

Statistically significant differences were found only for facial emotion recognition ($U = 464.0$, $p = .006$) with a moderate effect size (.31), with the controls obtaining a higher score than the patients. Analyzing each emotion separately, DM1 patients scored significantly lower than controls on the recognition of anger and disgust items. Emotion recognition did not correlate with genetic load, but did correlate negatively with age. No differences were found between patients and controls in any of the other variables related to Theory of Mind (ToM) and empathy.

Conclusion

DM1 does not manifest specific impairments in ToM since difficulties in this area predominantly rely on the cognitive demand of the tasks employed. However, a more basic process

such as emotion recognition appears as a core deficit. The role of this deficit as a marker of aging related decline is discussed.

Introduction

Myotonic dystrophy type 1 (DM1) is a slowly progressive muscular dystrophy characterized by multisystemic involvement [1]. As the most prevalent neuromuscular disorder, it shows a particularly high-prevalence focus in Gipuzkoa (North of Spain), reaching 300 cases per million inhabitants [2].

Previous genotype–phenotype correlation studies in DM1 have reported an association between the CTG repeat size (the molecular defect characterizing DM1) and certain clinical features, such as cognitive impairment and muscular weakness, as well as the age of onset [3–5]. In this regard, there are data supporting the possibility that the expansion size correlates with the cognitive impairment, particularly if the whole DM1 spectrum is taken into account, which ranges from intellectual disability (congenital DM1) to the presence of subtle cognitive impairments [6,7].

Whilst the existence of cognitive impairment in DM1 is not in question, there is yet no consensus on the exact pattern of alteration. Some authors point to attentional, memory, and language deficits [3,8,9], suggesting a pattern of frontal lobe degeneration. Others have found results consistent with a pattern of visuospatial/constructive and executive function impairment [6,7,10,11].

In comparison with other neuromuscular diseases, patients with DM1 have a more serious functional disability and greater dependency in daily activities [12]. In addition, these patients show lower social engagement, more psychosocial problems, and poorer psychosocial well-being [13–16]. Likewise, in the DM1 population, particularly among congenital and childhood forms, comorbidity has been described with Autism Spectrum Disorder (ASD) [17], a disorder where social function is known to be compromised due to documented social cognition deficits.

Social cognition refers to a set of cognitive functions that enable a person to properly understand and interact in social situations. In other words, social cognition includes all processes implicated in the perception, interpretation, and generation of a response when facing the behaviours, interests, or intentions of others. It includes emotion perception, empathy, Theory of Mind (ToM) or mentalizing and attributional style. The theoretical framework of social cognition is still to be settled, but a trend exists towards distinguishing a more affective form of processing (ability to perceive and understand others' emotional states) from another, more cognitive, form of processing (ToM and perspective-taking) [18]. In this regard, it is common to find studies assessing social cognition where a clear distinction is made, not only in conceptual terms but also based on neurobiological facts; emotion recognition (ventral stream) and the so-called Theory of Mind (dorsal pathway) [19]. ToM refers to the ability to perceive and understand other people's behaviour, knowledge, intentions, emotions, and beliefs. A neuroimaging study has found damage to the dorsolateral frontal cortex and amygdala in DM1 [20], with other authors suggesting that in social cognition tasks in the general population, both structures are involved, along with other frontal structures [21].

In this regard, to date there have been relatively few studies addressing the role of social cognition in DM1, with most of this work having focused on facial emotion recognition [22–24]. Beyond the recognition of facial emotions, two studies have examined ToM in DM1

[20,25], both of which have led to the conclusion that ToM is affected in patients with DM1. Further, difficulties in facial memory ability associated with reduced visuo-constructive and visual memory ability have also been described in DM1 [26].

However, it is still unclear whether these difficulties are symptoms of the disease *per se* or are secondary to a more global general cognitive impairment [6]. In some conditions, such as intellectual disability, problems with social judgement, risk assessment, self-management, emotions or interpersonal relationships have been reported [27]. Though, social abilities in this group are associated with a more global impairment rather than being a specific characteristic of the illness. Indeed, a recent review points out that the high comorbidity found between DM1 and ASD might partly be explained by the underlying low IQ of patients in the sample [28], and therefore, the authors recommend to methodologically control this variable.

The aim of this study is to explore social cognition in DM1, taking into account certain cognitive mediator variables such as IQ, in order to clarify whether difficulties in this area are specific to DM1 or secondary to difficulties in other areas. Clarification of this issue will have important implications for the educational and social interventions directed towards improving the quality of life in this population.

Method

This study has the approval of the Ethics Committee of the Donostia University Hospital, and written informed consent for participation and publication has been obtained from all participants.

Participants

This study included a total of 38 volunteer patients with DM1 (19 women and 19 men) who were invited to participate in the study according to the consecutive order in which they attended the Neuromuscular Unit of the Neurology Service at Donostia Hospital. All patients had a molecular confirmation of the illness, and the patients' clinical form was determined on the basis of the age of onset [29]. A control group of 38 subjects was recruited, paired in age (maximum allowed deviation ±5 years) and gender with the patient group. This group was composed of non-affected relatives of patients and healthy volunteers.

To address the objective of the study, inclusion criteria related to a normal IQ was set, in order to avoid the impact of an overall cognitive impairment on the outcome of social cognition tasks.

The inclusion criteria for both groups were (1) to be aged between 18 and 68 years (2) to have an IQ in the normal range (equal or over 85) at the time of assessment (3) the absence of any other neurological or psychiatric illness, and (4) abstinence from drugs or alcohol consumption.

Materials

Neuromuscular assessment.

1. Muscular Impairment Rating Scale [30], is a 5 point ordinal scale aimed at assessing the severity of distal and proximal muscular weakness in DM1. Patients' data on this scale were retrieved from medical records.

Neuropsychological assessment. The neuropsychological assessment lasted approximately one hour. Some of the tests were used in an abbreviated form of the original test, in order to prevent the patients from the well-documented fatigue they suffer [31], which is

known to have an impact on cognitive outcomes. Both a measure of overall cognitive functioning and a measure of attention performance were included:

1. Kaufman Brief Intelligence Test (K-BIT) [32]. This brief IQ estimation test was administered through its two scales: verbal (vocabulary subtest) and non-verbal (Matrices subtest) intelligence.
2. Digit span subtest from the Wechsler Adult Intelligence Scale (WAIS-III) [33]. This is a measure of immediate attention in which participants are requested to repeat sequences of numbers in both forward and backward recall conditions.

The tests below were used to assess three aspects of social cognition: emotion recognition using the Pictures of Facial Affect test, ToM using the Faux Pas test, and empathy using the Test of Cognitive and Affective Empathy.

1. Pictures of Facial Affect (POFA) [34]. This test was administered in order to assess the recognition of facial emotions. An abbreviated form of the original test, previously used in DM1 patients and composed of 28 pictures with a high rate of identification (80%) was employed in this study [24]. The stimuli used in this task included six men and seven women, expressing six basic emotions (happiness, sadness, fear, anger, disgust, surprise) and a neutral state, with each emotion being shown four times. All responses were elicited from the participants in a forced choice manner. Each correct response received 1 point. The psychometric properties of the POFA are largely assumed, since its construction was based on an extremely elaborate and highly reliable and valid system for determining and labelling the intensity of facial expressions of affect (FACS) [35].
2. The Faux Pas test [36]. This test assesses the ability of subjects to identify situations in which someone mistakenly says something they should not have ("*faux pas*"). Half of the 20 original stories were used; 5 faux pas and 5 control stories. Firstly, participants were requested to state whether a faux pas occurred or not. In the case of an affirmative answer, five open-ended questions were asked in order to assess subjects' comprehension of the inappropriateness, intentions, and beliefs that a story character holds as well as his or her feelings. In the case of a negative answer, two open-ended control questions were asked. A correct response to each question received 1 point. Regarding the validity of the Faux-Pas Test, research has indicated that patients suffering mental illnesses or frontal lesions are typically impaired in their understanding of faux pas [37–40].
3. The Test of Cognitive and Affective Empathy (TECA) [41]. This is a 33-item self-report scale measuring both cognitive and affective aspects of empathy on a Likert-type five-point response format that ranged from 1 (totally disagree) to 5 (totally agree). The instrument assesses two affective dimensions of empathy (empathetic distress and empathetic joy, with 16 items) and two cognitive dimensions (perspective taking and emotional understanding, with 17 items). Scores on affective and cognitive empathy range from 16 to 80 and from 17 to 85 respectively. Regarding the validity of the TECA, it has shown strong convergent validity with high correlations with the Questionnaire Measure of Emotional Empathy (QMEE; [42]) and with the Spanish adaptation of the Interpersonal Reactivity Index (IRI; [43]).

Statistical analysis

Firstly, group equivalence regarding age, years of study, and neuropsychological functioning was tested. Secondly, inter-group comparisons were conducted on social cognition measures.

To do so, medians, interquartile ranges, Mann-Whitney *U* test and r effect size [44] were calculated when data distributional properties did not meet the assumptions for parametric statistical methods. When assumptions were met, means, standard deviations (SD), Student's *t* test and Cohen's d effect size [45] were calculated. We consider .20, .50, and .80 as small, medium, and large effect sizes when interpreting Cohen's *d*, and .10, .30, .50 as small, moderate, and large effect sizes when interpreting *r*.

Correlation analyses were carried out for those social cognition measures with statistically significant differences between groups, in order to check for relations between those measures and CTG expansion size.

Additionally, correlations between age and social cognition measures were conducted separately in patients and controls, in order to look for any markers of aging related decline. Finally, correlations between IQ and social cognition measures were carried out for the whole sample, in order to analyse whether selected social cognition tests are sensitive to IQ in a sample with limited IQ range.

Correlations were calculated using Pearson's *r* when variables met the normality assumption and Spearman's rho when they did not.

Results

No significant differences were found between patients and controls in terms of age, educational level, and cognitive functioning (IQ and attentional span) (Table 1).

The patients had a CTG expansion size ranging from 65 to 1667 and a mean score in MIRS of 2.27 (SD: 0.932). In our sample, 12 (31.6%) of the DM1 cases were juvenile onset, 21 (55.3%) were adult onset, and 5 (13.2%) were partial or late onset. Regarding inheritance, 86.8% inherited the illness from the father.

According to the differences on the social cognition tasks, significant differences were found only in the overall score of the POFA test with a moderate effect size (r = 0.31), with the controls obtaining a higher score than the patients (Tables 2 and 3).

Further analysis of the participants' responses on the POFA revealed that patients showed a significantly lower score than the control group in the recognition of two emotions: anger and disgust ($U = 439.0$, $p < .001$, $r = 0.39$; $U = 430.0$, $p < .001$, $r = 0.37$, respectively). Table 4 shows frequencies and percentages of the number of correct answers given to each emotion by patients and controls. There we can see that 47.4% of healthy controls and 7.9% of patients correctly answered the four items of anger, whilst 63.2% of healthy controls and 31.6% of patients correctly answered the four items of disgust. An examination of the error pattern did not reveal a specific response pattern for these emotions in the DM1 group. In other words, anger and disgust were not systematically mistaken for another emotion. No correlation was

Table 1. Socio-demographic and cognitive functioning data.

	Control Group (n = 38) Median (IQR)	Patient Group (n = 38) Median (IQR)	Mann Whitney *U*	*p*	*r*
Socio-demographic data					
Age	45.50 (37.75–54.00)	45.50 (37.75–53.25)	698.0	.803	0.029
Years of study	15.00 (11.75–17.00)	15.50 (11.00–18.00)	648.5	.442	0.088
Cognitive functioning					
IQ (K-BIT)	114.50 (104.00–124.00)	108.00 (101.00–117.25)	542.5	.062	0.214
Digit span (WAIS-III)	11.00 (9.00–15.00)	10.00 (7.00–15.00)	633.0	.353	0.106

IQR: interquartile range. Raw scores are presented except for IQ (standardized score X = 100; SD = 15)

https://doi.org/10.1371/journal.pone.0204227.t001

Table 2. Differences in POFA and Faux Pas test between controls and patients.

	Control Group (n = 38) Median (IQR)	Patient Group (n = 38) Median (IQR)	Mann Whitney *U*	*p*	*r*
POFA	24.00 (23.00–26.00)	23.00 (22.00–24.00)	460.0**	.006	0.317
Faux Pas					
Control	38.00 (35.00–39.00)	37.50 (33.75–39.00)	712.5	.920	0.011
ToM	24.00 (19.00–31.50)	25.00 (21.00–30.50)	689.0	.731	0.039
Total	61.50 (56.00–67.00)	62.00 (56.00–68.25)	695.5	.783	0.032

IQR: interquartile range. Raw scores are presented.

**Statistically significant $p < 0.01$

https://doi.org/10.1371/journal.pone.0204227.t002

Table 3. Differences in TECA between controls and patients.

	Control Group (n = 38) Mean (SD)	Patient Group (n = 38) Mean (SD)	Student *t*	*p*	*d*
TECA					
Perspective taking	53.26 (9.55)	50.71 (11.22)	1.068	.289	0.245
Emotional understanding	51.13 (12.30)	50.42 (9.25)	0.285	.777	0.065
Empathetic distress	51.92 (10.35)	50.24 (8.45)	0.777	.440	0.178
Empathetic joy	55.21 (10.55)	52.00 (11.39)	1.275	.206	0.292
Total	54.24 (9.91)	50.61 (8.95)	1.677	.098	0.384

SD: Standard deviation. Standardized scores are presented (X = 50; SD = 10).

https://doi.org/10.1371/journal.pone.0204227.t003

found between the POFA total score and the number of CTG repeats (Spearman's rho = .146, $p = .380$).

Additionally, the results regarding the relationship between age and social cognition measures showed that there was a statistically significant negative correlation with POFA scores, but only in the patient group (Spearman's rho = -.35; $p = .03$ in the patient group; Spearman's rho = .15; $p = .37$ in the control group). No other outcome measure showed a statistically significant correlation with age, in either patients or in controls.

Finally, the results of the correlation between outcome measures and IQ revealed a statistically significant relationship between IQ and POFA scores (Spearman's rho = .25; $p = .028$) and Faux Pas Test scores (Spearman's rho = .26; $p = .022$), but not between IQ and TECA.

Table 4. Frequencies (f) and percentages (%) of correct answers for each emotion of the POFA test.

	Control group					Patient group				
	Number of correct answers					Number of correct answers				
Emotion	**0 f(%)**	**1 f(%)**	**2 f(%)**	**3 f(%)**	**4 f(%)**	**0 f(%)**	**1 f(%)**	**2 f(%)**	**3 f(%)**	**4 f(%)**
Happiness	0(0)	0(0)	0(0)	0(0)	38(100)	0(0)	0(0)	0(0)	0(0)	38(100)
Sadness	0(0)	2(5.3)	8(21.1)	15(39.5)	13(34.2)	0(0)	0(0)	7(18.4)	18(47.4)	13(34.2)
Fear	2(5.3)	2(5.3)	7(18.4)	7(18.4)	20(52.6)	2(5.3)	4(10.5)	4(10.5)	14(36.8)	14(36.8)
Anger	0(0)	2.6(1)	5.3(2)	44.7(17)	47.4(18)	0(0)	5.3(2)	7.9(3)	78.9(30)	7.9(3)
Disgust	0(0)	0(0)	10.5(4)	26.3(10)	63.2(24)	0(0)	5.3(2)	36.8(14)	26.3(10)	31.6(12)
Surprise	0(0)	0(0)	7.9(3)	23.7(9)	68.4(26)	0(0)	5.3(2)	13.2(5)	13.2(5)	68.4(26)
Neutral	0(0)	0(0)	2.6(1)	31.6(12)	65.8(25)	0(0)	0(0)	5.3(2)	28.9(11)	65.8(25)

https://doi.org/10.1371/journal.pone.0204227.t004

Discussion

The results of this study, which aimed to analyse social cognition in patients with DM1, have partially ruled out the presence of specific difficulties in these abilities for this group of patients, when including only participants whose IQ is in the normal range. DM1 patients did not show specific difficulties in social cognition tasks which demand higher order cognitive processes (i.e. ToM tasks), but performed worse than controls on a task where a less cognitively demanding inference is required (facial emotion recognition).

It has been suggested that the difficulties shown by the DM1 population in social participation may be related to social cognition abilities, and several studies have emerged examining this topic in DM1. In particular, limitations in the ability to recognize facial emotions [22–24,46], as well as ToM and empathy deficits [20,25], have been reported. Nonetheless, these studies have some methodological limitations (small sample sizes, lack of a control group, or absence of an overall cognitive impairment measure) that could explain the differences with respect to the results obtained in our study, in which we have tried to overcome these limitations.

The patients in our study did not show poorer performance than the controls on either the Faux Pas Test or the TECA. To the best of our knowledge, only a few studies have employed other tools apart from facial emotion recognition in DM1. These studies have assessed social cognition through the Faux Pas test [22,25] and the Theory of Mind test (a modified Italian version of the Happé's Strange Stories test) [20]. In the Faux Pas test, researchers found DM1 patients to be less sensitive to the emotional impact of a faux pas, whilst in contrast, patients failed on the more cognitive ToM test.

Similar to previous studies, our results confirm that DM1 patients have difficulties in facial emotion recognition tasks, specifically in anger and disgust, where patients' scores are significantly lower than controls. This same result was reported by Kobayakawa and colleagues [22,46], using the Reading the Mind in the Eyes Test (RMET). Similarly, Takeda et al. [23] and Winblad et al. [24] reported lower scores in the DM1 group for anger and disgust, and additionally found differences in the recognition of fear.

Contrary to the findings of Winblad et al. [24], the failure to find a correlation between scores on the POFA test and CTG expansion size in our study could be taken to suggest that facial emotion recognition deficit is a specific deficit affecting DM1 patients as a whole group and is not dependent on genetic load. This idea is also supported by the results of Kobayakawa et al. [25] who found no correlation between CTG and RMET. With respect to the relationship between POFA scores and IQ, in our study we found this correlation to be statistically significant (see also Kobayakawa et al., who found a correlation between RMET and IQ [25]). Nonetheless, variations in IQ are unable to account for the significant differences in POFA scores that emerged between the patients and controls in our study. Thus, although sensitive to IQ level, facial emotion recognition impairment is not secondary to cognitive functioning, but instead appears to be inherent to the disease itself.

Taken together, our results—and most of those found in the reviewed literature—point towards a clear impairment in affectively loaded social cognition tasks (emotion recognition), whilst inconsistent results are found when using more cognitively loaded tasks (ToM). Moreover, the latter are usually story-based, which demands the functioning of other processes such as attention, text comprehension, and working memory—functions usually affected in patients with cognitive impairment. Taking into account that the DM1 population has, as a group, a lower IQ in comparison with the general population, one could hypothesize that the social difficulties identified in previous studies are secondary to their limitations at cognitive level [5,6,11]. The lack of results suggesting cognitive social cognition impairment in this study may

possibly be due to the fact that the patients' IQ was normal. This allowed us to avoid the bias that can be produced by general cognitive damage when performing such tasks. Indeed, the self-report was the only measure that did not correlate with IQ, suggesting a high cognitive load in the remaining assessment tools employed.

When looking for an aging effect, a negative correlation was found only between the POFA score and the age of the patients. In the last decade, longitudinal studies supporting the notion of an age-related decline in the DM1 population have emerged [47,48]. More specifically, an age-related decline of frontal and temporal functions has been described [3,8]. Facial emotion recognition impairment is a core and early developing feature in patients with fronto-temporal dementia [49,50]. Our finding that POFA scores are worse in older patients could reinforce the notion of a decline in fronto-temporal functions.

Nevertheless, this study also has certain limitations. First, although excluding low IQ patients from the study has allowed us to analyse social cognition whilst avoiding the bias that this implied, it has also reduced the representativeness of our sample (despite our sample being representative in terms of molecular damage). Additionally, the ecological validity of the tests used is arguable, given that these may not be representative of daily life situations where social cognition skills are required. However, this is a largely unavoidable issue when measuring social abilities with the tools currently available in such artificial contexts. A more natural way of studying the concept may be achieved by including reports or questionnaires from relatives who could provide more information about the adaptability and functionality of the patients being studied.

In summary, the results of this study suggest a more specific impairment of affective aspects of social cognition in DM1, while those social skills with greater cognitive load (at least ToM) may rely on intellectual processes. Therefore, in patients with cognitive deficit, impairment on these tasks could be secondary; a possibility that has already been put forward by other authors who have suggested a role for symptoms such as apathy, which is linked to overall cognitive status [14]. In this regard, our findings raise the question of whether the growing body of evidence on DM1 social cognition impairment should be regarded as a possible reflection of their overall cognitive difficulties.

In future work, it could be of interest to include other possible confounding or mediator variables such as those related to comorbid clinical symptomatology (apathy, depressive symptoms, etc.) or executive dysfunction, as suggested by a recent meta-analysis [51]. This would provide the scientific community with a greater understanding of the reasons why DM1 patients have such difficulties integrating family, work, and social life, and such information could consequently help towards raising their quality of life. Finally, our results suggest the possibility of opening a research discussion on this topic by considering facial emotion recognition as a possible marker of aging-related decline in DM1, as has been established in other types of dementia with which DM1 appears to share features.

Acknowledgments

We are grateful to all the participants in this study, and would like to thank all the staff from the Donostia University Hospital who made this study feasible.

Author Contributions

Conceptualization: Garazi Labayru, Irati Arenzana, Adolfo López de Munain, Andone Sistiaga A.

Data curation: Garazi Labayru, Irati Arenzana, Jone Aliri, Miren Zulaica.

Formal analysis: Garazi Labayru, Jone Aliri.

Investigation: Garazi Labayru, Irati Arenzana, Miren Zulaica.

Methodology: Garazi Labayru, Jone Aliri, Adolfo López de Munain, Andone Sistiaga A.

Project administration: Adolfo López de Munain, Andone Sistiaga A.

Resources: Jone Aliri, Andone Sistiaga A.

Software: Jone Aliri.

Supervision: Adolfo López de Munain, Andone Sistiaga A.

Validation: Andone Sistiaga A.

Visualization: Jone Aliri.

Writing – original draft: Garazi Labayru, Irati Arenzana, Andone Sistiaga A.

Writing – review & editing: Garazi Labayru, Irati Arenzana, Jone Aliri, Miren Zulaica, Adolfo López de Munain, Andone Sistiaga A.

References

1. Harper PS. Myotonic Dystrophy. 2nd ed. Oxford University Press; 2009.
2. López de Munain A, Blanco A, Emparanza JI, Poza JJ, Martí-Massó JF, Cobo AM, et al. Prevalence of myotonic dystrophy in Guipúzcoa (Basque Country, Spain). Neurology. 1993; 43(8):1573–6. PMID: 8351014
3. Modoni a, Silvestri G, Pomponi MG, Mangiola F, Tonali P a, Marra C. Characterization of the pattern of cognitive impairment in myotonic dystrophy type 1. Arch Neurol [Internet]. 2004; 61:1943–7. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15596617
4. Novelli G, Gennarelli M, Menegazzo E, Mostacciuolo M, Pizzuti A, Fattorini C, et al. (CTG)n Triplet mutation and phenotype manifestations in Myotonic Dystrophy patients. Biochem Med Metab Biol. 1993; 50:85–92. PMID: 8373638
5. Perini GI, Menegazzo E, Ermani M, Zara M, Gemma A, Ferruzza E, et al. Cognitive Impairment and (CTG) n Expansion in Myotonic Dystrophy Patients. Biol Psychiatry. 1999; 46:425–31. PMID: 10435210
6. Sistiaga A, Urreta I, Jodar M, Cobo AM, Emparanza J, Otaegui D, et al. Cognitive/personality pattern and triplet expansion size in adult myotonic dystrophy type 1 (DM1): CTG repeats, cognition and personality in DM1. Psychol Med [Internet]. 2010; 40(3):487. Available from: http://www.journals.cambridge.org/abstract_S0033291709990602
7. Winblad S, Lindberg C, Hansen S. Cognitive deficits and CTG repeat expansion size in classical myotonic dystrophy type 1 (DM1). Behav brain Funct [Internet]. 2006; 2:16. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1475858&tool=pmcentrez&rendertype=abstract
8. Modoni A, Silvestri G, Vita MG, Quaranta D, Tonali PA, Marra C. Cognitive impairment in myotonic dystrophy type 1 (DM1): A longitudinal follow-up study. J Neurol. 2008; 255(11):1737–42. https://doi.org/10.1007/s00415-008-0017-5 PMID: 18821050
9. Rubinsztein S, Rubinsztein DC, Mckenna PJ, Goodburn S, Holland AJ. Mild myotonic dystrophy is associated with memory impairment in the context of normal general intelligence. J Med Genet. 1997; 34:229–33. PMID: 9132495
10. Bird TD, Follett C, Griep E, Bird T. Cognitive and personality function in myotonic muscular dystrophy. J Neurol Neurosurgery, Psychiatry. 1983; 46(3):971–80.
11. Malloy P, Mishra SK, Adler SH, Mishra S H Adler SK. Neuropsychological deficits in myotonic muscular dystrophy. J Neurol Neurosurgery, Psychiatry. 1990; 53:1011–3.
12. Nätterlund B, Ahlström G. Activities of daily living and quality of life in persons with muscular dystrophy. J Rehabil Med. 2001; 33(5):206–11. PMID: 11585151
13. Gagnon C, Mathieu J, Jean S, Laberge L, Perron M, Veillette S, et al. Predictors of Disrupted Social Participation in Myotonic Dystrophy Type 1. Arch Phys Med Rehabil. 2008; 89(7):1246–55. https://doi.org/10.1016/j.apmr.2007.10.049 PMID: 18586127

14. Gallais B, Montreuil M, Gargiulo M, Eymard B, Gagnon C, Laberge L. Prevalence and correlates of apathy in myotonic dystrophy type 1. BMC Neurol [Internet]. BMC Neurology; 2015; 15(1):148. Available from: http://bmcneurol.biomedcentral.com/articles/10.1186/s12883-015-0401-6

15. Laberge L, Mathieu J, Auclair J, Gagnon É, Noreau L, Gagnon C. Clinical, psychosocial, and central correlates of quality of life in myotonic dystrophy type 1 patients. Eur Neurol. 2013; 70(5–6):308–15. https://doi.org/10.1159/000353991 PMID: 24158106

16. Laberge L, Veillette S, Mathieu J, Auclair J, Perron M. The correlation of CTG repeat length with material and social deprivation in myotonic dystrophy. Clin Genet. 2007; 71(1):59–66. https://doi.org/10.1111/j.1399-0004.2007.00732.x PMID: 17204048

17. Ekström AB, Hakenäs-Plate L, Samuelsson L, Tulinius M, Wentz E. Autism spectrum conditons in myotonic dystrophy type 1: A study on 57 individuals with congenital and childhood forms. Am J Med Genet Part B Neuropsychiatr Genet. 2008; 147(6):918–26.

18. Badoud D, Schneider M, Menghetti S, Glaser B, Debbané M, Eliez S. Understanding others: a pilot investigation of cognitive and affective facets of social cognition in patients with 22q11.2 deletion syndrome (22q11DS). J Neurodev Disord [Internet]. Journal of Neurodevelopmental Disorders; 2017; 9 (1):35. Available from: http://jneurodevdisorders.biomedcentral.com/articles/10.1186/s11689-017-9216-7 PMID: 28946869

19. Brüne M. Emotion recognition, "theory of mind," and social behavior in schizophrenia. Psychiatry Res. 2005; 133(2–3):135–47. https://doi.org/10.1016/j.psychres.2004.10.007 PMID: 15740990

20. Serra L, Cercignani M, Bruschini M, Cipolotti L, Mancini M, Silvestri G, et al. "I know that you know that I know": Neural substrates associated with social cognition deficits in DM1 patients. PLoS One. 2016; 11 (6):1–13.

21. Adolphs R. The neurobiology of social cognition. Curr Opin Neurobiol. 2001; 11:231–9. PMID: 11301245

22. Kobayakawa M. Social Cognitive Impairment in Myotonic Dystrophy Type 1. Brain Nerve. 2016; 68 (2):137–44. https://doi.org/10.11477/mf.1416200363 PMID: 26873233

23. Takeda A, Kobayakawa M, Suzuki A, Tsuruya N, Kawamura M. Lowered sensitivity to facial emotions in myotonic dystrophy type 1. J Neurol Sci [Internet]. Elsevier B.V.; 2009; 280(1–2):35–9. Available from: http://dx.doi.org/10.1016/j.jns.2009.01.014

24. Winblad S, Hellstrom P, Lindberg C, Hansen S. Facial emotion recognition in myotonic dystrophy type 1 correlates with CTG repeat expansion. J Neurol Neurosurg Psychiatry [Internet]. 2006; 77(2):219–23. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16421126

25. Kobayakawa M, Tsuruya N, Kawamura M. Theory of mind impairment in adult-onset myotonic dystrophy type 1. Neurosci Res [Internet]. Elsevier Ireland Ltd and Japan Neuroscience Society; 2012; 72 (4):341–6. Available from: http://dx.doi.org/10.1016/j.neures.2012.01.005

26. Kleberg JL, Lindberg C, Winblad S. Facial memory deficits in myotonic dystrophy type 1. Acta Neurol Scand. 2014; 130(5):312–8. https://doi.org/10.1111/ane.12228 PMID: 24527956

27. Asociación Americana de Psiquiatría (APA). Manual Diagnóstico y Estadístico de los Trastornos Mentales (DSM-5). 5ª. Ed. Arlington; 2014.

28. Angeard N, Huerta E, Jacquette A, Cohen D, Xavier J, Gargiulo M, et al. Childhood-onset form of myotonic dystrophy type 1 and autism spectrum disorder: Is there comorbidity? Neuromuscul Disord [Internet]. Elsevier B.V.; 2017; 28(3):216–21. Available from: https://doi.org/10.1016/j.nmd.2017.12.006

29. De Antonio M, Dogan C, Hamroun D, Mati M, Zerrouki S, Eymard B, et al. Unravelling the myotonic dystrophy type 1 clinical spectrum: A systematic registry-based study with implications for disease classification. Rev Neurol (Paris). 2016; 172(10):572–80.

30. Mathieu J, Boivin H, Meunier D, Gaudreault M, Bégin P. Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. Neurology. 2001; 56(April 1998):336–40.

31. Kalkman JS, Schillings ML, van der Werf SP, Padberg GW, Zwarts MJ, van Engelen BG, et al. Experienced fatigue in facioscapulohumeral dystrophy, myotonic dystrophy, and HMSN-I. J Neurol Neurosurg Psychiatry [Internet]. 2005; 76(10):1406–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16170086%5Cnhttp://jnnp.bmj.com/content/76/10/1406.full.pdf

32. Kaufman AS. K-BIT: Test breve de inteligencia de Kaufman. Madrid: TEA Ediciones; 1997.

33. Wechsler D. WAIS-III: Escala de Inteligencia de Wechsler para Adultos III. Madrid: TEA Ediciones; 1999.

34. Ekman P, Friesen W V. Pictures of facial affect. Palo Alto: Consulting psychologists press; 1976.

35. Hall JA, Bernieri FJ, Carney DR. Nonverbal behavior and interpersonal sensitivity. In: Harrigan JA, Rosenthal R, Scherer KR, editors. The new handbook of methods in nonverbal behavior research. Oxford: University Press; 2005. p. 237–81.

36. Baron-Cohen S, O'Riordan M, Stone V, Jones R, Plaisted K. Recognition of Faux Pas by Normally Developing Children and Children with Asperger Syndrome or High-Functioning Autism. J Autism Dev Disord. 2000; 30(6):622–3. PMID: 11261477

37. Konstantakopoulos G, Oulis P, Ploumpidis D, Patrikelis P, Nikitopoulou S, Papadimitriou GN, et al. Self-rated and performance-based empathy in schizophrenia: The impact of cognitive deficits. Soc Neurosci. 2014; 9(6):590–600. https://doi.org/10.1080/17470919.2014.934395 PMID: 24968720

38. Spek AA, Scholte EM, Van Berckelaer-Onnes IA. Theory of mind in adults with HFA and asperger syndrome. J Autism Dev Disord. 2010; 40(3):280–9. https://doi.org/10.1007/s10803-009-0860-y PMID: 19763808

39. Zalla T, Sav AM, Stopin A, Ahade S, Leboyer M. Faux pas detection and intentional action in asperger syndrome. A replication on a French sample. J Autism Dev Disord. 2009; 39(2):373–82. https://doi.org/10.1007/s10803-008-0634-y PMID: 18726150

40. Yeh ZT, Lo CY, Tsai MD, Tsai MC. Mentalizing ability in patients with prefrontal cortex damage. J Clin Exp Neuropsychol. 2015; 37(2):128–39. https://doi.org/10.1080/13803395.2014.992864 PMID: 25692803

41. López-Pérez B, Fernández-Pinto I, Abad FJ. TECA: Test de empatía cognitiva y afectiva. Madrid: TEA Ediciones; 2008.

42. Mehrabian A, Epstein N. A measure of emotional empathy. J Pers. 1972; 40(4):525–43. PMID: 4642390

43. Pérez-Albéniz A, De Paúl J, Etxeberría J, Montes MP, Torres E. Adaptación de Interpersonal Reactivity Index (IRI) al español. Psicothema. 2003; 15(2):267–72.

44. Rosenthal R. Meta-Analytic Procedures for Social Research. Newbury Park, C.A.: Sage; 1991.

45. Cohen J. Statistical power analysis for the behavioural sciences. 2nd ed. New York: Academic Press; 1998.

46. Kobayakawa M, Tsuruya N, Takeda A, Suzuki A, Kawamura M. Facial emotion recognition and cerebral white matter lesions in myotonic dystrophy type 1. J Neurol Sci [Internet]. Elsevier B.V.; 2010; 290(1–2):48–51. Available from: http://dx.doi.org/10.1016/j.jns.2009.11.011

47. Winblad S, Samuelsson L, Lindberg C, Meola G. Cognition in myotonic dystrophy type 1: a 5-year follow-up study. Eur J Neurol. 2016; 23(9):1471–6. https://doi.org/10.1111/ene.13062 PMID: 27323306

48. Gallais B, Gagnon C, Mathieu J, Richer L. Cognitive decline over time in adults with myotonic dystrophy type 1: A 9-year longitudinal study. Neuromuscul Disord [Internet]. Elsevier B.V.; 2017; 27(1):61–72. Available from: http://dx.doi.org/10.1016/j.nmd.2016.10.003

49. Bora E, Velakoulis D, Walterfang M. Meta-Analysis of Facial Emotion Recognition in Behavioral Variant Frontotemporal Dementia. J Geriatr Psychiatry Neurol [Internet]. 2016; 29(4):205–11. Available from: http://journals.sagepub.com/doi/10.1177/0891988716640375

50. Goodkind MS, Sturm VE, Ascher EA, Miller BL. Emotion Recognition in Frontotemporal Dementia and Alzheimer's Disease: A New Film-Based Assessment. Emotion. 2015; 15(4):416–27. https://doi.org/10.1037/a0039261 PMID: 26010574

51. Okkersen K, Buskes M, Groenewoud J, Kessels RPC, Knoop H, van Engelen B, et al. The cognitive profile of myotonic dystrophy type 1: A systematic review and meta-analysis [Internet]. Cortex. Elsevier Ltd; Vol. 95 2017. 143–155 p. Available from: http://dx.doi.org/10.1016/j.cortex.2017.08.008

Annex 2

NeuroImage: Clinical 24 (2019) 102078

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Regional brain atrophy in gray and white matter is associated with cognitive impairment in Myotonic Dystrophy type 1

Garazi Labayru[a,b,m,*], Ibai Diez[c,d,e], Jorge Sepulcre[c,d], Esther Fernández[a,f,g], Miren Zulaica[a,b], Jesús M. Cortés[h,i,j], Adolfo López de Munain[a,b,k,l], Andone Sistiaga[a,b,m]

[a] Neuroscience Area, Biodonostia Research Institute, San Sebastián, Gipuzkoa, Spain
[b] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Institute Carlos III, Madrid, Spain
[c] Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States
[d] Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States
[e] Neurotechnology Laboratory, Tecnalia Health Department, Derio, Spain
[f] Osatek, Donostia University Hospital, Donostia-San Sebastian, Gipuzkoa, Spain
[g] Radiolody Department, University of the Basque Country (UPV/EHU), Donostia-San Sebastian, Gipuzkoa, Spain
[h] Biocruces Health Research Institute. Hospital Universitario de Cruces, Barakaldo, Spain
[i] Cell Biology and Histology Department, University of the Basque Country (UPV/EHU), Leioa, Spain
[j] IKERBASQUE, The Basque Foundation for Science, Bilbao, Spain
[k] Neurology Department, Donostia University Hospital, Donostia-San Sebastian, Gipuzkoa, Spain
[l] Neurosciences Department, University of the Basque Country (UPV/EHU), Donostia-San Sebastian, Gipuzkoa, Spain
[m] Personality, Assessment and psychological treatment department; Psychology Faculty, University of the Basque Country (UPV/EHU), San Sebastian, Gipuzkoa, Spain

ARTICLE INFO

Keywords:
Myotonic Dystrophy Type 1
MRI
VBM
DTI
neuropsychology

ABSTRACT

Background: Myotonic Dystrophy type 1 (DM1) is a slowly progressive myopathy characterized by varying multisystemic involvement. Several cerebral features such as brain atrophy, ventricular enlargement, and white matter lesions (WMLs) have frequently been described. The aim of this study is to investigate the structural organization of the brain that defines the disease through multimodal imaging analysis, and to analyze the relation between structural cerebral changes and DM1 clinical and neuropsychological profiles.

Method: 31 DM1 patients and 57 healthy controls underwent an MRI scan protocol, including T1, T2 and DTI. Global gray matter (GM), global white matter (WM), and voxel-level Voxel Based Morphometry (VBM) and voxel-level microstructural WM abnormalities through Diffusion Tensor Imaging (DTI) were assessed through group comparisons and linear regression analysis with age, degree of muscular impairment (MIRS score), CTG expansion size and neuropsychological outcomes from a comprehensive assessment.

Results: Compared with healthy controls, DM1 patients showed a reduction in both global GM and WM volume; and further regional GM decrease in specific primary sensory, multi-sensory and association cortical regions. Fractional anisotropy (FA) was reduced in both total brain and regional analysis, being most marked in frontal, paralimbic, temporal cortex, and subcortical regions. Higher ratings on muscular impairment and longer CTG expansion sizes predicted a greater volume decrease in GM and lower FA values. Age predicted global GM reduction, specifically in parietal regions. At the cognitive level, the DM1 group showed significant negative correlations between IQ estimate, visuoconstructive and executive neuropsychological scores and both global and regional volume decrease, mainly distributed in the frontal, parietal and subcortical regions.

Conclusions: In this study, we describe the structural brain signatures that delineate the involvement of the CNS in DM1. We show that specific sensory and multi-sensory — as well as frontal cortical areas — display potential vulnerability associated with the hypothesized neurodegenerative nature of DM1 brain abnormalities.

1. Introduction

Myotonic Dystrophy type 1 (DM1) is an autosomal dominant disease that is the most common adult-onset muscular dystrophy. The disease is characterized by severe neuromuscular defects, including myotonia and progressive muscle weakness and wasting (atrophy), leading to

* Corresponding author.
E-mail address: garazi.labayru@biodonostia.org (G. Labayru).

https://doi.org/10.1016/j.nicl.2019.102078
Received 3 July 2019; Received in revised form 28 September 2019; Accepted 4 November 2019
Available online 06 November 2019

disability as the disease progresses, and respiratory distress either from primary muscle failure or from cardio-pulmonary complications. It is also characteristically multisystemic and degenerative, affecting the heart and the brain, among other body systems (reviewed in: Harper, 2001; Thornton, 2014). The molecular basis of the disease is the pathogenic expansion of an unstable CTG (cytosine-thymine-guanine) microsatellite in the 3'UTR of the DM protein kinase (DMPK) gene. Epidemiologically, it is the most frequent neuromuscular disorder with a reported prevalence of 1/7400 people worldwide (Harper, 2001), having a significantly higher prevalence in Gipuzkoa (North of Spain), reaching 300 cases per million inhabitants (López de Munain et al., 1993). Although CNS involvement in DM1 was reported a long time ago, implicated brain structures and the cognitive profile of DM1 is still a matter of debate.

Neuroimaging studies have recently emerged, reporting brain abnormalities in DM1 patients. On the whole, these studies have reported that a reduction in the volume of both gray and white matter and increased WM hyperintensities are of widespread occurrence in DM1 brains, along with other functional abnormalities such as abnormal connectivity patterns or reduced cerebral perfusion (Okkersen et al., 2017). However, results have so far failed to characterize the exact pattern of CNS involvement in DM1, and its association with clinical and neuropsychological features of the disease is still inconclusive.

Voxel based morphometry (VBM) is one of the most commonly employed techniques for measuring the loss of gray matter (GM) volume. In DM1, a widespread decrease has been reported across all cortical surface (with the frontal lobe being reduced in all cases) and in some subcortical structures (Antonini et al., 2004; Baldanzi et al., 2016; Caso et al., 2014; Minnerop et al., 2011; Ota et al., 2006; Schneider-Gold et al., 2015; Serra et al., 2015; Weber et al., 2010; Zanigni et al., 2016). Moreover, Diffusion Tensor Imaging (DTI) is been widely documented as a tool for quantifying WM damage. The structural integrity of all major association, projection, and commissural fibers has been reported to be altered in DM1 (Baldanzi et al., 2016; Caso et al., 2014; Fukuda et al., 2005; Minnerop et al., 2011; Ota et al., 2006; Serra et al., 2015; van Dorst et al., 2019; Wozniak et al., 2014, 2013, 2011; Zanigni et al., 2016). The study of the relationships between gray and white matter abnormalities in DM1 will help to elucidate the mechanisms of CNS involvement in the disease, which some have suggested to rely on a disconnection of cortical structures due to WM interruptions (Caso et al., 2014; Minnerop et al., 2011; Zanigni et al., 2016).

From a neuropsychological point of view, various patterns of cognitive impairment have been found across studies, possibly due to methodological discrepancies (such as assessment battery or representativeness of the DM1 population). Overall, studies have reported deficits in attention, memory, and language (Modoni et al., 2008; Rubinsztein et al., 1997), as well as in visuospatial/constructive and executive functions (Sistiaga et al., 2010; Winblad et al., 2006a) and facial emotion recognition (Kobayakawa et al., 2010; Labayru et al., 2018; Winblad et al., 2006b). Some authors have also suggested a pattern of frontal lobe degeneration, whilst others have found results consistent with a pattern of fronto-parietal impairment. Although clinical impressions as well as some recent findings suggest a cognitive decline over time (Gallais et al., 2017; Modoni et al., 2008; Sansone et al., 2007; Winblad et al., 2016), this issue still remains unclear.

The study of neuroimaging correlations with clinical and neuropsychological data is still scarce and the literature has yielded mixed results. Moreover, variations in cognitive assessment tests limit their comparison. The aim of this study is to determine the pattern of structural abnormalities in gray and white matter in juvenile and adult onset DM1 and to analyze the relationship between this pattern and muscular, molecular, and neuropsychological data.

2. Methods

2.1. Participants

The DM1 patients analyzed in this work were selected from those attending the outpatient consultancies at the Neurology Department of the Donostia Hospital (San Sebastian), a tertiary public hospital that serves a population of 650,000 inhabitants (almost all of the Guipuzcoa province). All patients were examined by a neurologist and underwent a neuropsychological assessment close to the MRI acquisition date.

Inclusion criteria for DM1 patients included being between 18 and 70 years old along with molecular confirmation of the clinical diagnosis. Patients were excluded on the basis of the following criteria: congenital or childhood form (considered to be qualitatively distinct phenotypes), history of major psychiatric or somatic disorder (in accordance with DSM-IV criteria), acquired brain damage or alcohol or drug abuse and the presence of corporal paramagnetic body devices (Pacemaker etc.) that could impede MRI studies.

35 DM1 patient with genetically confirmed juvenile ($N = 10$) and adult ($N = 25$) onset DM1 were recruited. Of the sample, 4 patients were excluded after MRI acquisition due to cerebral anomalies: 3 patients presented significant ventricular dilatation suggestive of hydrocephaly according to Evans´ index (44-year-old male with 333 CTG; 41-year-old female with 667 CTG; and 28-year-old female with 500 CTG) and 1 patient presented frontal hyperostosis (36-year-old female with 400 CTG). A final group of 31 DM1 patients (9 juvenile and 22 adult onset) was included for analysis. As a control group, 57 healthy controls (HC) were recruited including unaffected family members and healthy volunteers who met none of the exclusion criteria.

All participants were informed of the objectives and details of the study and gave informed written consent. The study was approved by the Ethics Committee of the Donostia University Hospital.

2.2. Clinical and neuropsychological assessment

Clinical data regarding CTG expansion size, Muscular Impairment Rating Scale (MIRS) score (Mathieu et al., 2001), disease form, and demographic data were extracted from medical records.

All patients underwent an assessment by an experienced neuropsychologist who was blind to the patient's clinical condition (CTG expansion size, clinical form, muscular impairment and MRI results). Neuropsychological assessment included several subtests from the Wechsler Adult Intelligence Scale III (WAIS III) (Wechsler, 1999), including: Digit span, Vocabulary, Block design, Object assembly, Arithmetic and Similarities; some of which were used for estimating IQ from a five-subtest short form based on López et al. (2003). Other cognitive tests used were: Stroop test (Golden, 2001), California Computerized Assessment Package (CALCAP) (Miller, 1990), Raven's progressive matrices (Raven et al., 2001), Rey Auditory Verbal Learning Test (RAVLT) (Lezak et al., 2004), Word Fluency (Casals-Coll et al., 2013; Peña-Casanova et al., 2009), Rey-Osterrieth Complex Figure test (ROCF) (Rey, 2009) and Benton's Judgement of Line Orientation (Benton et al., 1994). The patients' raw scores were converted into standardized T values according to the Spanish population-based norms for each test.

2.3. MRI acquisition

MR scanning was performed on a 1.5 Tesla scanner (Achieva Nova, Philips). The current results are based on a high-resolution volumetric "turbo field echo" (TFE) series (sagital 3D T1 weighted acquisition, TR = 7.2, TE = 3.3, flip angle = 8, matrix = 256 × 232, slice thickness 1 mm, voxel dimensions of 1 mm x 1 mm x 1 mm, NSA = 1, no slices 160, gap = 0, total scan duration 5´34¨).

Diffusion-weighted images were acquired using a Single Shot SPIR: 1.75 × 1.75 × 2 mm voxels; 60 axial slices; b-value of 800 s/mm^2; 32

direction diffusion-weighted and 1 baseline image; TR = 9967 ms, TE = 66 ms; angle 90, acquisition matrix size = 128 × 128.

All the scans were acquired on the same MR scanner and no hardware or software upgrades were carried out within the study period.

2.4. Data preprocessing

A detailed flowchart of the image-processing pipeline can be found in Supplementary Fig. 1. To study voxel based GM volume loss in DM1 patients and its association with different neuropsychological scales, FSL Voxel Based Morphometry (VBM) was used (Douaud et al., 2007), an optimized VBM protocol (Good et al., 2001) carried out with FSL tools (Smith et al., 2004). First, structural images were brain-extracted and GM-segmented before being registered to the MNI 152 standard space using non-linear registration (Andersson et al., 2007). The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific GM template. Second, all native GM images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated GM images were then smoothed with an isotropic Gaussian kernel with a sigma of 3.

To estimate global brain tissue volume, SIENAX tool was used (Smith et al., 2002). SIENAX is a FSL package for single-time-point analysis of brain atrophy (volumetric loss of brain tissue), which estimates total brain tissue volume from a single image, normalized for skull size. It first strips non-brain tissue, and then uses the brain and skull images to estimate the scaling between the subject's image and standard space. It then runs tissue segmentation to estimate the volume of brain tissue, and multiplies this by the estimated scaling factor to reduce head-size-related variability between subjects.

Additionally, anatomical T1 images were used to compute the cortical parcellation and volumetric segmentation of each subject's brain using FreeSurfer 6.0.0 (Dale et al., 1999). This pipeline included: removal of non-brain tissue using a hybrid watershed/surface deformation procedure; automated Talairach transformation; segmentation of the subcortical WM and deep GM structures (Fischl et al., 2002); intensity normalization; delineation of the GM/WM boundary; automated topology correction; and surface deformation following intensity gradients to optimally identify the gray/white and gray/cerebrospinal fluid boundaries. Surface based registration projected the Desikan-Killiany parcellation to individual subjects (Desikan et al., 2006).

FMRIB Software Library v5.0.7 (FSL) software was used for the preprocessing of Diffusion-weighted images. Following eddy current correction, gradient vectors were rotated to compensate for head motion. Local fitting of the diffusion tensor at each voxel was then computed and Fractional Anisotropy maps for each subject were generated. To use Desikan-Killiany parcellation and subcortical segmentation for the tractography analysis this atlas was projected from T1 space to diffusion space computing a non-linear transformation between the T1 and fractional anisotropy map. To model crossing fibers, the FSL BEDPOSTX (Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques) tool was used with default parameters, and probabilistic tractography was subsequently performed using the FSL PROBTRACKX2 (probabilistic tracking with crossing fibers) tool with 100 samples with loopcheck.

2.5. Statistical analysis

Demographic data were analyzed using the SPSS (IBM SPSS Statistics 24) statistical package. Intergroup comparisons were conducted to compare DM1 patients and HC, using contingency analysis (chi-square) for categorical data and parametric *t* test for interval data.

2.5.1. VBM between and within group statistical analysis

A general linear model was used to compute the between group statistical analysis using Matlab R2017a (see Supplementary File 1 for more detail). A two sample T-test was used, controlling for age and head size. All the results were corrected for multiple comparisons using Monte Carlo simulation cluster-wise correction from AFNI software (https://afni.nimh.nih.gov/) with 10,000 iterations to estimate the probability of false positive clusters with a p value < 0.05.

To study the brain region implicated in the severity of the disease, we used a general linear model and evaluated the relationship between GM volume and CTG and MIRS clinical scales. Once again, the results were adjusted for age and head size and corrected for multiple comparisons using Monte Carlo simulation. Additionally, partial correlation was used to evaluate the relationship between global volume loss and disease severity, as measured by MIRS and CTG (adjusting for age and head size).

To study the effect of age in comparison with HC, a general linear model was used to compare the relationship between GM volume and age between the two groups. In this case, Monte Carlo simulation was used to correct for multiple comparison adjusting only for head size.

Global volume loss was also correlated with neuropsychological variables using a partial correlation analysis, controlling for age and head size. To control for the number of associations with different neuropsychological tests, a false discovery rate (FDR) correction was applied.

Finally, a within-group analysis was conducted to study the association between neuropsychological variables and GM volume, using a regression analysis. For each neuropsychological variable, the imaging results were controlled for age and head size and corrected for whole brain multiple comparisons using Monte Carlo simulation cluster-wise correction with 10,000 iterations to estimate the probability of false positive clusters with a p value < 0.05. After correcting for multiple comparisons, a mask with the results of the group difference was applied to look only for the associations in regions were DM1 patients show a significant GM volume loss compared with healthy controls. Only clusters with a spatial extent of 100 adjacent voxels are reported.

2.5.2. DTI between and within group statistical analysis

DTI data were used to examine the cortical and subcortical regions affected by the disintegration of the WM. First, the brain was divided into 86 regions: 68 cortical areas defined by Freesurfer Desikan parcellation and 18 subcortical regions obtained with Freesurfer segmentation. For each individual subject and brain region probabilistic tractography was used with the probtrackx2 tool, taking 100 samples from the range of possible principal diffusion directions within each voxel. The probabilistic tracking output was a density map defining the number of fibers passing through each voxel when tracking from a given seed. To remove false positives from these maps, voxels with fewer fibers than 0.1% of all the tracked fibers were thresholded. The thresholded density maps of each region were used to compute the weighted FA values as a proxy of WM integrity throughout different areas (high values of FA representing high integrity). The FA values of the voxels in the path of the fibers starting in each region, defined in the density map, were extracted and weighted based on the probability of fibers passing through each voxel; the weighted mean FA gives more importance to voxels with higher probability. DTI images were available in 23 patients and 44 HC.

To examine between-group differences of the mean weighted FA values of the 86 regions in each individual, a general linear model was performed adjusting for age using Matlab (see Supplementary File 1 for more detail). This analysis identified cortical and subcortical brain areas showing differential fiber integrity in patients with DM1 compared with healthy controls. To correct for multiple comparisons, both FDR with $q = 0.05$ and Bonferroni with $\alpha = 0.05$ corrections were applied.

To study the relationship between fiber integrity and both disease severity and neuropsychological variables, a general linear model was used, controlling for age. The results were corrected for multiple

Table 1
Socio-demographic, clinical, and molecular characteristics according to group.

	DM1 N = 31	HC N = 57	DM1 vs HC Statistic	*p*
Sex N (%)				
Male	13 (41.9%)	27 (47.4%)	X^2 = .24	.625
Female	18 (58.1%)	30 (52.6%)		
Age				
Mean (SD)	43.94 (11.59)	45.14 (12.96)	t = −.43	.667
Min-max	22–61	18–70		
Muscular weakness (MIRS)				
Mean (SD)	2.8 (1.22)	-		
Min-max	1–5	-		
Molecular defect (CTG)				
Mean (SD)	667.77 (473.97)	-		
Min-max	63–1833	-		
Gray matter volume (mm^3)				
Mean (SD)	750765.77 (54664.48)	792954.13 (45787.84)	F = 14.84	.000
White matter volume (mm^3)				
Mean (SD)	686896.86 (43162.82)	711533.98 (42684.27)	F = 6.64	.011

Note: DM1, Myotonic Dystrophy Type 1; HC, Healthy Controls; SD, Standard deviation; MIRS, Muscular Impairment Rating Scale; CTG, triplet expansion size.

comparison using FDR, with q = 0.05. The resulting ROI that were not present in the group difference correcting for FDR were removed, to focus only on those regions showing decreased diffusivity compared to HC.

Using the same approach as in GM volume, the effect of age was compared between DM1 and HC, using FDR to correct for multiple comparisons.

3. Results

3.1. Demographic, clinical, and neuropsychological outcomes

The main demographic characteristics of the sample are shown in Table 1. No statistical differences were found between the patient group and the HC group in terms of mean age and sex ratio. The neuropsychological outcomes of the DM1 patients are summarized in Supplementary Table 1. The profile is characterized by slow processing speed, mild executive impairment (abstract reasoning, planning and flexibility) and visuoconstructive impairment, and a mean IQ of 84.29 (standard deviation, SD: 12.67). The patients as a group scored below the normative mean on every cognitive subtest and obtained a mean score below -1 SD on nine measures and below -2 SD on one subtest (ROCF copy).

3.2. VBM and DTI– between group comparison

VBM analysis revealed statistically significant differences in both total gray and white matter volume between DM1 and HC subjects. (Table 1 and box plot Fig. 1a for GM).

When analyzing the location of GM volume differences between groups in VBM corrected for age and head size, specific areas emerged as showing a significant decrease in DM1 compared with the HC group. The brains in Fig. 1b show a widespread decrease in volume in comparison with healthy subjects in primary regions (visual, somatomotor, and auditive), multimodal integration regions, the ventromedial and dorsolateral prefrontal cortex, intraparietal sulcus (IPS), striatum, thalamus, hypothalamus, periaqueductal gray and cerebellum.

DTI group comparison between DM1 patients and HC revealed a significant decrease in total FA (Fig. 1c). Locally, using Bonferroni correction, a significantly decreased FA was found in 54.02% of all studied tracts (going to both cortical and subcortical areas, Fig. 1d). Areas with affected connectivity were bilaterally but non-symmetrically (higher number of affected connectivity areas in the left hemisphere) distributed across the entire cortical surface, with the exception of the cerebellum and brainstem. The regions with the highest percentage of affected connectivity areas were the frontal cortex (90.9% of the frontal areas with affected fibers), paralimbic cortex (64.2%), temporal cortex (50%) and subcortical regions (43.75%).

3.3. VBM and DTI – association with clinical and neuropsychological data

Associations between GM volumes and CTG, MIRS, and age are shown in Fig. 2a–c. All the three statistical analyses were corrected for head size, and additionally corrected for age in the case of MIRS and CTG correlations. Scatter plots in the lower part of the figure indicate that total brain volume is significantly correlated with MIRS outcome, CTG expansion size, and age (higher ratings on MIRS, CTG repeat size and older age correlate with lower volume). Areas with atrophies that correlate with increased CTG expansion size are the orbitofrontal area, anterior and posterior cingulate cortex, left sensorimotor areas, right temporoparietal junction and precuneus, visual association areas, thalamus, striatum and subcallosal cortex (Fig. 2a). Areas with atrophies correlated with increased MIRS are primary visual and sensorimotor regions, prefrontal ventromedial and orbitofrontal areas, anterior cingulate cortex, IPS and precuneus, left thalamus and bilateral striatum (Fig. 2b). Finally, the correlation between GM volume and age was compared between DM1 patients and HC. The areas in which volume loss related to age was greater in DM1 patients than healthy controls are the precuneus, left latero-occipital and superior parietal areas (Fig. 2c).

Further, DTI analysis showed that whole brain FA correlated only with MIRS (higher MIRS was associated with lower FA). Regionally, patients with higher CTG showed decreased FA in the fibers starting in bilateral prefrontal areas, anterior cingulate, temporal cortex (superior, inferior and banks of the superior temporal sulculs), insula and putamen (Fig. 2d). Patients with higher MIRS showed altered FA in the fibers starting in the frontal cortex (except the orbitofrontal areas), cingulate cortex, primary sensory cortex, insula and precuneus, ventral temporal areas (Fusiform gyrus, lingual cortex, and para-hippocampus), brainstem and ventral diencephalon (Fig. 2e). No correlations were found between FA measures and age.

The correlation analysis between neuropsychological performance and total volume loss in GM is presented in Table 2. Only those correlations that remained statistically significant after correcting for multiple comparisons (FDR) are presented. Lower scores on neuropsychological tests predicted lower total GM volume. Only the color subtest of the Stroop test correlated with total FA, showing an inverse correlation (r = −.72, p = .000).

To obtain an association between neuropsychological outcomes and affected brain areas after correcting for whole brain multiple comparisons, we focused on regions defined by the mask of group differences between DM1 and HC. Associations between different neuropsychological tests and the GM intensity are shown in Table 3 (and in Supplementary Figure 2). All associations except one were negative (lower score on cognitive tests correlated with greater GM volume loss). Regarding WM integrity, an isolated negative association was found between regional FA (parietal lobe, precuneus, postcentral gyrus and supramarginal cortex) and one subtest of the Stroop (Supplementary Figure 2).

4. Discussion

The current paper presents a thorough analysis of structural abnormalities in both gray and white matter in a juvenile and adult onset DM1 sample compared with an extensive healthy control group. GM VBM analysis was performed using the highest standards of statistical

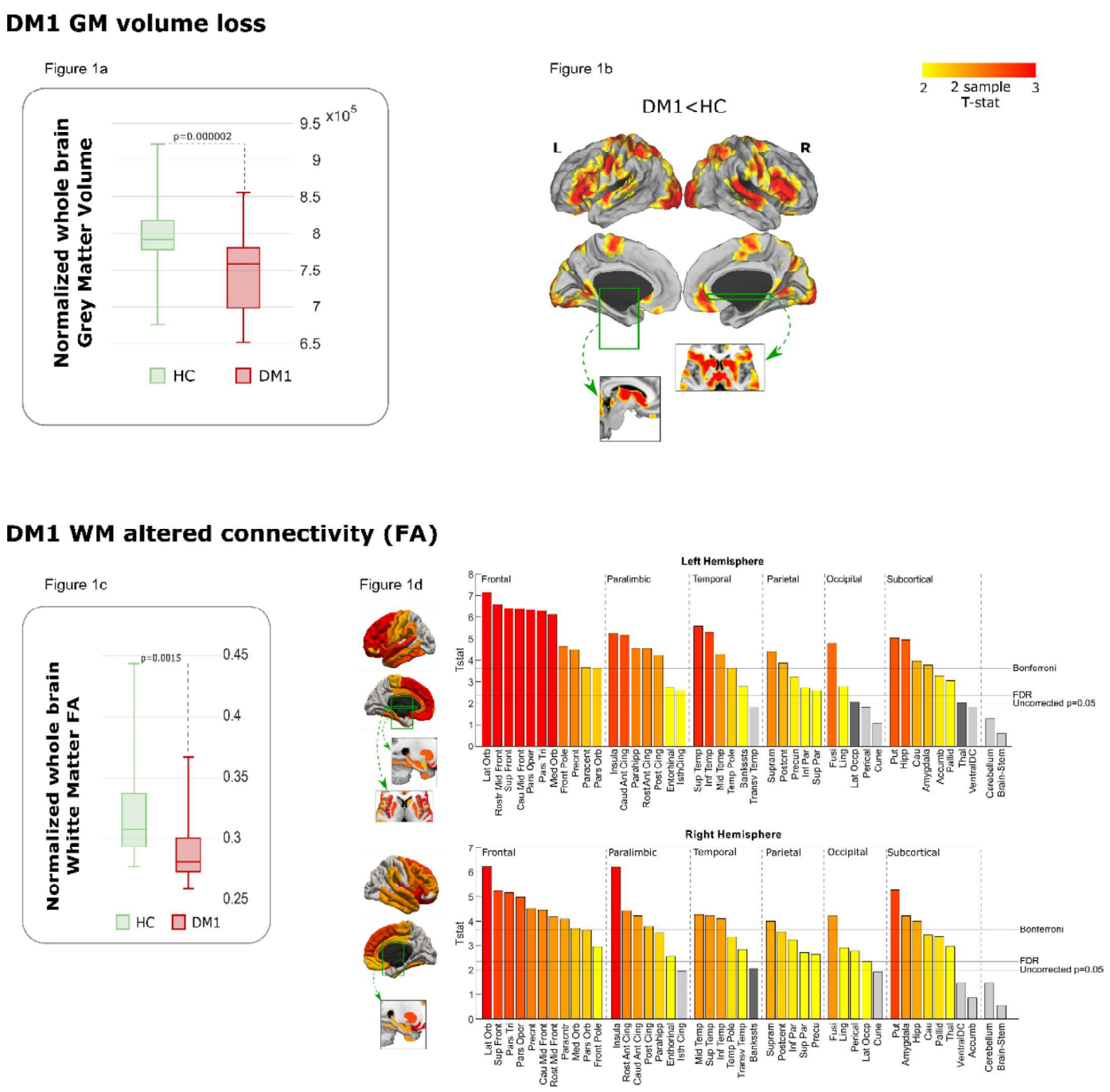
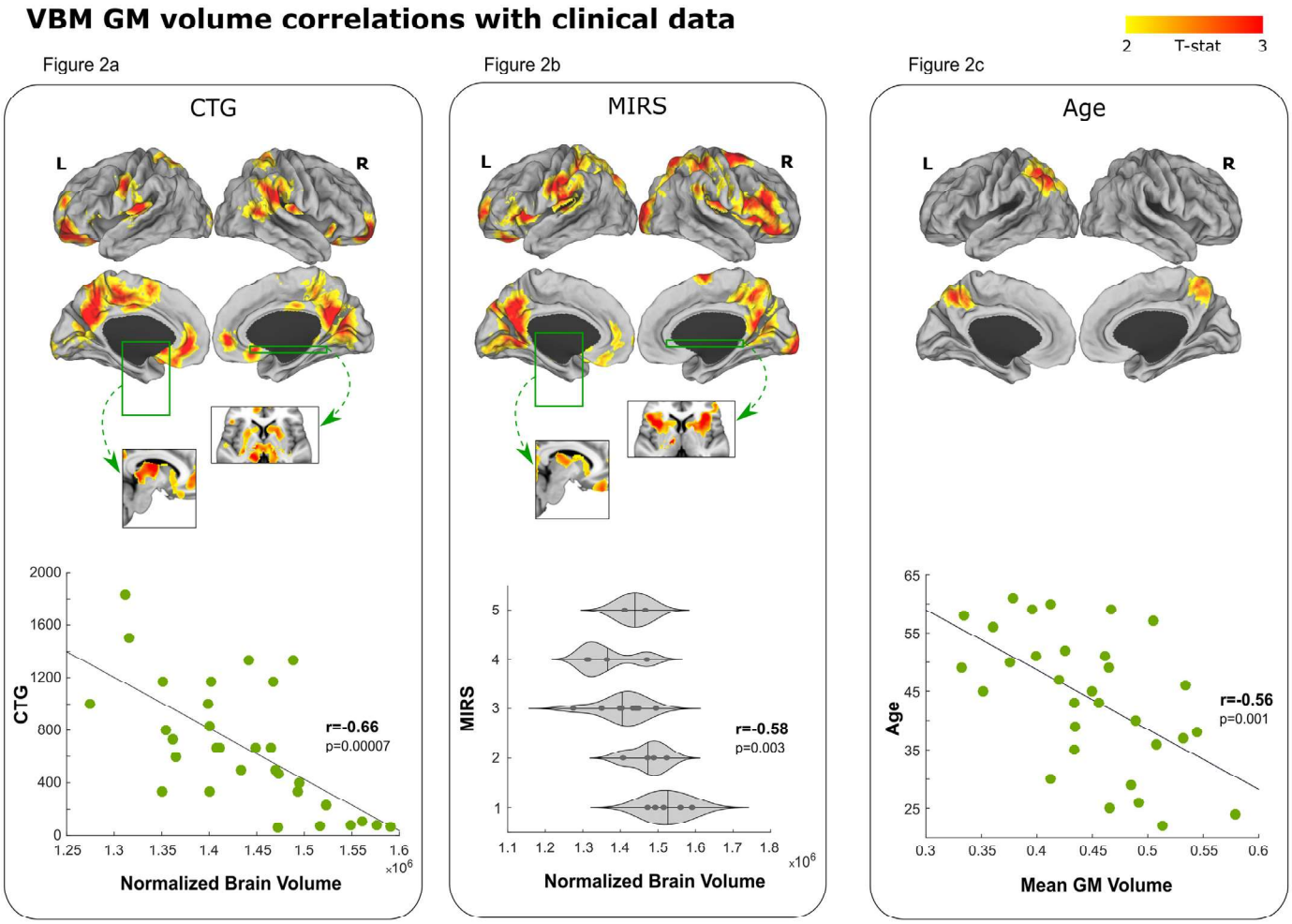


Fig. 1. Gray matter (GM) volume loss and decrease in white matter (WM) integrity (FA, fractional anisotropy) in DM1 patients. T statistic maps with the results of two sample T test comparing DM1 vs healthy controls (HC) are displayed. Only results surviving multiple comparison adjusting for age and brain size are shown for GM volume. The box plot in Fig. 1a shows the comparison of whole brain GM volume between the DM1 and HC group adjusting for age and head size. Fig. 1b shows areas of greater decrease in DM1 compared with HC. The cortical results were projected on a brain surface and were complemented with subcortical slices; the green boxes and arrows represent the position of these slices in the brain surface. The box plot in Fig. 1c shows the comparison of whole brain WM FA between the DM1 and the HC group adjusting for age. Fig. 1d shows areas of major FA decrease in DM1 vs healthy subjects according to brain region. The bar plots display the T statistics results of the group difference (the magnitude of the difference between the two groups) for all the brain regions.

control, and correlation analysis was conducted to explore the relationships with clinical parameters. Volume reduction was not limited to the whole brain volume, but was extended to specific regions. In this work WM integrity was assessed with a statistical threshold corrected for multiple comparisons. In addition, while many of the DTI studies have focused on Tract-Based Spatial Statistics (TBSS, analyzing the diffusion parameters in the skeleton of the WM tracts), the present study used probabilistic tractography to assess structural connectivity alterations in DM1 patients. Whereas some studies used tractography to focus on a small number of hypothesized and predefined tracts, in this work we conducted whole brain tractography and studied connectivity to all cortical areas, obtaining new and valuable information regarding the cortical signature of the disease due to the disruption of WM connections.

Further, this study comprises a comprehensive neuropsychological assessment of DM1 patients, and the most relevant clinical features were correlated with MRI findings. The DM1 sample in this study showed a neuropsychological profile that is representative of the DM1



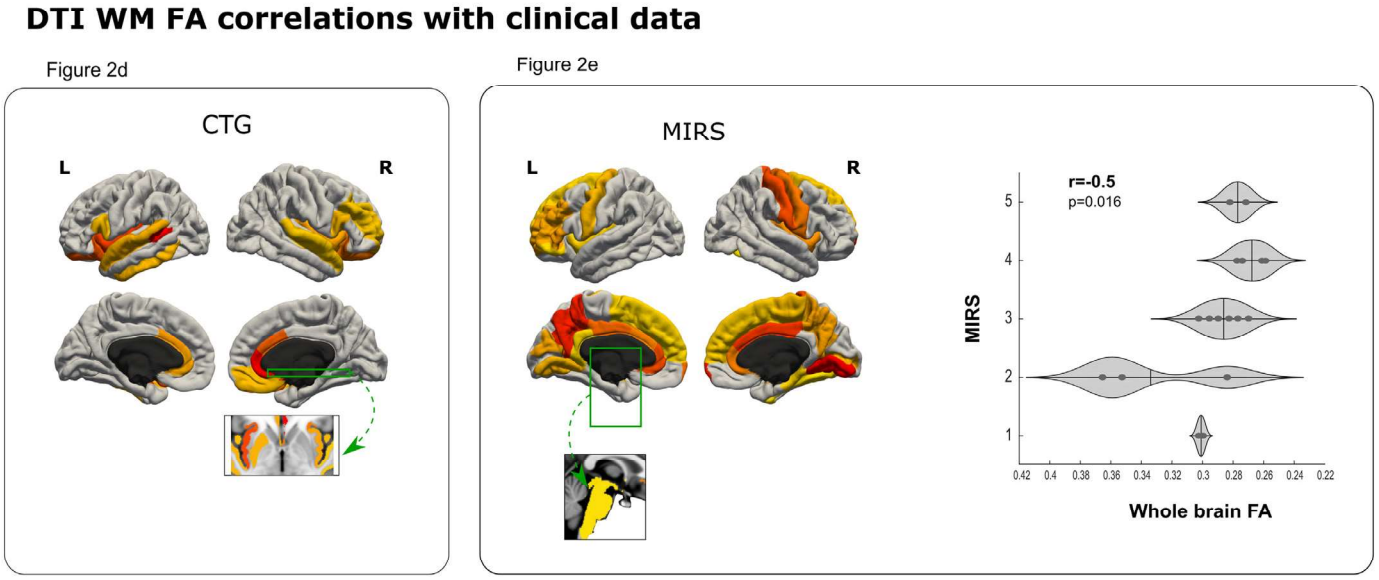


Fig. 2. Gray matter (GM) volume loss and decrease in white matter (WM) integrity (FA, fractional anisotropy) associated with severity of DM1 patients. The T-statistic showing the relationship between GM volume and WM integrity in DM1 patients and various disease severity markers is displayed. Only results surviving multiple comparisons are shown, correcting for age and head size in GM volume and for age in WM integrity. Fig. 2a shows the areas significantly decreased in relation to CTG in DM1 patients. The scatter plot shows the association between whole brain volume and CTG value. Fig. 2b shows the areas significantly decreased in relation to MIRS scale in DM1 patients. The violin plot shows the association between whole brain volume and MIRS scale. Fig. 2c shows the areas where greater volume loss occurs in DM1 patients with age in comparison with healthy controls (HC). The scatter plots show the effect of age and volume loss in this specific region. Fig. 2d shows the areas with significantly disrupted connectivity in relation to CTG in DM1 patients. Fig. 2e shows the areas with significantly disrupted connectivity in relation to MIRS scale in DM1 patients. The violin plot shows the association between whole brain WM FA and MIRS scale. The cortical results were projected on a brain surface and were complemented with subcortical slices; the green boxes and arrows represent the position of this slices in the brain surface.

Table 2
Association between total GM atrophy and neuropsychological outcomes in DM1

	Total GM atrophy	
Neuropsychological measure	r	p
Block design	.49	.006
Raven's progressive matrices	.52	.006
ROCF copy	.55	.001
IQ estimate	.46	.010

Note: GM, Gray matter; ROCF, Rey-Osterrieth Complex Figure test; IQ, Intelligence Quotient. All the correlations are performed with standardized T scores or standard scores (IQ estimate only) of the neuropsychological outcome measures.

population according to the current literature (Jean et al., 2014).

4.1. VBM and DTI – group differences

Consistent with the findings of the most recent studies (Okkersen et al., 2017), overall the MRI analysis showed widespread bilateral alteration in both gray and white matter, which could partially be explained by the heterogeneous effect of genetics upon a variety of tissues in DM1.

Cortical GM atrophy was found to be more pronounced in certain areas of every cerebral lobe. The involvement of subcortical structures has been confirmed in previous work, reporting lower volumes in striatum, thalamus, hypothalamus and periaqueductal gray, with a bilateral and symmetric involvement. Both cortical and subcortical atrophy fits well with the histopathological findings in DM1, including RNA nuclear inclusions or neurofibrillary tangles found in the cortex

Table 3
Gray matter volume loss associated with neuropsychological outcome of DM1 patients.

	Cluster				MNI coordinates		
	Size	Mean T	*p*	Peak ROI Structure	X	Y	Z
IQ estimate	133	3	.005	Left Frontal Pole	−40	40	−8
Arithmetic	181	2.28	.030	Right Cuneal Cortex	4	−84	24
Block design	132	2.66	.013	Left Frontal Pole	−46	44	−8
	257	2.47	.020	Right Precuneus Cortex	10	−64	52
ROCF copy	413	2.65	.013	Left Frontal Orbital Cortex	−20	20	−22
	860	2.64	.013	Left Caudate	−16	−8	20
	180	2.66	.013	Left Precentral Gyrus	−52	6	16
	427	2.62	.014	Left Precentral Gyrus	−52	0	34
	597	2.59	.015	Left Superior Frontal Gyrus	−8	−6	72
ROCF memory	705	2.9	.007	Right Subcallosal Cortex	4	30	−30
	250	2.39	.024	Left Insular Cortex	−30	0	14
	136	2.52	.018	Left Precentral Gyrus	−54	8	38
	116	2.49	.019	Left Inferior Frontal Gyrus	−38	16	26
	328	2.96	.006	Left Precentral Gyrus	−16	−12	76
Object assembly	543	2.47	.020	Right Lingual Gyrus	6	−74	−8
	337	2.67	.012	Right Precentral Gyrus	34	−18	56
	269	2.78	.009	Right Superior Frontal Gyrus	18	−6	68
Stroop Interference	521	2.54	.018	Left Frontal Medial Cortex	−2	32	−28
	197	2.41	.024	Right Putamen	24	8	−6
	232	−2.57	.017	Right Precentral Gyrus	50	4	40
Stroop Word	992	2.63	.015	Left Putamen	−30	6	−8
	872	3.08	.005	Right Caudate	8	16	10
Stroop Color-Word	694	2.61	.015	Left Frontal Orbital Cortex	−18	12	−12
	241	2.52	.019	Right Putamen	24	10	−8
Raven's progressive matrices	147	2.5	.019	Left Inferior Frontal Gyrus	−52	34	−16
	142	2.62	.015	Left Middle Frontal Gyrus	−50	22	32
	390	2.67	.013	Left Precentral Gyrus	−48	−8	46
	251	2.52	.019	Left Superior Parietal Lobule	−40	−40	54
	154	2.58	.016	Left Precuneus Cortex	−10	−54	52
	163	2.74	.011	Right Precuneus Cortex	12	−50	60
Election RT	352	2.58	.017	Left Occipital Pole	−16	−94	0
Sequence 2 RT	469	2.72	.012	Right Frontal Orbital Cortex	10	24	−28
	272	2.37	.026	Left Middle Frontal Gyrus	−46	30	24
	107	2.39	.026	Left Parietal Operculum Cortex	−48	−24	16
	196	2.32	.029	Left Postcentral Gyrus	−44	−16	30

Note. DM1: Myotonic Dystrophy Type 1; MNI: Montreal Neurosciences Institute; ROI: Regions of Interest; IQ: Intelligence Quotient; ROCF: Rey-Osterrieth Complex Figure; RT: Reaction Time. Only results surviving multiple comparisons are shown, correcting for age and head size. The results were then masked to display only those results in the regions where DM1 patients showed a significant decrease in volume compared with healthy controls. For each neuropsychological measure the table shows the cluster size representing the total number of voxels that comprise the cluster; the mean T represents the magnitude of the association between the mean grey matter measure in the cluster and the p-value of this association (Higher T indicates a stronger association).

and several deep GM structures (Caillet-Boudin et al., 2014).

When analyzing WM FA alteration related to connectivity areas, anterior cortical areas and again subcortical regions appeared to be the most affected. Interestingly, all the anterior GM areas with decreased volume resulted in altered WM integrity. Conversely, there was no correspondence between gray-white matter impairment in the case of more posterior and deep gray structures (i.e. IPS, thalamus, periaqueductal gray, and cerebellum). DM1 has been proposed to be a predominantly WM disease. However, based on the extent to which our data on GM damage exceeds that of WM integrity loss, this idea could be questioned (Schneider-Gold et al., 2015). So far, DM1 should be considered a disease with both gray and white matter involvement, without any clearly established trend towards a greater involvement of either type of tissue.

4.2. VBM and DTI – correlation with clinical data

Although not consistently reported in the literature, there is evidence in support of the possibility that longer CTG could be a predictor of structural abnormalities. Similar to other studies (Park et al., 2018), we found a significant correlation between muscular and genetic impairment and GM volume loss.

CTG expansion size determined in peripheral blood cells has been questioned for genotype-phenotype correlation analysis due to tissue and temporal variations (Martorell et al., 1998). However, in this study, patients with greater CTG expansion sizes showed reduced GM volumes, primarily in fronto-parietal areas and subcortical regions, according to the functional cognitive profile described in some neuropsychological studies (Peric et al., 2017, 2014; Sistiaga et al., 2010). WM abnormalities related to longer CTG also appeared in both cortical and subcortical regions. Taken together, CTG-related associations, along with brain imaging findings, encourage us to maintain the idea that genetic features of DM1 are target variables in the study of possible mediators between CNS involvement and actual symptoms.

Muscular impairment also appeared to be associated with total FA reduction, regional FA reduction and with GM volume loss, but more widely dispersed throughout the cortical tissue than genetic features, which is suggestive of this being a good global severity marker of the disease. Consistent with this proposal is the fact that previous studies have reported not only evidence of regional atrophy in motor-related structures, but also correlations between MIRS and global brain atrophy (Schneider-Gold et al., 2015) and with altered integrity in the majority of WM tracts (Wozniak et al., 2014). This reinforces the notion of central, and not merely muscular, motor dysfunction in adult DM1.

In DM1, an association between age and MRI findings has been reported (Minnerop et al., 2018). Although studies assessing disease progression are still scarce, the results so far indicate a premature aging effect in DM1. Accordingly, in this study, age predicted global GM volume loss and was associated with particular vulnerability to parietal GM regions, suggesting an age-related higher vulnerability of that specific cortical lobe. Follow up studies including both neuropsychological and neuroimaging data are strongly needed in order to clarify the neurodegenerative hypothesis that has repeatedly been suggested. In view of the parietal vulnerability to age indicated in this study, the inclusion of visuoconstructive/visuospatial tasks in longitudinal assessments of cognition should be considered for characterization of the patients.

Neither total FA reduction nor FA decrease in specific connectivity areas was found to be related to age, which could reflect the neurodevelopmental nature of WM abnormalities in DM1, rather than a degenerative process. Indeed, diffuse WM integrity disruption has been reported in early stages in young DM1 patients (Wozniak et al., 2013). However, only longitudinal data with a baseline set at disease onset could clarify this hypothesis.

4.3. VBM and DTI – correlation with neuropsychological data

The lack of consistent results on neuropsychological correlates with neuroimaging results have been reported to date in the DM1 literature. Our findings, however, suggest that the selected neuropsychological measures are highly sensitive for detecting structural changes in the brain, as quantified in the numerous correlations found primarily in cortical areas. Nevertheless, a lack of specificity could still be an issue, since anatomo-functional correspondence of the results found might not be those that were expected. The network-wise organization for cognitive functions (as opposed to a greater localizationist approach) could be behind this lack of correspondence. Moreover, considering the large global atrophy consistently reported in DM1, functional reorganization could be playing a role.

Overall, visuoconstructive and executive performance correlated with both total GM atrophy and with some of the specific areas of decreased volume in DM1 compared with healthy controls. Taken together, the results point to the possibility that these cognitive domains are good markers of brain abnormalities in the DM1 population.

This study is not without limitations. Considering the clinical heterogeneity of the disease, the sample size is relatively small in this study. The inclusion of larger sample sizes will allow for a more in-depth analysis of MRI abnormalities, and could provide accurate and evidence-based information to suggest alternative classifications of DM1 patients. Another limitation in the present study is that congenital and childhood forms were not included, and therefore, the results cannot be generalized to the whole DM1 population. Additionally, there are other measures of DTI apart from FA that were not analyzed in the present work in an effort to simplify the quantity of results presented. Whilst other measures correlate more strongly with features such as cellularity or membrane diameter, FA is more sensitive to tissue integrity, which represents a main focus of the present work. Finally, in DTI, tractography algorithms are not able to reconstruct single axons. While deterministic tractography does not account for crossing fibers, probabilistic tracking generates a distribution of fiber directions in each voxel to deal with this problem, although this produces a large number of false positive fibers. To minimize this error, less probable fibers were removed and the weighted mean FA was calculated. In the future, the inclusion of higher resolution protocols and new probabilistic tractography algorithms, including anatomical information, could lead to more accurate and reproducible fiber estimation.

4.4. Conclusion

Global GM atrophy and WM integrity compromise is confirmed in DM1. A pattern of greater fronto-temporo-parietal and subcortical impairment is proposed, with a gray-related WM integrity loss. On balance, our results, together with those previously reported, suggest the existence of a complex neuronal network damage-related disease as opposed to the idea that focal structural degeneration is responsible for CNS symptoms in DM1.

Neuropsychological assessment emerges as an unspecific but strong predictor of GM abnormalities. Age-related vulnerability of certain regions suggests a possible neurodegenerative process in DM1 that remains to be thoroughly studied. Longitudinal studies including both GM and WM structural data in cognitive and clinically well characterized patients are needed. This will allow for a more complete characterization of the interrelations between gray and white matter in DM1, and will also allow us to elucidate whether a progression over time is confirmed in relation to cognitive decline.

Declaration of Competing Interest

Authors declare that this work was supported by the Institute of Health Carlos III co-founded by Fondo Europeo de Desarrollo Regional-FEDER (grant numbers PI17/01231 and PI17/01841), CIBERNED

(grant number: 609), the Basque Government (grant numbers SAIO08-PE08BF01 and PRE_2016_1_0187) and the postdoctoral fellowship program from the Basque country Government.

Acknowledgements

We are grateful to all the patients, their families and the healthy volunteers who participated in this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nicl.2019.102078.

References

Andersson, J.L.R., Jenkinson, M., Smith, S., 2007. Non-linear registration, aka spatial normalisation. FMRIB Technial Report TR07JA2. Oxford Cent. Funct. Magn. Reson. Imaging Brain, Dep. Clin. Neurol. Oxford Univ. Oxford, UK 22. https://doi.org/10.1016/j.neuroimage.2008.10.055.

Antonini, G., Mainero, C., Romano, A., Giubilei, F., Ceschin, V., Gragnani, F., Morino, S., Fiorelli, M., Soscia, F., Di Pasquale, A., Caramia, F., 2004. Cerebral atrophy in myotonic dystrophy: a voxel based morphometric study. J. Neurol. Neurosurg. Psychiatry 75, 1611–1613. https://doi.org/10.1136/jnnp.2003.032417.

Baldanzi, S., Cecchi, P., Fabbri, S., Pesaresi, I., Simoncini, C., Angelini, C., Bonuccelli, U., Cosottini, M., Siciliano, G., 2016. Relationship between neuropsychological impairment and grey and white matter changes in adult-onset myotonic dystrophy type 1. NeuroImage Clin. 12, 190–197. https://doi.org/10.1016/j.nicl.2016.06.011.

Benton, A., Sivan, A., Hamsher, K., Varney, N.R., Spreen, O., 1994. Contributions to Neuropsychological Assessment, 2nd ed. Oxford University Press, New York.

Caillet-Boudin, M.L., Fernandez-Gomez, F.J., Tran, H., Dhaenens, C.M., Buee, L., Sergeant, N., 2014. Brain pathology in myotonic dystrophy: when tauopathy meets spliceopathy and RNAopathy. Front. Mol. Neurosci. 6, 1–20. https://doi.org/10.3389/fnmol.2013.00057.

Casals-Coll, M., Sánchez-Benavides, G., Quintana, M., Manero, R.M., Rognoni, T., Calvo, L., Palomo, R., Aranciva, F., Tamayo, F., Peña-Casanova, J., 2013. Estudios normativos españoles en población adulta joven (proyecto NEURONORMA jóvenes): normas para los test de fluencia verbal. Neurología 28, 33–40. https://doi.org/10.1016/j.nrl.2012.02.010.

Caso, F., Agosta, F., Peric, S., Rakočević-Stojanović, V., Copetti, M., Kostic, V.S., Filippi, M., 2014. Cognitive impairment in myotonic dystrophy type 1 is associated with white matter damage. PLoS One 9, 1–8. https://doi.org/10.1371/journal.pone.0104697.

Dale, A.M., Fischl, B., Sereno, M.I., 1999. Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 9, 179–194. https://doi.org/10.1006/nimg.1998.0395.

Desikan, R.S., Ségonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S., Killiany, R.J., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31, 968–980. https://doi.org/10.1016/j.neuroimage.2006.01.021.

Douaud, G., Smith, S., Jenkinson, M., Behrens, T., Johansen-Berg, H., Vickers, J., James, S., Voets, N., Watkins, K., Matthews, P.M., James, A., 2007. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. Brain 130, 2375–2386. https://doi.org/10.1093/brain/awm184.

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., Van Der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355. https://doi.org/10.1016/S0896-6273(02)00569-X.

Fukuda, H., Horiguchi, J., Ono, C., Ohshita, T., Takaba, J., Ito, K., 2005. Diffusion tensor imaging of cerebral white matter in patients with myotonic dystrophy. Acta Radiol. 46, 104–109. https://doi.org/10.1080/02841850510015974.

Gallais, B., Gagnon, C., Mathieu, J., Richer, L., 2017. Cognitive decline over time in adults with myotonic dystrophy type 1: a 9-year longitudinal study. Neuromuscul. Disord. 27, 61–72. https://doi.org/10.1016/j.nmd.2016.10.003.

Golden, C.J., 2001. STROOP. Test de Colores y Palabras, 3rd ed. TEA Ediciones, Madrid.

Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N.A., Friston, K.J., Frackowiak, R.S.J., 2001. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage 14, 21–36. https://doi.org/10.1006/nimg.2001.0786.

Harper, P.S., 2001. Myotonic Dystrophy, 3rd ed. Saunders, London.

Jean, S., Richer, L., Laberge, L., Mathieu, J., 2014. Comparisons of intellectual capacities between mild and classic adult-onset phenotypes of myotonic dystrophy type 1 (DM1). Orphanet J. Rare Dis. 9, 1–10. https://doi.org/10.1186/s13023-014-0186-5.

Kobayakawa, M., Tsuruya, N., Takeda, A., Suzuki, A., Kawamura, M., 2010. Facial emotion recognition and cerebral white matter lesions in myotonic dystrophy type 1. J. Neurol. Sci. 290, 48–51. https://doi.org/10.1016/j.jns.2009.11.011.

Labayru, G., Arenzana, I., Aliri, J., Zulaica, M., López de Munain, A., Sistiaga, A., 2018. Social cognition in Myotonic Dystrophy Type 1 : specific or secondary impairment? PLoS One 13, 1–11. https://doi.org/10.1371/journal.pone.0204227.

Lezak, M., Howieson, D.B., Loring, D., 2004. Neuropsychological Assessment, 4th ed. Oxford University Press, New York.

López, J.M., Rodríguez, J.M., Santín, C., Torrico, E., 2003. Utilidad de las formas cortas de la Escala de Inteligencia de Wechsler para Adultos (WAIS). An. Psicol 19, 53–63.

López de Munain, A., Blanco, A., Emparanza, J.I., Poza, J.J., Martí-Massó, J.F., Cobo, A.M., Martorell, L., Baiget, M., Martínez-Lage, J., 1993. Prevalence of myotonic dystrophy in Guipúzcoa (Basque Country, Spain). Neurology 43, 1573–1576.

Martorell, L., Monckton, D.G., Gamez, J., Johnson, K.J., Gich, I., Lopez De Munain, A., Baiget, M., 1998. Progression of somatic CTG repeat length heterogeneity in the blood cells of myotonic dystrophy patients. Hum. Mol. Genet. 7, 307–312. https://doi.org/10.1093/hmg/7.2.307.

Mathieu, J., Boivin, H., Meunier, D., Gaudreault, M., Bégin, P., 2001. Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. Neurology 56, 336–340. https://doi.org/10.1212/WNL.56.3.336.

Miller, E.N., 1990. CalCAP: California Computerized Assessment Package.

Minnerop, M., Gliem, C., Kornblum, C., 2018. Current progress in CNS imaging of myotonic dystrophy. Front. Neurol. 9, 1–21. https://doi.org/10.3389/fneur.2018.00646.

Minnerop, M., Weber, B., Schoene-Bake, J.C., Roeske, S., Mirbach, S., Anspach, C., Schneider-Gold, C., Betz, R.C., Helmstaedter, C., Tittgemeyer, M., Klockgether, T., Kornblum, C., 2011. The brain in myotonic dystrophy 1 and 2: evidence for a predominant white matter disease. Brain 134, 3527–3543. https://doi.org/10.1093/brain/awr299.

Modoni, A., Silvestri, G., Vita, M.G., Quaranta, D., Tonali, P.A., Marra, C., 2008. Cognitive impairment in myotonic dystrophy type 1 (DM1): a longitudinal follow-up study. J. Neurol. 255, 1737–1742. https://doi.org/10.1007/s00415-008-0017-5.

Okkersen, K., Monckton, D.G., Le, N., Tuladhar, A.M., Raaphorst, J., van Engelen, B.G.M., 2017. Brain imaging in myotonic dystrophy type 1. A systematic review. Am. Acad. Neurol. 89, 960–969. https://doi.org/10.1212/WNL.0000000000004300.

Ota, M., Sato, N., Ohya, Y., Aoki, Y., Mizukami, K., Mori, T., Asada, T., 2006. Relationship between diffusion tensor imaging and brain morphology in patients with myotonic dystrophy. Neurosci. Lett. 407, 234–239. https://doi.org/10.1016/j.neulet.2006.08.077.

Park, J.S., Song, H., Jang, K.E., Cha, H., Lee, S.H., Hwang, S.K., Park, D., Lee, H.J., Kim, J.Y., Chang, Y., 2018. Diffusion tensor imaging and voxel-based morphometry reveal corticospinal tract involvement in the motor dysfunction of adult-onset myotonic dystrophy type 1. Sci. Rep. 8, 1–11. https://doi.org/10.1038/s41598-018-34048-9.

Peña-Casanova, J., Quiñones-Úbeda, S., Gramunt-Fombuena, N., Quintana-Aparicio, M., Aguilar, M., Badenes, D., Cerulla, N., Molinuevo, J.L., Ruiz, E., Robles, A., Barquero, M.S., Antúnez, C., Martínez-Parra, C., Frank-García, A., Fernández, M., Alfonso, V., Sol, J.M., Blesa, R., 2009. Spanish multicenter normative studies (NEURONORMA Project): norms for verbal fluency tests. Arch. Clin. Neuropsychol. 24, 395–411. https://doi.org/10.1093/arclin/acp042.

Peric, S., Mandic-Stojmenovic, G., Stefanova, E., Savic-Pavicevic, D., Pesovic, J., Ilic, V., Dobricic, V., Basta, I., Lavrnic, D., Rakocevic-Stojanovic, V., 2014. Frontostriatal dysexecutive syndrome: a core cognitive feature of myotonic dystrophy type 2. J. Neurol. 262, 142–148. https://doi.org/10.1007/s00415-014-7545-y.

Peric, S., Racoceviz-Stojanovic, V., Mandic-Stojmenovic, G., Ilic, V., Kovacevic, M., Parojcic, A., Pesovic, J., Mijajlovic, M., Savic-Pavicevic, D., Meola, G., 2017. Clusters of cognitive impairment among different phenotypes of myotonic dystrophy type 1 and type 2. Neurol. Sci. 38, 415–423. https://doi.org/10.1007/s10072-016-2778-4.

Raven, J.C., Court, J.H., Raven, J., 2001. Raven: Standard Progressive Matrices. TEA Ediciones, Madrid.

Rey, A., 2009. Test de copia y de reproducción de memoria de figuras geométricas complejas. TEA Ediciones, Madrid.

Rubinsztein, S., Rubinsztein, D.C., Mckenna, P.J., Goodburn, S., Holland, A.J., 1997. Mild myotonic dystrophy is associated with memory impairment in the context of normal general intelligence. J. Med. Genet. 34, 229–233. https://doi.org/10.1136/jmg.34.3.229.

Sansone, V., Gandossini, S., Cotelli, M., Calabria, M., Zanetti, O., Meola, G., 2007. Cognitive impairment in adult myotonic dystrophies: a longitudinal study. Neurol. Sci. 28, 9–15. https://doi.org/10.1007/s10072-007-0742-z.

Schneider-Gold, C., Bellenberg, B., Prehn, C., Krogias, C., Schneider, R., Klein, J., Gold, R., Lukas, C., 2015. Cortical and subcortical grey and white matter atrophy in myotonic dystrophies type 1 and 2 is associated with cognitive impairment, depression and daytime sleepiness. PLoS One 10, 1–20. https://doi.org/10.1371/journal.pone.0130352.

Serra, L., Petrucci, A., Spanò, B., Torso, M., Olivito, G., Lispi, L., Costanzi-Porrini, S., Giulietti, G., Koch, G., Giacanelli, M., Caltagirone, C., Cercignani, M., Bozzali, M., 2015. How genetics affects the brain to produce higher-level dysfunctions in myotonic dystrophy type 1. Funct. Neurol. 30, 21–31. https://doi.org/10.11138/FNeur/2015.30.1.021.

Sistiaga, A., Urreta, I., Jodar, M., Cobo, A.M., Emparanza, J., Otaegui, D., Poza, J.J., Merino, J.J., Imaz, H., Martí-Massó, J.F., López de Munain, A., 2010. Cognitive/personality pattern and triplet expansion size in adult myotonic dystrophy type 1 (DM1): CTG repeats, cognition and personality in DM1. Psychol. Med. 40, 487–495. https://doi.org/10.1017/S0033291709990602.

Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E.J., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23, 208–219. https://doi.org/10.1016/j.neuroimage.2004.07.051.

Smith, S.M., Zhang, Y., Jenkinson, M., Chen, J., Matthews, P.M., Federico, A., De Stefano, N., 2002. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage 17, 479–489. https://doi.org/10.1006/nimg.2002.1040.

Thornton, C.A., 2014. Myotonic dystrophy. Neurol. Clin 32, 705–719. https://doi.org/10.

1016/j.ncl.2014.04.011.
van Dorst, M., Okkersen, K., Kessels, R.P.C., Meijer, F.J.A., Monckton, D.G., Engelen, B.G.M.Van, Tuladhar, A.M., Raaphorst, J., 2019. Structural white matter networks in myotonic dystrophy type 1. NeuroImage Clin 21, 101615. https://doi.org/10.1016/j.nicl.2018.101615.
Weber, Y.G., Roebling, R., Kassubek, J., Hoffmann, S., Rosenbohm, A., Wolf, M., Steinbach, P., Jurkat-Rott, K., Walter, H., Reske, S.N., Lehmann-Horn, F., Mottaghy, F.M., Lerche, H., 2010. Comparative analysis of brain structure, metabolism, and cognition in myotonic dystrophy 1 and 2. Neurology 74, 1108–1117. https://doi.org/10.1212/WNL.0b013e3181d8c35f.
Wechsler, D., 1999. WAIS-III: Escala de Inteligencia de Wechsler para Adultos III. TEA Ediciones, Madrid.
Winblad, S., Hellstrom, P., Lindberg, C., Hansen, S., 2006b. Facial emotion recognition in myotonic dystrophy type 1 correlates with CTG repeat expansion. J. Neurol. Neurosurg. Psychiatry 77, 219–223. https://doi.org/10.1136/jnnp.2005.070763.
Winblad, S., Lindberg, C., Hansen, S., 2006a. Cognitive deficits and CTG repeat expansion size in classical myotonic dystrophy type 1 (DM1). Behav. brain Funct. 2, 16. https://doi.org/10.1186/1744-9081-2-16.
Winblad, S., Samuelsson, L., Lindberg, C., Meola, G., 2016. Cognition in myotonic dystrophy type 1: a 5-year follow-up study. Eur. J. Neurol. 23, 1471–1476. https://doi.org/10.1111/ene.13062.
Wozniak, J.R., Mueller, B.A., Lim, K.O., Hemmy, L.S., Day, J.W., 2014. Tractography reveals diffuse white matter abnormalities in Myotonic Dystrophy Type 1. J. Neurol. Sci. 341, 73–78. https://doi.org/10.1016/j.jns.2014.04.005.
Wozniak, J.R., Mueller, B.A., Ward, E.E., Lim, K.O., Day, J.W., 2011. White matter abnormalities and neurocognitive correlates in children and adolescents with myotonic dystrophy type 1: A diffusion tensor imaging study. Neuromuscul. Disord. 21, 89–96. https://doi.org/10.1016/j.nmd.2010.11.013.
Wozniak, J.R., Mueller, B.aA, Bell, C.J.B., Muetzel, R.L., Lim, K.O., Day, J.W., 2013. Diffusion tensor imaging reveals widespread white matter abnormalities in children and adolescents with myotonic dystrophy type 1. J. Neurol. 260, 1122–1131. https://doi.org/10.1038/cdd.2010.172.MicroRNAs.
Zanigni, S., Evangelisti, S., Giannoccaro, M.P., Oppi, F., Poda, R., Giorgio, A., Testa, C., Manners, D.N., Avoni, P., Gramegna, L.L., De Stefano, N., Lodi, R., Tonon, C., Liguori, R., 2016. Relationship of white and gray matter abnormalities to clinical and genetic features in myotonic dystrophy type 1. NeuroImage Clin. 11, 678–685. https://doi.org/10.1016/j.nicl.2016.04.012.

Annex 3

Journal of Neuropsychology (2019)

The British Psychological Society

www.wileyonlinelibrary.com

Age-related cognitive decline in myotonic dystrophy type 1: An 11-year longitudinal follow-up study

Garazi Labayru[1,2,3]*, Jone Aliri[4], Miren Zulaica[1], Adolfo López de Munain[1,3,5,6] and Andone Sistiaga[1,2,3]

[1]Neuroscience Area, Biodonostia Health Research Institute, Donostia-San Sebastian, Gipuzkoa, Spain

[2]Personality, Assessment and Psychological Treatment Department, Psychology Faculty, University of the Basque Country (UPV/EHU), Donostia-San Sebastian, Gipuzkoa, Spain

[3]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Institute Carlos III, Madrid, Spain

[4]Department of Social Psychology and Methodology of Behavioral Sciences, University of the Basque Country (UPV/EHU), Donostia-San Sebastian, Gipuzkoa, Spain

[5]Neurology Department, Donostia University Hospital, Donostia-San Sebastian, Gipuzkoa, Spain

[6]Neurosciences Department, University of the Basque Country (UPV/EHU), Donostia-San Sebastian, Gipuzkoa, Spain

Background. Myotonic dystrophy type 1 (DM1) is an inherited multi-systemic disease involving the central nervous system (CNS) and is consequently characterized by a range of cognitive impairments. However, whether this cognitive profile progresses over time is still a matter of debate. The aim of this study was to longitudinally assess a DM1 sample, in order to compare, for the first time, this progression with that of a control group. Clinical and socio-demographic predictive factors potentially implicated in this possible decline are analysed.

Method. Seventy-five DM1 patients with childhood, juvenile, adult, and late-onset, and 54 control participants were re-assessed in an 11-year follow-up with a comprehensive neuropsychological battery. The analyses employed were mixed ANOVA for repeated measures to test intergroup comparisons over time and multiple linear regression for predictive variable analysis.

Results. Myotonic dystrophy type 1 patients significantly worsened in visuospatial/visuoconstructive abilities and visual memory compared with controls. Multiple linear

**Correspondence should be addressed to Garazi Labayru, Biodonostia Health Research Institute, Paseo Doctor Beguiristain, s/n. 20014 Donostia-San Sebastian, Spain (email: garazi.labayru@biodonostia.org).*

DOI:10.1111/jnp.12192

regression revealed that progression of cognitive impairment measured by copy of the Rey–Osterrieth complex figure was predicted by muscular impairment, whilst on the block design test age predicted the change with a cut-off at 31 years of age.

Discussion. A domain-specific progressive cognitive decline was found in DM1, with visuospatial/visuoconstructive abilities showing the greatest vulnerability to the passage of time. In addition to important clinical implications, these results suggest the need for the scientific community to delve deeper into the potential mechanisms underlying early cognitive decline in this population.

Myotonic dystrophy type 1 (DM1) is an autosomal dominant inherited disorder caused by an unstable CTG (cytosine–thymine–guanine) trinucleotide repeat expansion in the DM1 protein kinase (DMPK) gene on chromosome 19 (Brook *et al.*, 1992). In the normal population, this CTG expansion does not exceed 50 repeats, whilst DM1 ranges from 50 to 2,500, and larger expansion sizes are associated with greater disease severity. This is the most common form of adult muscular dystrophy with a reported prevalence of 1/7,400 people worldwide (Harper, 2001). However, the prevalence is significantly higher in Gipuzkoa (North of Spain), reaching 300 cases per million inhabitants (López de Munain *et al.*, 1993 and unpublished data).

Myotonic dystrophy type 1 is considered to be a progressive and chronic multisystem disorder causing muscular weakness (to both skeletal and smooth muscles) and impairment to organs such as the eyes, heart, endocrine system, and central nervous system (CNS) (Wenninger, Montagnese, & Schoser, 2018). CNS involvement has been studied through brain MRI studies, with several brain abnormalities reported in DM1 patients, such as increased prevalence of white matter hyperintensities, global and regional brain atrophy or alteration in the integrity of normal appearing white matter (Minnerop, Gliem, & Kornblum, 2018). Although the adult-onset type is the most typical form of DM1, the disease can be classified into five typical phenotypes depending on age of onset: congenital, childhood, juvenile, adult, and late-onset. Although each of them leads to distinct severity grades, disease course, and clinical features, including different forms of CNS involvement, the congenital form is considered to be a qualitatively distinct phenotype, and not merely a more severe form of the disease (Turner & Hilton-Jones, 2010).

Whilst cognitive impairments in DM1 have been characterized in recent decades, studies have still yielded mixed results. A recent meta-analysis on the cognitive profile of DM1 found larger effect sizes in global cognition, intelligence, visual memory, visuospatial and visuoconstructive abilities, psychomotor speed, and social cognition (Okkersen *et al.*, 2017). The present study is preceded by a previous study (2005–2007) including what at that time was the largest sample under neuropsychological assessment. The results revealed a CTG correlating dysexecutive and visuoconstructive impairment, suggesting fronto-parietal involvement (Sistiaga *et al.*, 2010).

Beyond the recognized cognitive impairments in DM1, rather less is known about how the cognitive profile evolves over time in comparison with the progression of cognitive outcomes related to normal ageing. To the best of our knowledge, only one longitudinal study with congenital and childhood onset in a young population (up to 28 years old at follow-up) (Lindeblad, Kroksmark, & Ekström, 2019) and five longitudinal studies in adult population have been carried out to date and the results have prompted differing conclusions. For instance, some studies have found no decline over time (Tuikka, Laaksonen, & Somer, 1993), whilst others have found a decrease in attention (Sansone *et al.*, 2007), memory (Gallais, Gagnon, Mathieu, & Richer, 2017), executive functions

(Modoni *et al.*, 2008), language (Winblad, Samuelsson, Lindberg, & Meola, 2016), information processing speed (Gallais *et al.*, 2017), and visuospatial abilities (Winblad *et al.*, 2016). In spite of the fact that in general, the results suggest a decline over time, some of these studies have also found improvements, primarily in general cognitive measures (i.e., MMSE or IQ) or in certain cognitive domains, such as executive functions (Gallais *et al.*, 2017), memory, and attention (Modoni *et al.*, 2008). Moreover, the extent to which clinical severity markers (i.e., molecular defect, muscular impairment, age, and disease duration) relate to cognitive decline over time is unclear.

The variety of results yielded by previous studies could be due to their methodological differences and constraints, including small sample sizes, limited follow-up duration, the exclusion of certain forms of disease (i.e., childhood or late-onset), the absence of comparable control groups, and a lack of information regarding other potentially relevant clinical variables.

The extensive neuropsychological data collected more than 10 years ago on a large sample of DM1 and control participants allows us to analyse the evolution of the DM1 cognitive profile over a long period of time. The aim of the present study is to analyse the progression of cognitive outcomes in a DM1 sample in comparison with a control group, where an age-related and domain-dependent cognitive decline is expected in patients. Moreover, this study aims to determine the possible predictive factors of such a decline, where a main significant effect of age is expected, whilst other factors (socio-demographic and clinical) will be considered in an exploratory manner.

Method

Participants

For this follow-up study, only participants who had attended neuropsychological assessment at baseline (DM1: $N = 145$, Controls: $N = 76$) and who still met the same inclusion criteria were eligible to take part. Exclusion criteria included congenital forms of the disease, a history of major psychiatric or somatic disorder (in accordance with DSM-IV criteria), acquired brain damage, and alcohol or drug abuse. Inclusion criteria were being aged 16 years or above, molecular confirmation of DM1 diagnosis, and being able to complete a neuropsychological assessment. Figure 1 displays a flow chart representing participants who were lost at follow-up and those who were re-tested. The retention rate for DM1 patients was 51.72% of those assessed at baseline, reaching 73.53% once decease-related drop out had been excluded. For controls, the retention rate was 71.05% of those assessed at baseline and 72.97% once deceased-related drop out had been excluded.

Considering the risk of bias due to selective attrition in cognitive longitudinal studies (Yao, Stawski, Hultsch, & Macdonald, 2016), the characteristics of patients and controls who failed to follow-up and those who were re-tested were statistically checked for equivalence, and only the same participants at baseline and follow-up were used for all other analyses (i.e., complete case analysis). Hence, participants with both baseline and follow-up assessment included in the statistical analysis comprised 75 DM1 patients (childhood [age of onset 1–10]: $n = 7$, 9.3%; juvenile [10–20]: $n = 20$, 26.7%; adult [20–40]: $n = 35$, 46.7%; late [>40 years]: $n = 13$, 17.3%) and 54 control participants (healthy relatives: $n = 32$, 59.3%; healthy non-relatives accompanying patients: $n = 10$, 18.5%; limb-girdle muscular dystrophy type 2: $n = 12$, 22.2%). The limb-girdle muscular dystrophy type 2 group was selected as being a neuromuscular disease in which CNS involvement has been ruled out (Miladi, Bourguignon, & Hentati, 1999). All participants

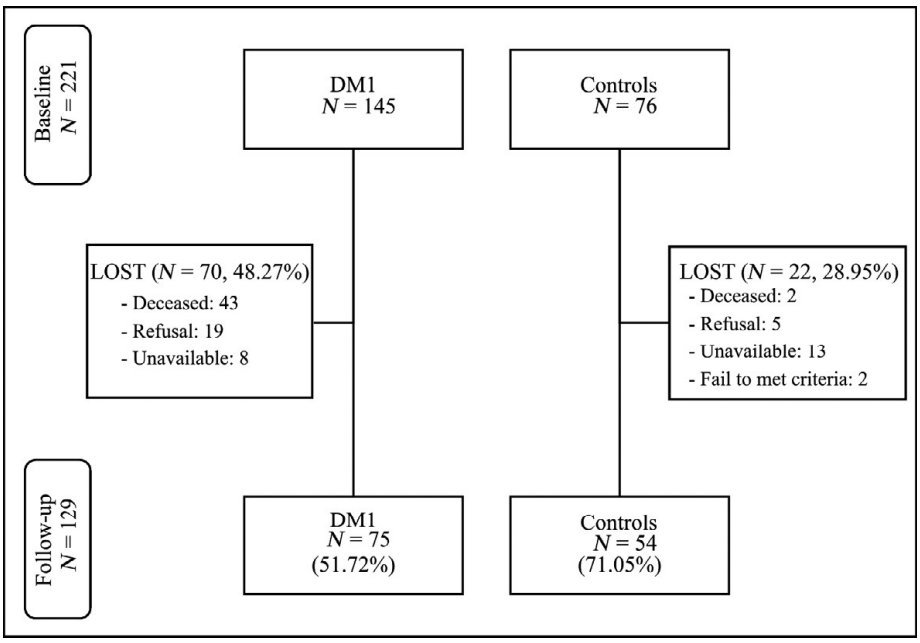


Figure 1. Flow chart showing the initial and follow-up samples.

were recruited from the outpatient service of the Neurology Department and gave written informed consent. The study was approved by the Ethics Committee of the Hospital.

Neuropsychological assessment

All patients were examined by two experienced neuropsychologists who were blind to the patient's clinical condition (CTG expansion size, clinical form, and muscular impairment). Neuropsychological assessment included the following subtests from the Wechsler Adult Intelligence Scale III (WAIS III) (Wechsler, 1999): Block design, Digit span, and Vocabulary. An estimated IQ score was calculated from a two subtest short form (block design and vocabulary) with high reliability (r_{xx} = .93) and validity (r = .87) based on Sattler and Ryan (Sattler & Ryan, 2001). Other cognitive tests used were as follows: Stroop test (Golden, 2001), California Computerized Assessment Package (CALCAP) (Miller, 1990), Rey Verbal Learning Test (RVLT) (Lezak, Howieson, & Loring, 2004), phonemic (P) and semantic (animals) verbal fluency test (Casals-Coll *et al.*, 2013; Peña-Casanova *et al.*, 2009), Rey–Osterrieth complex figure test (ROCF) (Rey, 2009), and Raven's progressive matrices (Raven, Court, & Raven, 2001).

Muscular impairment assessment

Muscular impairment was recorded by an experienced neurologist through the Muscular Impairment Rating Scale (MIRS) (Mathieu, Boivin, Meunier, Gaudreault, & Bégin, 2001) both at baseline and at follow-up. This scale evaluates muscular impairment severity according to five grades: (1) no muscular impairment, (2) minimal signs, (3) distal weakness, (4) mild to moderate proximal weakness, and (5) severe proximal weakness.

Genetic assessment

Cytosine–thymine–guanine expansion size was obtained through genetic assessment of the DMPK gene isolated from circulating leucocyte DNA. PCR was used to measure repeat

length in DMPK alleles up to approximately 100 CTG repeats and Southern blot analysis for larger expansions. At both baseline and follow-up, genetic assessment was conducted only for the patients who had no recent data (>5 years) on CTG expansion size. Accordingly, up to 97.33% of the patients repeated the assessment at follow-up.

Statistical analysis

Data were analysed using the SPSS (IBM SPSS Statistics 24, IBM, Madrid, Spain) statistical package. Intergroup comparisons, using contingency analysis (chi-square), parametric (*t* test), or non-parametric (Mann–Whitney *U* test) statistical methods where appropriate, were conducted to compare those that failed to re-test at follow-up and those who were re-tested. Analyses were carried out separately for controls and DM1 patients in order to rule out the possibility of there being a higher functioning sample in the re-tested groups (selective attrition).

In order to compare intergroup socio-demographic characteristics between controls and patients, we carried out contingency analysis (chi-square) for categorical data and a parametric *t* test for interval data. To assess possible intra-group differences between baseline and follow-up in socio-demographic (years of education) and clinical (CTG expansion size and MIRS) variables, the Wilcoxon signed-rank test was carried out.

Intergroup non-parametric comparisons (data not normally distributed) of neuropsychological outcomes at baseline were carried out in order to characterize the cognitive profile of the studied sample.

To compare the development of differences at follow-up between controls and DM1 patients on neuropsychological measures, a mixed ANOVA for repeated measures was conducted. Variables for the main effects were labelled as (1) 'group' (with two levels: DM1 patients and controls); (2) 'time' (with two levels: baseline and follow-up); and (3) time × group interaction. However, the main effect of time was not analysed here, since this was not considered to be informative for the purposes of this study. Pairwise comparisons with Bonferroni adjustment were used to interpret significant interactions.

In order to construct a regression model, only in DM1 patients were Pearson Correlation analyses were carried out between potential predictive factors at baseline and delta scores of the cognitive variables in which the time × group interaction had already been found. Sex, age, years of education, disease form, inheritance pattern (maternal or paternal), CTG expansion size, and MIRS score were selected as potential predictive factors. From the potentially predictive variables, only those with a significant correlation with the delta scores were finally included in the model, in order to determine their predictive capacity. Listwise deletion was used to deal with missing values.

Effect sizes were calculated and interpreted, *r* was calculated when non-parametric tests were used, and were interpreted as small (.10), medium (.30), and large (.50); Cohen's *d* was used when *t* test was used and was interpreted as small (.20), medium (.50), and large (.80) (Cohen, 1988).

Results

There was no difference between those controls that failed to re-test at follow-up (excluded controls) and those who were re-tested (included controls) in age, sex, and IQ. Statistically significant differences were found between the excluded and the included controls in years of education ($U = 376$; $p = .01$), with the excluded controls (mean

rank = 28.59) scoring lower than the included controls (mean rank = 42.54), with a small effect size (r = .28). Similarly, there was no difference between the excluded and included DM1 patients in sex, years of education, inheritance pattern, IQ, and CTG expansion size. Statistically significant differences were found between the excluded and the included DM1 patients in age, excluded DM1 older: $t(143) = 4.45$; $p = < .01$; Cohen's d = .73, and MIRS score (excluded DM1 greater muscular impairment: $U = 742.5$; $p = .00$; $r = .51$).

In the final re-tested sample (75 DM1 and 54 controls), there were no statistically significant intergroup differences in the follow-up in terms of sex (DM1: 45.33% male, controls: 38.88% male; x^2 (1, N = 129) = 0.53; p = .46; V = .06), or age, $t(127) = 1.41$; p = .16; Cohen's d = .25, whilst there was a statistically significant difference—although with a small effect size—in years of education, $t(127) = 2.12$; p = .04; *Cohen's d* = .37. Descriptive data and intergroup and intra-group follow-up comparisons for the main socio-demographic and clinical variables are displayed in Table 1. Only CTG expansion size showed a statistically significant difference in repeated measures. The mean duration between baseline and follow-up was 11.62 years (SD = 0.81).

The intergroup comparisons of cognitive outcome (baseline) of the re-tested sample revealed statistically significant differences in block design ($U = 1307.00$; $p = .00$; $r = .29$), Raven total score ($U = 1021.50$; $p = .00$; $r = .27$), all measures of the Stroop test (word: $U = 1184.00$; $p = .00$; $r = .27$; colour: $U = 1212.00$; $p = .00$.; $r = .25$; word-colour: $U = 1039.00$; $p = .00$; $r = .34$; interference: $U = 1267.00$; $p = .01$; $r = .23$), vocabulary ($U = 1510.00$; $p = .02$; $r = .20$), two measures of the CALCAP (Election RT: $U = 904.50$; $p = .01$; $r = .23$; Sequential 1 RT: $U = 952.00$; $p = .04$; $r = .20$) and IQ estimate ($U = 1228.50$; $p = 00$.; $r = .32$) (see Table S1 for full results).

Cognitive follow-up in DM1 vs. controls

Table 2 shows that when comparing DM1 and control participants as groups (taking into account both baseline and follow-up), the intergroup main effect reached significance for

Table 1. Baseline and follow-up data on age and comparisons for years of education, CTG expansion size, and MIRS outcome per group

	Baseline Mean (*SD*)	Follow-up Mean (*SD*)	*Z*	*p*
Age				
Control	42 (13.59)	53.44 (13.51)	–	–
DM1	38.65 (11.105)	50.40 (11.00)	–	–
Years of education				
Control	16.28 (4.45)	16.35 (4.99)	−0.34	.73
DM1	14.45 (3.95)	14.55 (4.59)	−0.03	.98
CTG				
DM1	531.54 (437.26)	651.12 (531.21)	−4.39***	.00
MIRS				
DM1	2.34 (0.883)	2.51 (1.00)	−1.40	.16

Notes. CTG = triplet expansion size; DM1 = myotonic dystrophy type 1; MIRS = Muscular Impairment Rating Scale; *SD* = standard deviation.
***$p < .001$.

Table 2. Baseline and follow-up comparison between groups (controls and DM1 patients)

		Baseline	Follow-up	Group		Time × group	
	N	Mean (*SD*)	Mean (*SD*)	*F*	*p*	*F*	*p*
WAIS III							
Block design							
Control	54	38.78 (13.29)	36.3 (13.68)	17.35***	.00	4.25*	.04
DM1	73	31.1 (11.86)	25.93 (11.93)				
Vocabulary							
Control	54	41.22 (11.48)	42.06 (9.49)	6.65*	.01	0.94	.33
DM1	73	36.77 (12.09)	36.56 (11.04)				
Digit span							
Forward							
Control	35	8.43 (1.75)	7.91 (1.94)	3.90	.05	0.16	.69
DM1	52	7.62 (2.12)	7.27 (1.85)				
Backward							
Control	35	5.97 (1.74)	5.77 (2.00)	1.20	.28	0.07	.79
DM1	52	5.63 (1.97)	5.33 (1.74)				
Total							
Control	35	14.4 (3.11)	13.69 (3.38)	2.78	.10	0.01	.92
DM1	52	13.25 (3.70)	12.6 (3.25)				
IQ estimate							
Control	54	102.85 (14.74)	108.18 (13.06)	21.99***	.00	1.09	.30
DM1	73	91.55 (16.59)	95.26 (14.84)				
RAVLT							
Immediate							
Control	51	6.41 (1.96)	6.12 (1.86)	0.01	.98	0.01	.90
DM1	75	6.44 (2.05)	6.11 (2.10)				
Total (1–5)							
Control	51	51.33 (9.51)	48.24 (9.67)	0.14	.71	0.66	.42
DM1	75	50.17 (10.15)	48.09 (10.93)				
Delayed							
Control	51	10.55 (2.67)	9.39 (3.03)	0.01	.98	0.10	.75
DM1	75	10.63 (3.01)	9.33 (3.33)				
RAVEN							
Control	45	46.36 (8.49)	43.38 (9.92)	12.50***	.00	0.21	.64
DM1	67	39.54 (12.00)	36.04 (11.36)				
CALCAP							
Simple RT							
Control	38	318.68 (54.65)	393.82 (79.07)	3.65	.06	0.22	.64
DM1	65	340.25 (74.12)	426.11 (118.22)				
Election RT							
Control	38	438 (92.96)	486.13 (66.19)	2.46	.12	0.60	.44
DM1	65	467.98 (97.06)	502.26 (73.61)				
Sequential 1 RT							
Control	38	557.61 (107.91)	614.21 (100.70)	3.76	.05	1.27	.26
DM1	65	607.26 (116.42)	643.26 (106.72)				
Sequential 2 RT							
Control	38	649.05 (104.56)	662.21 (107.87)	2.28	.13	1.04	.31
DM1	65	667.74 (137.2)	705.43 (104.52)				

Continued

Table 2. (*Continued*)

	N	Baseline Mean (*SD*)	Follow-up Mean (*SD*)	Group		Time × group	
				F	*p*	*F*	*p*
ROCF							
Copy							
Control	50	31.13 (4.4)	32.79 (3.83)	6.96**	.01	14.64***	.00
DM1	74	30.12 (5.31)	28.96 (6.76)				
Delayed recall							
Control	49	17.15 (5.94)	19.11 (5.03)	2.01	.16	9.74**	.00
DM1	74	17.34 (6.62)	15.99 (6.95)				
FLUENCY							
Semantic							
Control	50	24.16 (5.90)	23.12 (5.78)	2.08	.15	0.05	.82
DM1	74	22.85 (6.23)	21.55 (6.69)				
Phonetic							
Control	50	16.06 (6.57)	15.50 (4.82)	0.38	.54	1.15	.29
DM1	74	15.03 (5.50)	15.38 (5.59)				
STROOP							
Word							
Control	48	108.52 (17.40)	109.92 (18.59)	12.13***	.00	3.63	.06
DM1	71	99.63 (16.91)	96.94 (18.20)				
Colour							
Control	48	72.02 (12.70)	72.50 (12.21)	10.03**	.00	1.35	.25
DM1	70	65.76 (13.55)	64.27 (13.20)				
Word-Colour							
Control	48	45.40 (10.75)	44.98 (10.22)	16.31***	.00	0.01	.95
DM1	70	37.86 (10.90)	37.34 (11.53)				
Interference							
Control	48	2.27 (6.84)	1.46 (6.47)	6.83*	.01	0.74	.39
DM1	70	−1.56 (8.64)	−1.06 (7.89)				

Notes. CALCAP = California Computerized Assessment Package; DM1 = myotonic dystrophy type 1; IQ = Intelligence Quotient; RAVLT = Rey Auditory Verbal Learning Test; ROCF = Rey–Osterrieth complex figure; *SD* = standard deviation; WAIS III = Wechsler Adult Intelligence Scale III.
*$p < .05$; **$p < .01$; ***$p < .001$.

the block design test, vocabulary, estimated IQ, RAVEN's progressive matrices, copy of the ROCF, and all Stroop test variables.

In terms of cognitive change over time, the comparison of control and DM1 patients in terms of raw score progression in the repeated measures analysis revealed a statistically significant difference (time × group interaction) on the block design test, copy of the ROCF, and delayed recall of the ROCF. There were no statistically significant interactions between any other measures.

Explanatory factors of cognitive decline

Correlation analysis (Table 3) revealed no correlation between any of the delta criterion variables and sex and disease form. The statistically significant variables in the correlation analysis were introduced in the model for multiple linear regression analyses (Table 4),

Table 3. Pearson correlation analysis between significant decline delta scores and potentially predictive variables in DM1 patients

Delta scores	Sex	Years of education	Age	CTG	MIRS	Inheritance	Disease form
Block design	.05	.00	−.27*	.05	−.14	−.28*	−.09
ROCF copy	.02	.24*	−.11	−.29*	−.50**	.02	.15
ROCF delayed recall	.15	.23*	−.05	−.11	−.13	−.11	.11

Notes. CTG = triplet expansion size; DM1 = myotonic dystrophy type 1; MIRS = Muscular Impairment Rating Scale; ROCF = Rey–Osterrieth complex figure.
** $p < .01$; * $p < .05$.

Table 4. Multiple linear regression analyses for delta scores in DM1 patients

	β	*t*	*p*	Adjusted R^2	*F*	*p*
Block design						
Years of education	−.15	−0.99	.33	.12	2.55*	.04
Age	−.33	−2.19	.03			
CTG	−.13	−0.75	.45			
MIRS	−.13	−0.88	.38			
Inheritance	−.27	−1.93	.06			
ROCF copy						
Years of education	.22	1.67	.10	.30	5.68***	.00
Age	−.23	−1.67	.10			
CTG	.02	0.12	.91			
MIRS	−.45	−3.37	.00			
Inheritance	.01	0.07	.94			
ROCF delayed recall						
Years of education	.33	2.08	.04	.04	1.51	.20
Age	−.02	−0.14	.88			
CTG	−.00	−0.01	.99			
MIRS	−.10	−0.63	.53			
Inheritance	−.14	−0.99	.33			

Notes. CTG = triplet expansion size; DM1 = myotonic dystrophy type 1; MIRS = Muscular Impairment Rating Scale; ROCF = Rey–Osterrieth complex figure.
*$p < .05$; ***$p < .001$.

revealing a statistically significant effect of age and MIRS score in predicting the progressive decline in DM1 patients when measured by the block design test and copy of the ROCF, respectively. An increase in age predicted higher delta scores (greater decline) in block design, and increase in MIRS score predicted higher delta scores on ROCF copy.

Additionally, the effect of age on block design decline was further analysed using locally weighted scatterplot smoothing (LOWESS) (Figure 2). The graph indicates a cut-off point at 31 years of age for greater decline.

Discussion

To the best of our knowledge, this is the first longitudinal study that compares DM1 and control subjects in neuropsychological terms. Although it was not the main aim of the

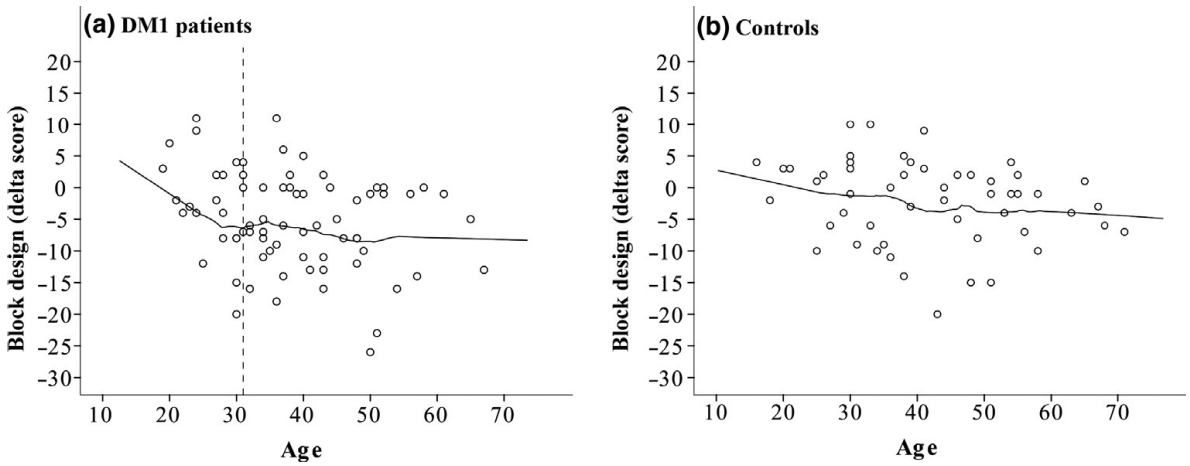


Figure 2. Locally weighted scatterplot smoothing for age on block design test delta scores in myotonic dystrophy type 1 patients (a) and controls (b).

present study to analyse the cognitive profile of DM1, a transversal comparison of both groups confirmed that the cognitive profile of our sample is in line with the pattern of dysexecutive and visuoconstructive deficits described previously in DM1, suggesting the involvement of fronto-parietal areas (Peric *et al.*, 2014).

From a longitudinal point of view, only some of the difficulties described in the cognitive profile of DM1 showed a decline over time greater than that expected for normal ageing. We failed to find a greater decline in patients compared with controls in attention, processing speed, verbal memory, language, executive functions, or IQ. These are the functions for which other studies have found statistically significant differences at follow-up, although both decline and improvements have been reported, depending on the study. Whilst some authors found improvements on naming and fluency tests (Gallais *et al.*, 2017), others have found a decline in language (Modoni *et al.*, 2008). Similarly, some authors have found a decrease in verbal memory (Gallais *et al.*, 2017) whilst others have found the opposite pattern of results (Modoni *et al.*, 2008).

However, our results appear to suggest a domain-specific pattern of cognitive decline in DM1. In particular, visuoconstructive functioning emerged as the only cognitive domain to be vulnerable to the passage of time. In our sample, there was a significant decline in block design and in the copy and delayed recall of the ROCF. Among the most recent four longitudinal studies, two of these employed the block design test and both found a decline over time (Gallais *et al.*, 2017, non-significant; Winblad *et al.*, 2016), which is in line with the results found in this study. Although all the longitudinal studies mentioned used the ROCF, consensus regarding the outcomes of this test is rather less clear. Whilst Gallais *et al.* (2017) and Sansone *et al.* (2007) found a non-significant decline in the copy subtest, Modoni *et al.* (2008) and Winblad *et al.* (2016) found no changes.

Several reasons could account for the discrepancies found between our results and those of others. Apart from the variations regarding sample sizes or the elapsed time between baseline and follow-up, the fact that disease duration and mean age at baseline and at follow-up are different across studies is of special interest. Thus, each study covers a different point in the life span, which could be directly related to the worsening (or absence of it) found. Moreover, DM1 is a neurologic condition that may affect the course of development of cognitive function and decline. Indeed, as already reported in DM1 patients (Gallais *et al.*, 2017), it could be hypothesized that the decline in various cognitive functions occurs at different points during the life span.

Regarding the domain-specific cognitive decline found in the present study, predictive factors were analysed in order to search for possible markers of this early deterioration in DM1 patients. The predictive model employed in this study was able to explain up to 12.4% of the variance in change in the performance on block design between the first and second neuropsychological test, with age emerging as the only statistically significant predictor. Further analysis of this effect revealed a cut-off point at 31 years of age, suggesting that the decline in this cognitive ability in DM1 starts early in life and continues into the thirties. From this age onwards, this decline remains stable with the higher delta scores, which is in line with the hypothesis of an accelerated ageing process that has already been suggested in DM1 (Mateos-Aierdi *et al.*, 2015). Functional sequential studies with functional RM could help to further deepen knowledge regarding the selective vulnerability of certain neuronal circuits. Further, muscular impairment was the only significant predictor for the decline in ROCF copy, reaching 29.9% of the variance explained by the model. Conversely, the model was not able to significantly explain the decline in visual memory.

In line with the involvement of visuospatial/visuoconstructive impairment in DM1, a recent meta-analysis on cognitive impairments in DM1 (Okkersen *et al.*, 2017) found the largest effect sizes on visuospatial perception tasks and one of the largest effects on visuoconstructive tasks. This suggests that visuospatial/visuoconstructive deficits could be among the most sensitive outcomes in the DM1 cognitive profile. Taken together, from a translational point of view these results provide clinicians and researchers with relevant information for selecting neuropsychological assessment tools for this population. Thus, the inclusion of tests such as block design and ROCF could be suggested as a first-line option, not only for describing the cognitive profile of this population, but also as possible markers of cognitive decline.

Moreover, although the use of time-dependent and graphomotor tasks can be questioned in the assessment of neuromuscular disorders with distal muscular involvement, the results of a recent study support the use of block design in DM1, since speed difficulties derived from peripheral muscle weakness have been ruled out (Hamilton *et al.*, 2018). Whilst ROCF has been extensively used in DM1, it is rather more difficult to rule out the possibility that distal muscle impairment could affect the copy, particularly when taking into account the fact that the MIRS score significantly predicted the change in performance between baseline and follow-up in copy of the ROCF. However, beyond the visuoconstructive load in ROCF, there is a considerable organizational component that could be compromised in this population. Indeed, in other neurological conditions with motor affectation such as traumatic brain injury, executive functions have been found to account for a large proportion of the performance on both copy and memory of ROCF, whilst fine motor ability failed to correlate with these measures (Schwarz, Penna, & Novack, 2009). Using an alternative scoring approach such as the Boston Qualitative Scoring System for the ROCF (Stern *et al.*, 1999) or the Developmental Scoring System for the ROCF (Bernstein & Waber, 1996) could help to better understand the underlying cognitive mechanisms involved.

Some limitations of the present study should be taken into account. Firstly—and inherent in all longitudinal studies—selective attrition must be considered. We believe we addressed this issue by employing all possible statistic control methods to minimize the effect. In any case, the intergroup differences found between those who failed to follow-up and those who were re-tested (the former were older and with greater muscular impairment) could be masking an even greater difference in decline between DM1 and controls. In fact, age and MIRS score were negatively correlated with target delta scores.

Moreover, given that clinical heterogeneity is a hallmark of DM1, a larger sample size would allow for comparing patterns of decline between groups according to different variables such as inheritance pattern or disease form. Finally, IQ had to be estimated from a reduced set of subtests instead of administering the whole WAIS III scale due to time constraints and in order to avoid the well-documented fatigue in patients (Kalkman *et al.*, 2005).

Nonetheless, this study was based on a large and carefully selected control group with both assessments at baseline and follow-up, which constitutes a notable strength of this work, since there are no previous DM1 longitudinal studies that include a control group. Further, the comprehensive and extensive neuropsychological battery employed complies with most of the latest recommended guidelines for cognitive assessment in DM1 (Gagnon *et al.*, 2013). Finally, whilst a high mortality rate was observed among our DM1 sample (29.65% of patients at baseline died), a re-assessment rate of almost 60% of the total sample is noteworthy, particularly for a follow-up of such long duration.

Taken together, the results of the present study show evidence for visuoconstructive decline as a reliable cognitive marker of ageing in DM1. However, the possible explanatory factors for cognitive decline remain an issue that requires further analysis. Topography related to the suggested cognitive dysfunction could be taken to indicate the existence of circuits and neurons that are particularly vulnerable to ageing processes. To this end, the inclusion of neuropathological/neuroimaging data in longitudinal studies is strongly recommended in order to clarify the accelerated ageing process in DM1 and its possible cerebral biomarkers.

Acknowledgements

The present study has been supported by funding from CIBERNED (Ref: 609), from the Institute of Health Carlos III co-founded by Fondo Europeo de Desarrollo Regional-FEDER (Ref: PI17/01231 and PI17/01841), and from the Basque Government (S-PE13UN030). G. Labayru was supported by a predoctoral grant from the Basque Government (PRE_2016_1_0187).

References

Bernstein, J. H., & Waber, D. P. (1996). *Developmental scoring system for the Rey-Osterrieth Complex Figure: Professional manual*. Lutz, FL: Psychological Assessment Resources Inc.

Brook, J. D., McCurrach, M. E., Harley, H. G., Buckler, A. J., Church, D., Aburatani, H., . . . Housman, D. E. (1992). Molecular basis of myotonic dystrophy: Expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. *Cell*, *68*, 799–808. https://doi.org/10.1016/0092-8674(92)90154-5

Casals-Coll, M., Sánchez-Benavides, G., Quintana, M., Manero, R. M., Rognoni, T., Calvo, L., . . . Peña-Casanova, J. (2013). Spanish normative studies in young adults (NEURONORMA young adults project): norms for verbal fluency tests. *Neurology*, *28*(1), 33–40. https://doi.org/10.1016/j.nrl.2012.02.010

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdate, NJ: LEA.

Gagnon, C., Meola, G., Hébert, L. J., Puymirat, J., Laberge, L., & Leone, M. (2013). Report of the first Outcome Measures in Myotonic Dystrophy type 1 (OMMYD-1) international workshop. Clearwater, Florida, November 30, 2011. *Neuromuscular Disorders*, *23*, 1056–1068. https://doi.org/10.1016/j.nmd.2013.07.004

Gallais, B., Gagnon, C., Mathieu, J., & Richer, L. (2017). Cognitive decline over time in adults with myotonic dystrophy type 1: A 9-year longitudinal study. *Neuromuscular Disorders*, *27*(1), 61–72. https://doi.org/10.1016/j.nmd.2016.10.003

Golden, C. J. (2001). *STROOP. Color and word test* (3rd ed.). Madrid, Spain: TEA Ediciones.

Hamilton, M. J., Mclean, J., Cumming, S., Ballantyne, B., Mcghie, J., Jampana, R., . . . Farrugia, M. E. (2018). Outcome measures for central nervous system evaluation in myotonic dystrophy type 1 may be confounded by deficits in motor function or insight. *Frontiers in Neurology*, *9*, 1–15. https://doi.org/10.3389/fneur.2018.00780

Harper, P. S. (2001). *Myotonic dystrophy* (3rd ed.). London, UK: Saunders.

Kalkman, J. S., Schillings, M. L., van der Werf, S. P., Padberg, G. W., Zwarts, M. J., van Engelen, B. G., & Bleijenberg, G. (2005). Experienced fatigue in facioscapulohumeral dystrophy, myotonic dystrophy, and HMSN-I. *Journal of Neurology, Neurosurgery and Psychiatry*, *76*, 1406–1409. https://doi.org/10.1136/jnnp.2004.050005

Lezak, M., Howieson, D. B., & Loring, D. (2004). *Neuropsychological assessment* (4th ed.). New York, NY: Oxford University Press.

Lindeblad, G., Kroksmark, A.-K., & Ekström, A. B. (2019). Cognitive and adaptive functioning in congenital and childhood forms of myotonic dystrophy type 1: A longitudinal study. *Developmental Medicine and Child Neurology*, 1–7. https://doi.org/10.1111/dmcn.14161

López de Munain, A., Blanco, A., Emparanza, J. I., Poza, J. J., Martí Massó, J. F., Cobo, A., . . . Martínez Lage, J. M. (1993). Prevalence of myotonic dystrophy in Guipúzcoa (Basque Country, Spain). *Neurology*, *43*, 1573–1576.

Mateos-Aierdi, A. J., Goicoechea, M., Aiastui, A., Torrón, R. F., Garcia-Puga, M., Matheu, A., & López de Munain, A. (2015). Muscle wasting in myotonic dystrophies: A model of premature aging. *Frontiers in Aging Neuroscience*, 7, 1–16. https://doi.org/10.3389/fnagi.2015.00125

Mathieu, J., Boivin, H., Meunier, D., Gaudreault, M., & Bégin, P. (2001). Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. *Neurology*, *56*, 336–340. https://doi.org/10.1212/WNL.56.3.336

Miladi, N., Bourguignon, J., & Hentati, F. (1999). Cognitive and psychological profile of a Tunisian population of limb girdle muscular dystrophy. *Neuromuscular Disorders*, *9*, 352–354.

Miller, E. N. (1990). *CalCAP: California Computerized Assessment Package*. Los Angeles, CA: Norland Software.

Minnerop, M., Gliem, C., & Kornblum, C. (2018). Current progress in CNS imaging of myotonic dystrophy. *Frontiers in Neurology*, *9*, 1–21. https://doi.org/10.3389/fneur.2018.00646

Modoni, A., Silvestri, G., Vita, M. G., Quaranta, D., Tonali, P. A., & Marra, C. (2008). Cognitive impairment in myotonic dystrophy type 1 (DM1): A longitudinal follow-up study. *Journal of Neurology*, *255*, 1737–1742. https://doi.org/10.1007/s00415-008-0017-5

Okkersen, K., Buskes, M., Groenewoud, J., Kessels, R. P. C., Knoop, H., van Engelen, B., & Raaphorst, J. (2017). The cognitive profile of myotonic dystrophy type 1: A systematic review and meta-analysis. *Cortex*, *95*, 143–155. https://doi.org/10.1016/j.cortex.2017.08.008

Peña-Casanova, J., Quiñones-Úbeda, S., Gramunt-Fombuena, N., Quintana-Aparicio, M., Aguilar, M., Badenes, D., . . . Blesa, R. (2009). Spanish Multicenter Normative Studies (NEURONORMA Project): Norms for verbal fluency tests. *Archives of Clinical Neuropsychology*, *24*, 395–411. https://doi.org/10.1093/arclin/acp042

Peric, S., Mandic-Stojmenovic, G., Stefanova, E., Savic-Paviceciv, D., Pesovic, J., Ilic, V., . . . Rakocevic-Stojanovic, V. (2014). Frontostriatal dysexecutive syndrome: A core cognitive feature of myotonic dystrophy type 2. *Journal of Neurology*, *262*(1), 142–148. https://doi.org/10.1007/s00415-014-7545-y

Raven, J. C., Court, J. H., & Raven, J. (2001). *Raven: Standard progressive matrices*. Madrid, Spain: TEA Ediciones.

Rey, A. (2009). *Copy and memory reproduction test of complex geometric figures*. Madrid, Spain: TEA Ediciones.

Sansone, V., Gandossini, S., Cotelli, M., Calabria, M., Zanetti, O., & Meola, G. (2007). Cognitive impairment in adult myotonic dystrophies: A longitudinal study. *Neurological Sciences*, *28*(1), 9–15. https://doi.org/10.1007/s10072-007-0742-z

Sattler, J. M., & Ryan, J. J. (2001). Wechsler Adult Intelligence Scale-III (WAIS-III): Description. In J. M. Sattler (Ed.), *Assessment of children. Cognitive applications* (4th ed.). San Diego, CA: Jerome M Sattler Publisher, Inc.

Schwarz, L., Penna, S., & Novack, T. (2009). Factors contributing to performance on the Rey Complex Figure Test in individuals with traumatic brain injury. *The Clinical Neuropsychologist*, *23*(2), 255–267. https://doi.org/10.1080/13854040802220034

Sistiaga, A., Urreta, I., Jodar, M., Cobo, A. M., Emparanza, J., Otaegui, D., . . . López de Munain, A. (2010). Cognitive/personality pattern and triplet expansion size in adult myotonic dystrophy type 1 (DM1): CTG repeats, cognition and personality in DM1. *Psychological Medicine*, *40*(3), 487–495. https://doi.org/10.1017/S0033291709990602

Stern, R. A., Javorsky, D. J., Singer, E. A., Harris, N. G. S., Somerville, J. A., Duke, L. M., . . . Kaplan, E. (1999). *BQSS: The Boston Qualitative Scoring System for the Rey-Osterrieth Complex Figure*. Odessa, FL: Psychological Assessment Resources Inc.

Tuikka, R. A., Laaksonen, R. K., & Somer, V. K. (1993). Cognitive function in myotonic dystrophy: A follow-up study. *European Neurology*, *33*, 436–441.

Turner, C., & Hilton-Jones, D. (2010). The myotonic dystrophies: Diagnosis and management. *Journal of Neurology, Neurosurgery and Psychiatry*, *81*, 358–367. https://doi.org/10.1136/jnnp.2008.158261

Wechsler, D. (1999). *WAIS-III: Wechsler Adult Intelligence Scale*. Madrid, Spain: TEA Ediciones.

Wenninger, S., Montagnese, F., & Schoser, B. (2018). Core clinical phenotypes in Myotonic Dystrophies. *Frontiers in Neurology*, *9*, 1–9. https://doi.org/10.3389/fneur.2018.00303

Winblad, S., Samuelsson, L., Lindberg, C., & Meola, G. (2016). Cognition in myotonic dystrophy type 1: A 5-year follow-up study. *European Journal of Neurology*, *23*, 1471–1476. https://doi.org/10.1111/ene.13062

Yao, C., Stawski, R. S., Hultsch, D. F., & Macdonald, S. W. S. (2016). Selective attrition and intraindividual variability in response time moderate cognitive change. *Journal of Clinical and Experimental Neuropsychology*, *38*, 227–237. https://doi.org/10.1080/13803395.2015.1102869

Received 31 January 2019; revised version received 24 July 2019

Supporting Information

The following supporting information may be found in the online edition of the article:

Table S1. Inter-group comparison on neuropsychological outcomes at baseline.

MANUSCRIPTS UNDER REVIEW

Annex 4

Neurodegeneration trajectory in pediatric and adult/late DM1: A follow-up MRI study across a decade

Running head: Neurodegeneration in DM1: a follow-up MRI study

Garazi Labayru MsC [1,2,3], Antonio Jimenez-Marin MsC [4,5], Esther Fernández MD [6], Jorge Villanua PhD [6], Miren Zulaica BSc [2,3], Jesus M. Cortes PhD [4,7,8], Ibai Díez PhD [9,10,11], Jorge Sepulcre PhD [9,10], Adolfo López de Munain PhD [2, 3,12,13], Andone Sistiaga PhD [1,2,3]

[1] Personality, Assessment and psychological treatment department; Psychology Faculty, University of the Basque Country (UPV/EHU), San Sebastián, Gipuzkoa (Spain)

[2] Neuroscience Area, Biodonostia Research Institute, Osakidetza, Donostia-San Sebastián, Gipuzkoa (Spain)

[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Institute Carlos III, Madrid, Spain

[4] Biocruces-Bizkaia Health Research Institute, Barakaldo, Bizkaia (Spain)

[5] Biomedical Research Doctorate Program, University of the Basque Country (UPV/EHU), Leioa (Spain)

[6] Osatek, Donostia University Hospital, Donostia- San Sebastián, Gipuzkoa, (Spain)

[7] Cell Biology and Histology Department, University of the Basque Country (UPV/EHU), Leioa, Spain

[8] IKERBASQUE, The Basque Foundation for Science, Bilbao, Spain

[9] Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States

[10] Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

[11] Neurotechnology Laboratory, Tecnalia Health Department, Derio, Spain

[12] Neurology Department, Donostia University Hospital, Donostia- San Sebastián, Gipuzkoa, (Spain)

[13] Neuroscience Department, University of the Basque Country (UPV/EHU), Donostia-San Sebastián, Gipuzkoa, Spain

Corresponding author: Garazi Labayru Isusquiza.

University of the Basque Country, Psychology Faculty

Avda. Tolosa, 70. 20018 Donostia- San Sebastián (Gipuzkoa); Spain.

garazi.labayru@ehu.eus Phone: +0034943018382

Number of characters in the title: 99

Number of characters in the running head: 47

Number of words in the abstract: 250

Number of words in the body of the manuscript: 4470

Number of figures, color figures and tables: 2,2,3, respectively

Abstract

Objective: To characterize the progression of brain structural abnormalities in pediatric and adult/late onset DM1, as well as to examine the potential predictive markers of such progression.

Methods: 21 DM1 patients (pediatric onset: N = 9; adult/late onset: N = 12) and 18 healthy controls (HC) were assessed longitudinally over 9.17 years through brain MRI. Additionally, patients underwent neuropsychological, genetic, and muscular impairment assessment. Inter-group comparisons of total and voxel-level regional brain volume were conducted through Voxel Based Morphometry (VBM); cross-sectionally and longitudinally, analyzing the associations between brain changes and demographic, clinical, and cognitive outcomes.

Results: Pediatric and adult/late onset patients showed lower total gray matter (GM) than HC (baseline and follow-up). Longitudinally, the percentage of GM loss did not differ in any of the groups compared with HC. Regional VBM analyses revealed subcortical GM damage in both DM1 groups, evolving to frontal regions in the pediatric onset patients. Muscular impairment and the outcomes of certain neuropsychological tests were significantly associated with follow-up GM damage, whilst visuoconstruction, attention, and executive function tests showed sensitivity to WM degeneration over time.

Interpretation: Distinct patterns of brain atrophy and its progression over time in pediatric and adult/late onset DM1 patients are suggested. Results indicate a possible neurodevelopmental origin of the brain abnormalities in DM1, along with the possible existence of an additional neurodegenerative process. Fronto-subcortical networks appear to be involved in the disease progression at young adulthood in pediatric onset DM1 patients. The involvement of a multimodal integration network in DM1 is discussed.

Keywords: Myotonic Dystrophy Type 1; Neurodegeneration; MRI; Voxel Based Morphometry; Follow-up

Introduction

Myotonic Dystrophy type 1 (DM1) is the most common form of adult muscular dystrophy. It is an autosomal dominant disorder affecting multiple systems. The severity of symptoms vary according to age of onset, with earlier onset patients being more severely affected.

Aside from the known progressive nature of the muscular impairment, many of the clinical symptoms have been suggested to be part of an accelerated aging process. CNS studies with a focus on brain pathology have defined DM1 as a combination of tauopathy, spliceopathy and RNAopathy, all of which contribute to neurodegeneration [1]. Further, from a neuropsychological perspective, recent findings suggest that cognitive functions suffer a decline in several areas, beyond that expected in normal aging [2–6].

Although neuroimaging data are thought to support the previously suggested hypothesis of neurodegenerative processes in DM1, to the best of our knowledge, there have been only two previous attempts to longitudinally study a hypothesized progressive impairment in brain structures. While Gliem et al. [7]did not find a greater volume loss over time in DM1 for either gray or white matter tissue, Conforti et al. found a progression in white matter lesions along with greater brain atrophy assessed by the ventricular/brain ratio [8].

The aim of this study was to longitudinally assess the structural brain changes of DM1 patients over a period of more than 9 years and to delineate the pathway of disease progression. Additionally, we aimed to examine the potential genetic, muscular, clinical, and cognitive markers of this progression.

Materials and methods

Participants

The DM1 patients analyzed in this work were selected from those attending the Neurology Department of the Donostia Universitary Hospital (Gipuzkoa, Spain). Healthy controls (HC) included accompanying relatives and non-relatives of DM1 patients using the service.

Inclusion criteria for DM1 patients included being older than 18 years with molecular confirmation of the clinical diagnosis. Patients were excluded if any of the following criteria were met both at baseline and at follow-up: congenital form, history of major psychiatric or somatic disorder, acquired brain damage or alcohol or drug abuse, presence of corporal paramagnetic body devices that could impede an MRI study, and the presence of cerebral anomalies that could affect the volumetric analysis. HC participants were required to satisfy the same inclusion criteria, except for the clinical diagnosis. The DM1 participants were classified into two groups according to their age of onset based on the most recently proposed classification (OMMYD-4): pediatric onset DM1 when age of onset was between 1-18 years old, and adult and late onset DM1 (from now on adult/late DM1) when patients had either adult onset (18-40 years old) or late onset (>40 years old).

Figure 1 shows the flow-chart of the recruitment process. From the healthy volunteers with a valid MRI at baseline, only those whose age, gender and years of education were equal or closely similar to any re-scanned patient were invited to participate in order to form demographically equivalent groups. The first 20 participants that agreed to be re-scanned were suitable for forming two groups equivalent in sex, age, and years of education with a valid sample size for purposes of comparison with each of the DM1 groups.

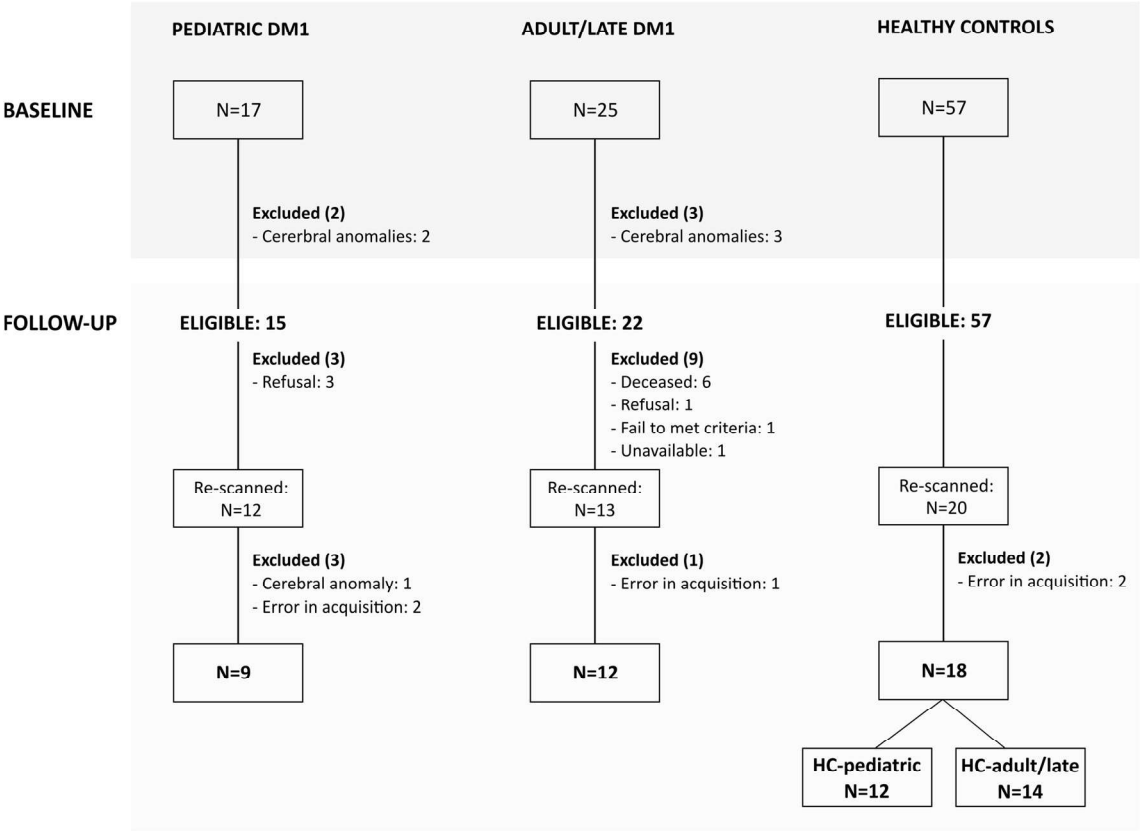


Figure 1. Flow-chart of sample recruitment from baseline to follow-up. DM1 = Myotonic Dystrophy Type 1; HC =healthy controls.

A final group of 21 DM1 patients (9 pediatric and 12 adult/late) and one of 18 HC were included for the analysis. The 18 HC controls were then subdivided to form the two comparison groups: one for comparisons with pediatric onset DM1 (HC-pediatric: N=12) and one for comparisons with adult/late onset DM1 (HC-adult/late: N=14). The healthy volunteers were included to form each control group in a controlled selection process, ensuring gender equivalence, and mean age differences between groups of less than 4 years, which were non-significant and with small effect sizes. All participants were informed of the objectives and details of the study and signed an informed consent form. The study was approved by the Ethics Committee of the Donostia University Hospital.

Clinical and Neuropsychological assessment

Clinical data were extracted from medical records. Additionally, both at baseline and at follow-up, all patients were clinically examined by a neurologist with the Muscular Impairment Rating Scale (MIRS) [9] and underwent a neuropsychological assessment.

All patients were examined by an experienced neuropsychologist who was blind to the patient's clinical condition (CTG expansion size, clinical form, maternal or paternal inheritance pattern, muscular impairment, and MRI results). Neuropsychological assessment included the following subtests from the Wechsler Adult Intelligence Scale III (WAIS III) [10]: Block design, Digit span, Object assembly, Arithmetic, Similarities and Vocabulary. An estimated IQ score was calculated from a two subtest short form (Block design and Vocabulary) with high reliability (r_{xx}=.93) and validity (r=.87) based on Sattler and Ryan [11]. Other cognitive tests used were: Stroop test [12], California Computerized Assessment Package (CALCAP) [13], Rey Auditory Verbal Learning Test (RAVLT) [14], phonemic (P) and semantic (animals) verbal fluency test [15,16], Rey-Osterrieth Complex Figure test (ROCF) [17], Raven's progressive matrices [18], Benton's Judgement of Line Orientation [19] and the Wisconsin Card Sorting Test [20]. Raw scores were converted into standardized T values according to Spanish norms for each test.

MRI acquisition and Data preprocessing

MR scanning was conducted on a 1.5 Tesla scanner (Achieva Nova, Philips). The current results are based on a high-resolution volumetric "turbo field echo" (TFE) series (Sagital 3D T1 weighted acquisition, TR = 7.2, TE = 3.3, flip angle = 8, matrix = 256 x 232, slice thickness 1mm, voxel dimensions of 1mm x 1mm x 1mm, NSA = 1, no slices 160, gap= 0, total scan duration 5´34¨). All the scans, both at baseline and at follow-up, were acquired on the same MR scanner.

To study voxel-based GM volume loss in DM1 patients and its association with different clinical and neuropsychological outcomes, FSL (version 6.01) Voxel Based Morphometry (VBM) was used [21], which is an optimized VBM protocol [22] carried out with FSL tools [23]. First, structural

images were brain-extracted and GM-segmented before being registered to the MNI 152 standard space using non-linear registration [24]. The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific GM template. Second, all native GM images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated GM images were then smoothed with an isotropic Gaussian kernel with a sigma of 3.

To estimate global brain tissue volume, normalized for subject head size, the SIENAX tool was used [25].

Statistical analysis

For all statistical analyses listwise deletion was used to deal with missing values. Demographic (sex, age at baseline, age at follow-up, years of education) and clinical (CTG repeats, inheritance pattern, and MIRS score) data were analyzed using the SPSS (IBM SPSS Statistics 24) statistical package. Inter-group comparisons were conducted to compare DM1 patients and HC, as well as the two DM1 groups, using contingency analysis (Chi-square) for categorical data and parametric (t-test) or non-parametric (Mann-Whitney U) for interval data, where appropriate. In order to dismiss the possibility of a higher functioning sample in the re-tested groups (selective attrition), the same analyses were employed to compare those patients that failed to re-test at follow-up and those who were retested. Intra-group analysis of the longitudinal evolution of clinical and neuropsychological data was conducted using the Wilcoxon signed-rank test.

In order to address the general objective of this study, three main analyses were carried out:

1. **Total GM and WM volume analyses: Cross-sectional and longitudinal: Intra-group and inter-group comparisons**

Total GM and WM volume comparisons, both inter-group and intra-group, were analyzed using the SPSS statistical package. All analyses were conducted by correcting the volume with the head size. Inter-group cross-sectional comparisons of total GM and WM volumes were conducted to compare DM1 patients and HC at baseline and at follow-up, using a parametric t-test (the data met the assumptions for parametric tests). Longitudinal analyses of GM and WM volumes were separately assessed for DM1 patients and HC using the Wilcoxon signed-rank test. Finally, the percentage of volume loss from baseline to follow-up in each group was calculated and comparisons were made between each DM1 group and their HC group, using a univariate ANOVA test corrected for time between the baseline and the follow-up scan.

2. Regional GM volume analysis from baseline to follow-up: VBM inter-group analysis

A general linear model was used to compute the inter-group statistical analysis using FSL, controlling for age and head size. All the results were obtained using two-tailed tests and corrected for multiple comparisons using the Monte Carlo simulation cluster-wise correction, as implemented in the AFNI software (version 19.3.00) (https://afni.nimh.nih.gov/) with 10,000 iterations to estimate the probability of false positive clusters with a p value<0.05.

3. Association between brain volume and demographic, clinical, and neuropsychological outcomes: VBM intra-group analysis

To study the potential predictive capacity of demographic, clinical, and neuropsychological variables at baseline, three separate analyses were conducted.

Firstly, in order to assess the predictive capacity of these variables at baseline (CTG, MIRS, years of education, disease inheritance and neuropsychological scores) for volume loss (volume variations between follow-up and baseline), partial correlation analyses controlling for age, head size and time span from baseline to follow-up scan were conducted. The results were further

corrected by multiple comparisons using the false discovery rate (FDR) strategy. These analyses were conducted separately for pediatric and adult/late onset DM1 groups.

Secondly, in order to assess the predictive capacity of the same variables for the image at follow-up, partial correlation analyses were conducted, controlling for age, head size and time from baseline assessment to follow-up scan, between predictive variables and global GM and WM volume at follow-up. These analyses were conducted separately for pediatric and adult/late onset DM1 groups.

Finally, to assess the association between the previous variables and regional GM volume at follow-up, a general linear model analysis was applied to evaluate the relationship between GM volume and CTG, MIRS clinical scale, years of education, and inheritance pattern of the disease, as well as the outcomes of neuropsychological tests. The results were controlled for age, head size and time span between the baseline scan and the follow-up (except for neuropsychological variables, which were adjusted for time between the baseline neuropsychological assessment and the follow-up scan). The statistical tests were two-tailed and corrected for multiple comparisons using Monte Carlo simulations with cluster-wise correction after 10,000 iterations to estimate the probability of false positive clusters with a p value<0.05. After corrections, a mask was applied using the results of the group difference to look for any overlap with the regions where DM1 patients show a significant GM volume loss compared with controls. Only clusters of more than 50 contiguous voxels were reported. Analyses were conducted separately for pediatric and adult/late onset DM1 groups.

Results

Statistically significant differences were found between the excluded and the included DM1 patients for the following variables: CTG expansion size at baseline ($t(34)= 3.276$; $p= .002$, $d= 1.1$) with more repetitions in the excluded DM1 (mean= 1002.88, SD= 432.88) than the included DM1 (mean= 534.4, SD= 421.21) and MIRS score at baseline ($U= 45.5$; $p= .001$; $r= 0.58$), with the

excluded DM1 presenting greater muscular impairment (mean= 3.62, SD= 0.87, mean rank= 24.5) than the included DM1 (mean= 2.29, SD= 0.96 mean rank= 13.17). No difference was found between the excluded and the included DM1 patients in terms of sex, inheritance pattern, years of education, age, and IQ estimate.

The demographic and clinical characteristics of the sample are summarized in Table 1 and Table 2. None of the DM1 patient groups differed from the corresponding HC groups in terms of sex, age at baseline, age at follow-up or years of education. Pediatric onset DM1 patients were significantly younger and had a greater CTG expansion at baseline than adult/late onset DM1 patients. Both groups showed a significant increase in MIRS score and CTG repeat size from baseline to follow up. The mean time from baseline to follow-up was 9.17 (SD=0.47) years for the complete sample. Neuropsychological outcomes of the DM1 groups are shown in Supplementary Table 1.

Table 1. Baseline and follow-up demographic characteristics of the sample divided into 4 subgroups: pediatric DM1, adult/late DM1, HC-pediatric, HC-adult/late.

		Pediatric DM1 (N=9)		**HC-pediatric (N=12)**				
		Mean/N(%)	**(SD)**	**Mean/N(%)**	**(SD)**	**Statistic**	***P***	**Effect size**
Sex	Male	4 (44.4%)		5 (41.7%)		X^2=.016	.899	*V*=.028
	Female	5 (55.6%)		7 (58.3%)				
Age at baseline		30	(6.59)	33.5	(8.32)	*t*=1.039	.312	*d*=.46
Age at follow up		39.67	(6.61)	42.5	(8.06)	*t*=0.858	.401	*d*=.36
Years of education		14.22	(4.26)	18.89	(6.49)	*U*=22.5	.111	*r*=.35
		Adult/late DM1 (N=12)		**HC-adult/late (N=14)**				
		Mean/N(%)	**(SD)**	**Mean/N(%)**	**(SD)**	**Statistic**	***P***	**Effect size**
Sex	Male	6 (50%)		8 (57.1%)		X^2=.133	.716	*V*=.071

	Female	6 (50%)		6 (42.9%)				
Age at baseline		45.83	(9.05)	43.64	(8.11)	*t*=-0.651	.521	*d*=.26
Age at follow-up		55.17	(8.89)	52.71	(8.27)	*t*=-0.728	.473	*d*=.29
Years of education		13.42	(6.68)	17.64	(5.93)	*t*=0.976	.340	d=.41

		Pediatric DM1 (N=9)		**Adult/late DM1 (N=12)**				
		Mean/N(%)	**(SD)**	**Mean/N(%)**	**(SD)**	**Statistic**	***P***	**Effect size**
Sex	Male	4 (44.4%)		6 (50%)		X^2=0.064	.801	*V*=.055
	Female	5 (55.6%)		6 (50%)				
Age at baseline		30	(6.59)	45.83	(9.05)	*t*=-4.427	.000	*d*=1.95
Age at follow-up		39.67	(6.61)	55.17	(8.89)	*t*=-4.387	.000	*d*=1.93
Inheritance	Maternal	6 (66.7%)		3 (27.3%)		X^2=3.104	.078	*V*=.394
	Paternal	3 (33.3%)		8 (72.7%)				
CTG at baseline		791.75	(434.60)	362.83	(325.54)	*t*=2.528	.021	*d*=.1.15
CTG at follow-up		933.29	(443.86)	500.75	(504.03)	*U*=19.500	.056	*r*=.44
MIRS at baseline		2,56	(0.88)	2.08	(1)	*U*=41.000	.382	*r*=.22
MIRS at follow-up		3.11	(1.17)	2.67	(1.37)	*U*=46.500	.602	*r*=.12

Note. DM1: Myotonic Dystrophy Type 1; HC: Healthy controls; SD: Standard Deviation. Descriptive data are shown as mean and SD for age at baseline, age at follow-up and years of education. Frequency (N) and percentage (%) are shown only for sex.

Table2. Longitudinal evolution of clinical features in pediatric DM1 and adult/late DM1 groups.

			Baseline		Follow-up				
		N	**Mean**	**(SD)**	**Mean**	**(SD)**	**Statistic**	***p***	**Effect size**
Pediatric DM1	CTG	6	605.67	(313.22)	866.67	(446.24)	*t*=-3.564	.016	*d*=.85
	MIRS	9	2.56	(0.88)	3.11	(1.17)	*t*=-3.162	.013	*d*=.75
Adult/late DM1	CTG	12	362.83	(325.54)	500.75	(504.03)	*Z*=-2.31	.021	*r*=.67
	MIRS	12	2.08	(0.99)	2.67	(1.37)	*Z*=-2.33	.021	*r*=.67

Note. DM1: Myotonic Dystrophy Type 1; HC: Healthy controls; SD: Standard Deviation.

1. **Total GM and WM volume analyses: cross-sectional and longitudinal: Intra-group and inter-group analyses**

With regard to cross-sectional comparisons of brain volume, patients in the pediatric onset group presented lower volumes of both GM and WM compared with controls, both at baseline and at follow up. Conversely, the adult and late onset group obtained lower volumes only for GM when compared with controls (Table 3). Longitudinal analyses of GM and WM volume loss over time showed that none of the groups suffered a significant decrease in GM volume, and only adult/late DM1 patients and their HC group suffered a significant loss in WM volume. Nonetheless, when comparing the longitudinal variations between groups, the percentage of volume loss did not differ between pediatric onset DM1 and their controls, or between adult/late onset DM1 and their controls. However, the global GM volume decrease in the pediatric group reached 3.86% compared with a 0.63% decrease in HC, and, despite being non-significant, the effect size was large (ES=0.94). Similarly, a medium effect size (ES=0.57) was found for the difference between the percentage of GM volume loss in adult/late onset DM1 (2.71%) and HC-adult/late (0.85%).

Table 3. Cross-sectional (inter-group) and longitudinal (intra-group and inter-group) comparisons for GM and WM volume

							Intra-group Follow-up - Baseline		Intergroup comparison of percentage of volume loss			
	N	Mean	SD	DM1vsHC	Mean	SD	DM1vsHC	*Z*	*p*	Mean	*F*	*p*	g_{Hedge}
		Baseline			Follow-up								
Pediatric DM1 vs HC-pediatric													
GM volume				*t*=4.208			*t*=5.071						
DM1	9	728492.55	(59435.88)	*p*=.000	703854.60	(53407.41)	*p*=.000	-1.836	.066	-3.86¶	3.497	.078	0.94
HC	12	829069.52	(50050.01)	*d*=1.86	819800.71	(50693.01)	*d*=2.24	-1.569	.117	-0.63¶			
WM volume													
DM1	9	656340.98	(45830.39)	*t*=4.334	658010.04	(28468.26)	*t*=4.643	-0.296	.767	-0.25¶	0.097	.759	0.16
HC	12	721993.51	(22597.64)	*p*=.000	712342.09	(25040.87)	*p*=.000	-1.412	.158	-0.77¶			
				d=1.91			*d*=2.05						
Adult/late DM1 vs HC-adult/late													
GM volume				*t*=2.172			*t*=2.324						
DM1	12	756500.85	(42058.37)	*p*=.040	737651.22	(52119.48)	*p*=.029	-1.961	.050	-2.71$	1.721	.203	0.57

HC	14	787456.64	(30440.24)	*d*=.85	779936.16	(40616.75)	*d*=.91	-1.475	.140	-0.85[$]			
WM volume													
DM1	12	703532.11	(40638.08)	*t*=1.582	685715.24	(36237.65)	*t*=1.256	-3.059	.002	-2.63[$]	0.106	.748	0.14
HC	14	727201.95	(35684.99)	*p*=.127	704947.47	(41037.58)	*p*=.221	-2.856	.004	-2.97[$]			
				d=.62			*d*=.49						

Note. DM1: Myotonic Dystrophy Type 1; HC: Healthy controls; SD: Standard Deviation. Longitudinal inter-group comparisons of percentage of volume loss are calculated using time to follow-up as a covariate. [¶]Covariate value: 9.1624; [$] Covariate value: 9.0973.

2. Regional GM volume analysis from baseline to follow-up: VBM inter-group analysis

Results from the VBM analyses are depicted in Figure 2 (and Supplementary Tables 2, 3 and 4 for a detailed report on significant clusters). At baseline, areas where pediatric onset DM1 showed a decrease in GM volumes compared with the HC-pediatric group were the bilateral thalamus, caudate, putamen, parahippocampal gyrus, right hippocampus, right lingual gyrus, left Rolandic operculum, left middle and superior temporal gyrus, left Heschl's gyrus, left parietal operculum, left supramarginal gyrus, and left postcentral gyrus. At follow-up, these areas were still decreased in patients and, additionally, new cortical areas were involved, particularly the left precentral gyrus, bilateral inferior frontal gyrus (triangular part and orbital part), right inferior frontal gyrus (opercular part), bilateral parietal operculum, and left insula. Adult/late onset DM1 patients at baseline showed GM decrease in the bilateral caudate, putamen and thalamus and left insula in comparison with the HC-adult/late group, and at follow-up, these areas expanded to adjacent regions such as the right hippocampus and para-hippocampal gyrus, together with the cerebellum. Additionally, a decrease was found in the HC-adult/late group compared with adult/late onset DM1 patients, located at baseline in the following areas of the left hemisphere: inferior, middle and superior temporal gyri, middle and superior temporal poles, fusiform gyrus, inferior and middle frontal gyri (orbital part), parahippocampal gyrus, and the cerebellum. At follow-up, a significant decrease remained in only some of these areas in the HC-adult/late group compared with adult/late DM1 patients, again in the following regions of the left hemisphere: the middle and superior temporal lobes, fusiform gyrus, and the parahippocampal gyrus.

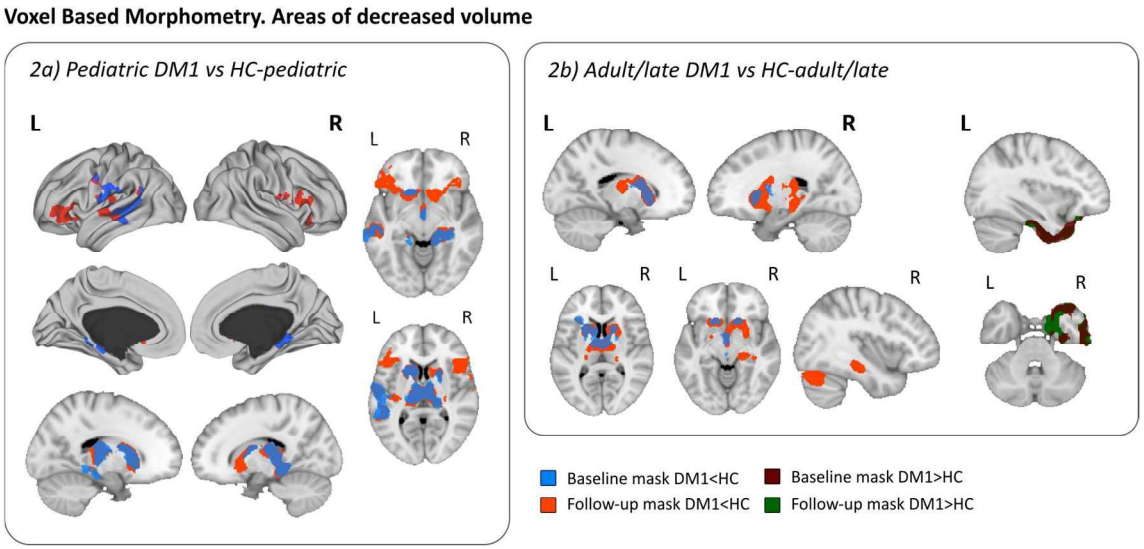


Figure 2. Voxel-Based Morphometry analyses showing significantly decreased regions in patients compared with HC at both baseline (blue) and follow-up (red). The depicted regions are those that survived multiple comparisons adjusted for age and brain size. Panel 2a) shows the masks where pediatric onset patients obtained lower gray matter values than their corresponding HC-pediatric group. Panel 2b) shows the masks where adult/late onset patients had lower gray matter values than their corresponding HC-adult/late group. The mask at baseline is represented with transparency in order to visualize the areas of overlap between baseline and follow-up (dark blue and dark brown) and the non-overlapping areas (light blue and light brown).

3. Association between brain volume and demographic, clinical, and neuropsychological outcomes: VBM intra-group analysis

For pediatric and adult/late onset DM1 patients, none of the clinical (MIRS score, CTG expansion size, inheritance pattern) or demographic (years of education) variables at baseline were significantly associated with the percentage of GM and WM volume loss from baseline to follow-up. However, among the neuropsychological tests, the ROCF copy and two CALCAP measures (Sequential 1 reaction time (RT) and Sequential 2 RT) were positively correlated with the

percentage of WM volume loss only in adult/late onset DM1 patients (Figure 3). Lower scores on neuropsychological tests were associated with a greater percentage of total WM volume loss.

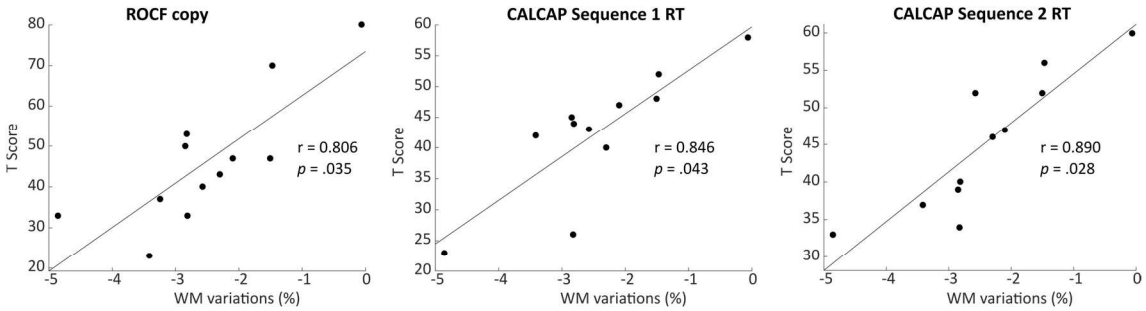


Figure 3. Partial correlation analyses of neuropsychological T scores with percentage of WM volume loss from baseline to follow-up in adult/late onset DM1 patients. Only the results that survived a false discovery rate (FDR) correction are presented.

No association was found between any clinical, demographic, or neuropsychological variable with the total GM and WM volume at follow-up in either DM1 group. However, the outcomes of some neuropsychological tests were significantly correlated with specific regions of reduced GM at follow-up. In the pediatric onset DM1 group the results were inconclusive due to the reduced sample size available in most of the neuropsychological tests, which strongly affected the sensitivity of the statistical procedure. Only the results of the Stroop task (color-word and interference) were regionally correlated with decreased GM at follow-up (data not shown). Results in the adult/late onset group are shown in Supplementary Figure 1 (see Supplementary Table 5 for a detailed report on significant clusters). To focus on the association between neuropsychological outcomes and affected brain areas, the regions defined by the follow-up mask of group differences between adult/late DM1 and HC-adult/late are indicated by black transparent shading.

The MIRS scale was the only clinical variable that significantly correlated with lower GM volume at follow-up. The specific areas where lower volumes at follow-up were associated with the MIRS score were the bilateral putamen, thalamus, left caudate, right amygdala, hippocampus,

left olfactory cortex, right anterior cingulum, left Rolandic operculum, left superior temporal gyrus, left Heschl's gyrus, left parietal operculum (particularly the OP4 area), left supramarginal gyrus, left postcentral gyrus, right medial and superior frontal orbital gyrus and bilateral gyrus rectus (see Figure 4 and Supplementary Table 6 for a detailed report on significant clusters).

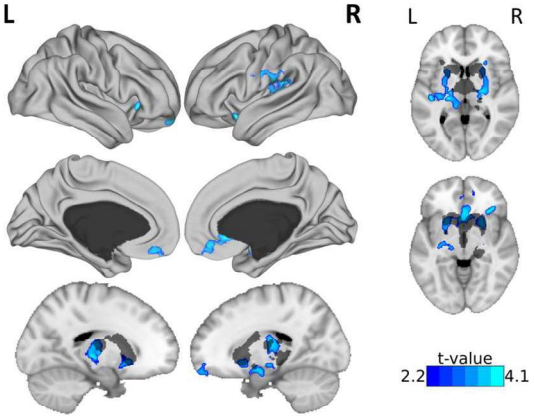


Figure 4. Gray matter (GM) volume decrease at follow-up associated with MIRS score of adult/late DM1 patients at baseline. The T-statistic showing the relationship between GM volume and MIRS score is displayed. Only results surviving multiple comparisons are shown, correcting for age, head size, and time from baseline assessment to follow-up scan. A black-transparency mask is displayed to show the damage mask of adult/late onset DM1 compared with HC-adult/late at follow-up.

Discussion

This study tests the hypothesis of a neurodegenerative process in DM1, examining for the first time, the different profiles of structural brain involvement in pediatric and adult/late DM1. For this purpose, the same patients and controls were followed across a timespan of almost a decade.

The categorization of DM1 patients is still a matter of debate, and the one employed in this study follows the recently proposed classification system agreed in the latest international workshop

(OMMYD-4) held in Sweden (June 2019). This study sheds light on biological signatures of brain deterioration that support this classification.

Regarding cross-sectional data, we confirmed that global GM atrophy occurs in DM1 patients, which has been well documented in other studies [26–29]. A detailed examination of the global volume results in each group yielded some interesting findings. In accord with normal expected aging, when inspecting the raw GM scores, the older HC group (HC-adult/late) showed a lower total volume score than the younger HC group (HC-pediatric). However, the raw scores of the pediatric patients showed, in all cases, lower total GM volumes than adult/late patients, even though the former are significantly younger. This reinforces the notion that CNS damage (at least with regard to GM volume atrophy) is greater in pediatric onset forms of the disease. Moreover, this result suggests a greater importance of the disease form itself over the mere age of the patients, and thus provides support for the newly-proposed classification of patients in DM1.

When analyzing the longitudinal data, some striking results emerged. In the pediatric onset group, the loss of volume over time was not significant when compared with that of their HC group. This could be taken to suggest that the brain structure of these patients is developmentally marked as opposed to suffering from an ongoing degenerative process. However, the results do not allow us to completely rule out the possibility of a neurodegenerative process in the pediatric patients, based on the large effect size. Moreover, these patients were not yet in their 40's at follow-up. Taken together, these data indicate a tendency towards a greater progressive loss than that expected in normal aging in the pediatric onset DM1 patients. However, the adult/late group did not suffer a significant decrease in their total brain volume over time compared with their HC group, but again, the effect size was moderate. Although the findings point more to the probable occurrence of brain abnormal maturation during neurodevelopment in adult/late DM1 patients, these data do not completely rule out the existence of such progressive degeneration. Indeed, it is important to consider that

adult/late patients are in their mid 40's at baseline and in their mid 50's at follow-up, meaning that, at most, the accelerated brain degeneration in adult/late onset DM1, if it does occur, does not start before the age of 55.

The results of this study show that the disease traces a brain signature in patients that develops in a way that is different to what might be expected during the normal aging process; leading to a disease-specific developmental trajectory regarding the CNS, as observed in other autosomal dominant diseases such as Neurofibromatosis type 1 [30] or Huntington's Disease [31,32]. These different trajectories are confirmed when observing the specific regions of decreased GM in the VBM analyses, and even allow for clearly showing that pediatric and adult/late onset patients display distinctive patterns of damage in a given time-spot and different trajectories from a longitudinal perspective. Specifically, pediatric DM1 patients showed reduced GM volume, mainly located at subcortical level at baseline, which additionally extended to cortical regions at follow-up, particularly the frontal lobe. Similar brain involvement is described in other neurodegenerative diseases, often classified as fronto-subcortical dementias (e.g., Huntington's disease or Parkinson's disease) [33]. Likewise, fronto-striatal atrophy has been described as a hallmark of the behavioral variant of frontotemporal dementia [34].

A further observation worth noting is that perisylvian areas such as the parietal operculum are affected in pediatric onset patients. The introduction of new techniques such as stepwise functional connectivity (SFC) [35,36], have allowed for identifying the above mentioned areas as key regions in the multimodal integration network, a cortical network where connectivity from somatosensory, auditory, motor, and visual primary cortices converge [35,36]. This involvement in pediatric onset DM1 patients could be associated with the observed difficulties in higher-order cognitive processes, for which these areas establish a link with basic sensorimotor regions. The impairment of such connectivity networks has been suggested in other clinical disorders. This is, for instance, the case for Autism Spectrum Disorder (ASD) [37], in which the disrupted connectivity

between primary sensory and sensory integration areas has been hypothesized to be at the basis of, among other features, the weak central coherence proposed in ASD. In fact, a higher than expected prevalence of autistic-like conditions has been reported in pediatric DM1 [38].

Further, adult/late onset patients showed a GM decrease that remained confined to the basal ganglia. Subcortical involvement in DM1 has repeatedly been reported [26,28,29,39–41] and this is confirmed in our study. Moreover, only in the adult/late subgroup was the opposite result found, primarily located in the temporal lobe. This result is in accordance with expected normal aging, in which there is a decreased temporal lobe volume[42,43].

Of the clinical variables assessed in this study, the MIRS score stands out as the only measure that was associated with lower GM volumes at follow-up in adult/late onset DM1. Further, the high correspondence between the regions implicated in this association and the affected areas (when compared with HC) indicates that this muscular impairment measure could be a potential marker of progressive GM impairment in adult/late onset patients. Muscular impairment has been reported to correlate with other brain volumetric measures, as well as WM integrity [29,40,44].

Neuropsychological correlates with neuroimaging findings are still scarce and inconclusive in the literature. In contrast, the results of this study highlight the potential capacity of certain neuropsychological tools for predicting the progressive loss of WM. Among these neuropsychological tests, ROCF emerges as a powerful measure that needs to be considered not only for characterizing the cognitive profiles of patients, but also as a sensitive prognostic measure. Performance on the ROCF has previously been shown to correlate with brain atrophy measures and WM microstructural damage [27,39,45]. Regionally, data obtained from a previous study in our laboratory [46] are compatible with the results of this study in the sense that neuropsychological tools appear to be sensitive but still unspecific measures of brain regional correlates. Considering the lack of topological specificity, a network-wise organization hypothesis must be considered. In this regard, the involvement of areas from the multimodal

integration network (such as the supplementary motor area, insula, Rolandic operculum, superior parietal cortex, and anterior cingulated cortex), in association with neuropsychological outcomes, is striking and could form the basis of impaired higher-order cognitive functions in DM1 patients due to disrupted connectivity between primary sensory regions and higher-order cortical hubs. Previous studies combining cognitive and imaging outcomes have often failed to find a systematic association between performance on neuropsychological tasks and GM or WM impairment, possibly due to variations in neuropsychological assessment protocols or sample characteristics. It is worth noting, however, that this is the first time that these tests have been employed as potential tools for predicting progressive brain degeneration in DM1. Future research studies should attempt to combine neuropsychological and neuroimaging longitudinal data in order to gain a deeper understanding of the capacity of such variables to serve as potential markers of neurodegeneration.

This study is not without limitations. The main flaw in this study is the small sample size, which forces us to be cautious when generalizing our findings, particularly those derived from regression analyses (associations between variables) where the sample size is a strong determinant of statistical power. A major strength, however, is the fact that, for the first time in a longitudinal study of DM1, a sex-age-years of education equivalent HC group was included for comparison purposes. Moreover, this work constitutes a longitudinal study that applies sophisticated brain volumetric techniques across the largest observation period reported to date. Other shortcomings of this study include the selective attrition bias inherent to any longitudinal study. It should be noted, however, that patients who did not remain in the study until follow-up had longer CTG expansion sizes, and exhibited greater muscular impairment compared with those who were re-scanned, which would have, in any case, led to an underestimation of the observed changes in brain structure over time. Finally, whilst a two-time-point longitudinal study can shed light on a trajectory, it is insufficient for delineating an established direction in disease progression. Therefore, successive scans (>2 records) of a given

cohort should be used in order to clarify whether the trajectories found in this study are maintained over time in a lineal or nonlinear course. Further, future studies are needed that include a broader scope of age-groups to clarify the trajectory of decline covering the whole life span, particularly older ages.

Conclusion

The findings obtained in this study contribute towards a characterization of the natural progression of a specific CNS feature, that is, brain structure, in DM1. The outcomes show the need to further investigate brain differences over time with a focus on potential neurodevelopmental anomalies preceding potential neurodegeneration, which, in light of the present results, appears to be more pronounced in pediatric onset DM1. Future studies should attempt to recruit larger cohorts of patients with (if feasible) the earliest possible onset of the disease, to allow for a more accurate depiction of the natural history of gray and white matter alterations as neurodevelopmental, neurodegenerative, or both. So far, our results support the recently proposed disease classification by identifying distinct patterns of brain atrophy and its progression over time, and therefore encourage upcoming research to more deeply analyze these differences and to extend the study to other potential variations associated with each phenotype, that is, neuropsychological profiles or biological correlates.

Acknowledgements: The present study has been supported by funding from the Institute of Health Carlos III co-founded by Fondo Europeo de Desarrollo Regional-FEDER [grant numbers PI17/01231 and PI17/01841], CIBERNED (grant number: 609) and the Basque Government [SAIO08-PE08BF01]. G. Labayru was supported by a predoctoral grant from the Basque Government [PRE_2016_1_0187]. A. Jiménez-Marín was supported by a predoctoral grant from the Basque Government [PRE_2019_1_0070].

Conflict of interest statement: Authors declare that there is no potential conflict of interest.

References

1. Caillet-Boudin ML, Fernandez-Gomez FJ, Tran H, et al. Brain pathology in myotonic dystrophy: when tauopathy meets spliceopathy and RNAopathy. Frontiers in Molecular Neuroscience 2014;6(57):1–20.
2. Sansone V, Gandossini S, Cotelli M, et al. Cognitive impairment in adult myotonic dystrophies: A longitudinal study. Neurological Sciences 2007;28(1):9–15.
3. Modoni A, Silvestri G, Vita MG, et al. Cognitive impairment in myotonic dystrophy type 1 (DM1): A longitudinal follow-up study. Journal of Neurology 2008;255(11):1737–1742.
4. Winblad S, Samuelsson L, Lindberg C, Meola G. Cognition in myotonic dystrophy type 1: a 5-year follow-up study. European Journal of Neurology 2016;23(9):1471–1476.
5. Gallais B, Gagnon C, Mathieu J, Richer L. Cognitive decline over time in adults with myotonic dystrophy type 1: A 9-year longitudinal study. Neuromuscular Disorders 2017;27(1):61–72.
6. Labayru G, Aliri J, Zulaica M, et al. Age-related cognitive decline in myotonic dystrophy type 1: An 11-year longitudinal follow-up study. Journal of Neuropsychology 2019;jnp.12192.
7. Gliem C, Minnerop M, Roeske S, et al. Tracking the brain in myotonic dystrophies: A 5-year longitudinal follow-up study. PLoS ONE 2019;14(3):e0213381.
8. Conforti R, de Cristofaro M, Cristofano A, et al. Brain MRI abnormalities in the adult form of myotonic dystrophy type 1: A longitudinal case series study. Neuroradiol J 2016;29(1):36–45.
9. Mathieu J, Boivin H, Meunier D, et al. Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. Neurology 2001;56(April):336–340.
10. Wechsler D. WAIS-III: Escala de Inteligencia de Wechsler para Adultos III. Madrid: TEA Ediciones; 1999.

11. Sattler JM, Ryan JJ. Wechsler Adult Intelligence Scale-III (WAIS-III): Description. In: Sattler JM, editor. Assessment of Children. Cognitive applications. San Diego, California: 2001

12. Golden CJ. STROOP. Test de Colores y Palabras. 3rd ed. Madrid: TEA Ediciones; 2001.

13. Miller EN. CalCAP: California Computerized Assessment Package. 1990;

14. Lezak M, Howieson DB, Loring D. Neuropsychological Assessment. 4th ed. New York: Oxford University Press; 2004.

15. Casals-Coll M, Sánchez-Benavides G, Quintana M, et al. Estudios normativos españoles en población adulta joven (proyecto NEURONORMA jóvenes): normas para los test de fluencia verbal. Neurología 2013;28(1):33–40.

16. Peña-Casanova J, Quiñones-Úbeda S, Gramunt-Fombuena N, et al. Spanish Multicenter Normative Studies (NEURONORMA Project): Norms for Verbal Fluency Tests. Archives of Clinical Neuropsychology 2009;24:395–411.

17. Rey A. Test de copia y de reproducción de memoria de figuras geométricas complejas. Madrid: TEA Ediciones; 2009.

18. Raven JC, Court JH, Raven J. Raven: Standard Progressive Matrices. Madrid: TEA Ediciones; 2001.

19. Benton A, Sivan A, Hamsher K, et al. Contributions to Neuropsychological Assessment. 2nd ed. New York: Oxford University Press; 1994.

20. Heaton RK, Chelune GJ, Talley JL, et al. Test de clasificación de tarjetas de Wisconsin. Madrid: 2001.

21. Douaud G, Smith S, Jenkinson M, et al. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. Brain 2007;130(9):2375–2386.

22. Good CD, Johnsrude IS, Ashburner J, et al. A voxel-based morphometric study of ageing in 465 normal adult human brains. NeuroImage 2001;14(1 I):21–36.

23. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 2004;23(SUPPL. 1):208–219.

24. Andersson JLR, Jenkinson M, Smith S. Non-linear registration, aka spatial normalisation. FMRIB Technial Report TR07JA2. Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, Department of Clinical Neurology, Oxford University, Oxford, UK 2007;(June):22.

25. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. NeuroImage 2002;17(1):479–489.

26. Caso F, Agosta F, Peric S, et al. Cognitive impairment in myotonic dystrophy type 1 is associated with white matter damage. PLoS ONE 2014;9(8):1–8.

27. Baldanzi S, Cecchi P, Fabbri S, et al. Relationship between neuropsychological impairment and grey and white matter changes in adult-onset myotonic dystrophy type 1. Neuroimage Clin 2016;12:190–197.

28. Sugiyama A, Sone D, Sato N, et al. Brain gray matter structural network in myotonic dystrophy type 1. PLoS ONE 2017;12(11):1–13.

29. Schneider-Gold C, Bellenberg B, Prehn C, et al. Cortical and subcortical grey and white matter atrophy in myotonic dystrophies type 1 and 2 is associated with cognitive impairment, depression and daytime sleepiness. PLoS ONE 2015;10(6):1–20.

30. Moore BD, Slopis JM, Jackson EF, et al. Brain volume in children with neurofibromatosis type 1: Relation to neuropsychological status. Neurology 2000;54(4):914–920.

31. Conforti P, Besusso D, Bocchi VD, et al. Faulty neuronal determination and cell polarization are reverted by modulating HD early phenotypes. Proceedings of the National Academy of Sciences of the United States of America 2018;115(4):E762–E771.

32. van der Plas E, Langbehn DR, Conrad AL, et al. Abnormal brain development in child and adolescent carriers of mutant huntingtin. Neurology 2019;93(10):e1021–e1030.

33. Bonelli RM, Cummings JL. Frontal-subcortical dementias. The Neurologist 2008;14(2):100–107.

34. Bertoux M, O'Callaghan C, Flanagan E, et al. Fronto-striatal atrophy in behavioral variant frontotemporal dementia and alzheimer's disease. Frontiers in Neurology 2015;6(JUL):1–9.

35. Sepulcre J. Integration of visual and motor functional streams in the human brain. Neuroscience Letters 2014;567:68–73.

36. Sepulcre J, Sabuncu MR, Yeo TB, et al. Stepwise connectivity of the modal cortex reveals the multimodal organization of the human brain. Journal of Neuroscience 2012;32(31):10649–10661.

37. Stevenson RA. Using functional connectivity analyses to investigate the bases of autism spectrum disorders and other clinical populations. Journal of Neuroscience 2012;32(50):17933–17934.

38. Ekström AB, Hakenäs-Plate L, Samuelsson L, et al. Autism spectrum conditons in myotonic dystrophy type 1: A study on 57 individuals with congenital and childhood forms. American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics 2008;147(6):918–926.

39. Weber YG, Roebling R, Kassubek J, et al. Comparative analysis of brain structure, metabolism, and cognition in myotonic dystrophy 1 and 2. Neurology 2010;74(14):1108–1117.

40. Minnerop M, Weber B, Schoene-Bake JC, et al. The brain in myotonic dystrophy 1 and 2: Evidence for a predominant white matter disease. Brain 2011;134(12):3527–3543.

41. Zanigni S, Evangelisti S, Giannoccaro MP, et al. Relationship of white and gray matter abnormalities to clinical and genetic features in myotonic dystrophy type 1. NeuroImage: Clinical 2016;11:678–685.

42. Vinke EJ, de Groot M, Venkatraghavan V, et al. Trajectories of imaging markers in brain aging: the Rotterdam Study. Neurobiology of Aging 2018;71:32–40.

43. Lockhart SN, DeCarli C. Structural Imaging Measures of Brain Aging. Neuropsychology Review 2014;24(3):271–289.

44. Wozniak JR, Mueller BA, Lim KO, et al. Tractography reveals diffuse white matter abnormalities in Myotonic Dystrophy Type 1. Journal of the Neurological Sciences 2014;341(1–2):73–78.

45. van Dorst M, Okkersen K, Kessels RPC, et al. Structural white matter networks in myotonic dystrophy type 1. NeuroImage: Clinical 2019;21:101615.

46. Labayru G, Diez I, Sepulcre J, et al. Regional brain atrophy in gray and white matter is associated with cognitive impairment in Myotonic Dystrophy type 1. NeuroImage: Clinical 2019;24(November):102078.

Supplementary material

Supplementary Table 1. Wilcoxon repeated measures analysis of neuropsychological outcomes at baseline and at follow-up in pediatric and adult/late onset DM1.

	Pediatric DM1								Adult/late DM1							
	Baseline			Follow-up					Baseline			Follow-up				
Neuropsychological test	**N**	**Mean**	**(SD)**	**N**	**Mean**	**(SD)**	**Z**	***p***	**N**	**Mean**	**(SD)**	**N**	**Mean**	**(SD)**	**Z**	***p***
IQ estimate	8	81.25	(13.47)	9	82.78	(17.05)	-0.931	.352	12	96,33	(12,74)	11	99,82	(11,03)	-0.666	.506
Block design	8	35.75	(8.19)	9	35.11	(12.33)	-0.105	.916	12	47,25	(7,93)	11	47,91	(10,46)	-0.255	.799
Digit span	5	40.60	(6.07)	9	37.56	(7.91)	-0.272	.785	8	52,88	(6,08)	11	50,73	(6,53)	-0.256	.798
Vocabulary	8	42.50	(10.17)	9	44.89	(7.90)	-1.263	.206	12	48,42	(8,49)	11	52,00	(5,35)	-1.589	.112
RAVLT 1	8	47.48	(11.89)	9	52.70	(11.06)	-1.400	.161	12	48,26	(12,88)	11	42,46	(12,85)	-1.334	.182
RAVLT Total	8	51.35	(13.38)	9	48.55	(11.79)	-0.560	.575	12	45,99	(16,56)	11	45,92	(10,08)	-0.622	.534
RAVLT delayed recall	8	51.91	(9.22)	9	47.26	(9.39)	-1.400	.161	12	46,55	(11,52)	11	48,54	(7,44)	-0.356	.722
ROCF copy	8	37.25	(10.38)	8	43.00	(16.74)	-1.355	.176	12	46,33	(15,93)	11	45,45	(12,36)	-0.070	.944
ROCF delayed recall	8	41.38	(10.21)	8	45.13	(14.12)	-1.265	.206	12	43,25	(12,34)	11	44,64	(9,43)	-0.561	.575
Semantic fluency	8	47.00	(8.14)	9	47.33	(9.30)	-0.933	.351	12	52,58	(17,33)	11	48,64	(13,64)	-1.074	.283

Phonemic fluency	8	41.00	(10.32)	9	42.89	(11.27)	-1.192	.233	12	46,58	(14,72)	11	49,45	(13,83)	-0.340	.734
RAVEN Total	8	39.20	(22.40)	9	31.48	(20.80)	-1.863	.063	11	37,59	(16,38)	11	32,31	(19,49)	-1.122	.262
Stroop Word	7	40.00	(18.18)	8	41.00	(16.90)	-1.084	.279	12	45,00	(7,36)	11	46,36	(6,38)	0.000	1.000
Stroop Color	7	35.71	(13.73)	8	37.25	(13.85)	-1.063	.288	12	37,67	(8,13)	11	37,45	(6,76)	-0.498	.618
Stroop Color-word	7	46.57	(7.46)	8	42.25	(10.05)	-1.084	.279	12	39,17	(9,16)	11	44,00	(8,53)	-1.485	.138
Stroop Interference	7	54.86	(9.01)	8	49.75	(8.10)	-2.226	.026	12	45,67	(5,58)	11	49,64	(5,35)	-1.330	.183
CALCAP simple RT	6	44.33	(8.69)	9	40.56	(16.06)	-0.271	.786	11	49,73	(10,62)	11	42,55	(7,37)	-1.580	.114
CALCAP election RT	6	21.67	(24.68)	9	30.67	(20.41)	-1.214	.225	11	34,73	(13,75)	11	25,45	(13,63)	-1.956	.050
CALCAP sequential 1 RT	6	38.67	(17.20)	9	40.78	(14.86)	-0.674	.500	11	42,55	(10,24)	11	39,18	(8,93)	-1.479	.139
CALCAP sequential 2 RT	6	39.17	(10.74)	9	44.22	(8.66)	-1.682	.093	11	45,09	(9,16)	11	40,55	(7,90)	-1.122	.262

Note. DM1: Myotonic Dystrophy Type 1; SD: Standard Deviation; IQ: Intelligence Quotient; RAVLT: Rey Auditory Verbal Learning Test; ROCF: Rey-Osterrieth Complex Figure; CALCAP: California Computerized Assessment Package; RT: Reaction Time

Supplementary Table 2. VBM analyses. Brain areas with reduced gray matter volume in pediatric onset DM1 patients compared with healthy controls, at baseline and at follow-up.

Baseline								**Follow-up**							
	MNI coord.								**MNI coord.**						
Region	**X**	**Y**	**Z**	***p***	**T**	**Num. voxels**	**Volume ($mm^{3)}$**	**Region**	**X**	**Y**	**Z**	***p***	**T**	**Num. voxels**	**Volume ($mm^{3)}$**
Temporal Mid L	-48	-36	4	0,000	2,716	867	6936	Temporal Mid L	-50	-36	-4	0,001	2,709	986	7888
Thalamus L	-18	-22	2	0,000	2,876	693	5544	Thalamus L	-16	-22	14	0,000	3,052	772	6176
Thalamus R	18	-20	14	0,000	2,920	683	5464	Thalamus R	18	-24	6	0,000	3,300	722	5776
Temporal Sup L	-46	-36	4	0,000	2,699	478	3824	Temporal Sup L	-52	-46	20	0,001	2,481	554	4432
Caudate L	-14	8	8	0,003	2,618	407	3256	* Frontal Inf Tri L	-46	32	2	0,001	2,628	525	4200
Postcentral L	-58	-12	14	0,001	2,720	402	3216	Caudate L	-10	20	-8	0,001	2,968	485	3880
Putamen L	-22	0	14	0,001	2,820	238	1904	Caudate R	10	20	-8	0,001	2,789	402	3216
Hippocampus R	18	-32	-6	0,000	2,659	206	1648	* Frontal Inf Orb L	-54	30	-6	0,003	2,527	359	2872
Para-Hippocampal R	20	-28	-12	0,002	2,779	155	1240	Putamen L	-22	14	8	0,000	3,130	296	2368
Rolandic Oper L	-60	-8	10	0,001	2,782	116	928	* Frontal Inf Tri R	48	24	6	0,001	2,654	235	1880

Supra-Marginal L	-60	-24	16	0,002	2,587	105	840	Postcentral L	-60	-2	36	0,002	2,385	219	1752
Caudate R	14	0	12	0,002	2,448	105	840	Hippocampus R	20	-30	-8	0,000	2,801	206	1648
Lingual R	18	-30	-6	0,000	2,951	100	800	Putamen R	16	12	-2	0,003	2,597	186	1488
Para-Hippocampal L	-18	-24	-16	0,000	2,505	90	720	* Frontal Inf Oper R	50	18	6	0,002	2,586	181	1448
Putamen R	24	0	14	0,005	2,587	75	600	* Insula L	-36	-20	10	0,004	2,418	170	1360
Heschl's L	-60	-10	10	0,002	2,654	63	504	* Frontal Inf Orb R	42	32	-10	0,008	2,345	167	1336
								Para-Hippocampal R	22	-32	-10	0,001	2,774	140	1120
								Precentral L	-58	-2	36	0,003	2,485	90	720
								Heschl's L	-38	-20	10	0,004	2,443	88	704
								Lingual R	14	-34	-4	0,001	2,810	86	688
								* Rolandic Oper R	56	0	16	0,001	2,498	84	672
								Rolandic Oper L	-62	-8	8	0,009	2,348	58	464

Note. Montreal Neurosciences Institute coordinates (MNI coord.) are given for the peak area of the region. Regions are named following the Automated Anatomical Labeling (AAL). Mean T and minimum p values are given for each region.

*Areas that showed a decrease at one timepoint but not at the other (either baseline or follow-up).

Supplementary Table 3. VBM analyses. Brain areas with reduced gray matter volume in adult/late onset DM1 patients compared with healthy controls, at baseline and at follow-up.

Baseline								**Follow-up**							
	MNI coord.								**MNI coord.**						
Region	**X**	**Y**	**Z**	***p***	**T**	**Num. voxels**	**Volume ($mm^{3)}$**	**Region**	**X**	**Y**	**Z**	***p***	**T**	**Num. voxels**	**Volume ($mm^{3)}$**
Caudate L	-6	16	-2	0,002	2,679	452	3616	* Cerebellum	42	-72	-26	0,000	3,660	730	5840
Caudate R	10	0	12	0,002	2,685	321	2568	Caudate L	-8	18	-6	0,000	2,939	572	4576
Putamen L	-18	6	6	0,001	2,855	240	1920	Caudate R	6	14	-4	0,000	3,106	525	4200
Thalamus L	-4	-8	10	0,001	2,656	157	1256	Thalamus R	12	-8	4	0,000	2,900	394	3152
Insula L	-28	24	8	0,004	2,441	115	920	Putamen R	22	20	-2	0,000	2,853	383	3064
Thalamus R	4	-8	6	0,001	2,748	96	768	Thalamus L	-4	-8	6	0,000	2,931	367	2936
Putamen R	26	2	14	0,004	2,509	90	720	Putamen L	-20	20	-6	0,000	2,954	337	2696
								* Hippocampus R	20	-26	-8	0,001	2,706	219	1752
								* Para-Hippocampal R	26	-26	-14	0,003	2,549	51	408

Note. Montreal Neurosciences Institute coordinates (MNI coord.) are given for the peak area of the region. Regions are named following the Automated Anatomical Labeling (AAL). Mean T and minimum p values are given for each region.

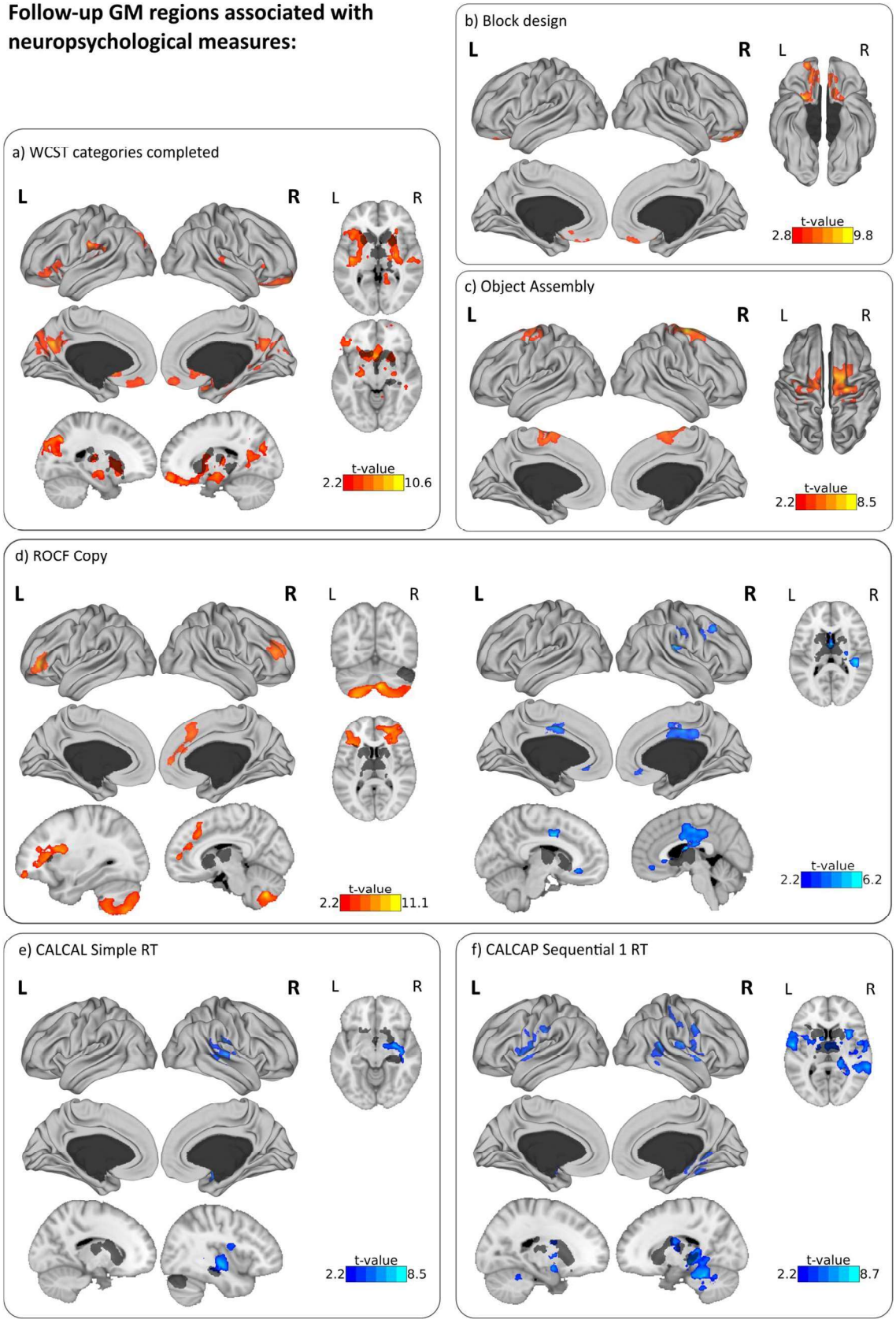
*Areas that showed a decrease at one timepoint but not at the other (either baseline or follow-up).

Supplementary Table 4. VBM analyses. Brain areas with reduced gray matter volume in healthy controls compared to adult/late onset DM1 patients, at baseline and at follow-up.

Baseline								**Follow-up**							
	MNI coord.								**MNI coord.**						
Region	**X**	**Y**	**Z**	***p***	**T**	**Num. voxels**	**Volume ($mm^{3)}$**	**Region**	**X**	**Y**	**Z**	***p***	**T**	**Num. voxels**	**Volume ($mm^{3)}$**
Temporal Inf L	-58	-22	-24	0,001	2,711	777	6216	Temporal Inf L	-26	0	-44	0,000	2,731	590	4720
Temporal Pole Mid L	-30	10	-40	0,001	2,746	431	3448	Temporal Pole Mid L	-18	4	-38	0,000	3,126	474	3792
Fusiform L	-28	2	-46	0,001	2,617	325	2600	Fusiform L	-24	0	-44	0,000	3,052	439	3512
Temporal Mid L	-66	-18	-6	0,000	2,891	260	2080	Temporal Pole Sup L	-18	4	-34	0,000	2,700	258	2064
Temporal Pole Sup L	-36	16	-30	0,001	2,584	240	1920	Para-Hippocampal L	-20	4	-34	0,000	2,923	141	1128
Frontal Inf Orb L	-36	30	-22	0,014	2,239	22	176								
Para-Hippocampal L	-18	-8	-36	0,006	2,305	17	136								
* Frontal Mid Orb L	-26	40	-20	0,031	2,150	8	64								
Cerebellum	-32	-30	-30	0,026	2,347	3	24								
* Temporal Sup L	-60	-16	2	0,041	2,116	2	16								

Note. Montreal Neurosciences Institute coordinates (MNI coord.) are given for the peak area of the region. Regions are named following the Automated Anatomical Labeling (AAL). Mean T and minimum p values are given for each region

*Areas that showed a decrease at one timepoint but not at the other (either baseline or follow-up).



Supplementary Figure 1. Gray matter (GM) volume decrease at follow-up associated with neuropsychological outcome of DM1 patients at baseline. The T-statistic showing the

relationship between GM volume and neuropsychological test is displayed. Only results surviving multiple comparisons are shown, correcting for age, head size and time from baseline assessment to follow-up scan. A black-transparency mask is displayed to show the damage mask of adult/late onset DM1 compared with HC-adult/late at follow-up. Panel a) shows the GM areas at follow-up with a significant decrease in relation to lower scores on the WCST (categories completed) at baseline. Panel b) shows the GM areas at follow-up with a significant decrease in relation to lower scores on Block design at baseline. Panel c) shows the GM areas at follow-up with a significant decrease in relation to lower scores on Object assembly at baseline. Panel d) shows the GM areas at follow-up with a significant decrease (red-yellow) or increase (blue) in relation to lower scores on ROCF at baseline. Panel e) shows the GM areas at follow-up with a significant increase in relation to lower scores on CALCAP simple RT at baseline. Panel f) shows the GM areas at follow-up with a significant increase in relation to lower scores on CALCAP Sequential 1 RT at baseline.

Supplementary Table 5. Gray matter volume at follow-up associated with baseline neuropsychological outcomes of adult/late DM1 patients.

		MNI coord.						
Neuropsychological test	**Region**	**X**	**Y**	**Z**	***p***	**T**	**Num. voxels**	**Volume ($mm^{3)}$**
Block design	Frontal Sup Orb R	22	14	-20	0,000	3,774	235	1880
	Rectus L	-10	16	-22	0,000	3,433	199	1592
	* Frontal Sup Orb L	-10	16	-24	0,000	3,719	136	1088
	Rectus R	18	18	-18	0,000	3,365	135	1080
	* Frontal Inf Orb L	-18	20	-18	0,002	3,203	71	568
Object assembly	Supp Motor Area R	16	-6	68	0,000	3,388	777	6216
	Frontal Sup R	18	-6	68	0,000	3,428	692	5536
	Supp Motor Area L	-2	-8	64	0,000	2,970	488	3904
	Precentral L	-28	-20	64	0,000	2,829	466	3728
	Precentral R	24	-16	74	0,000	3,343	362	2896
	Frontal Mid R	28	14	56	0,004	2,763	134	1072
	Postcentral R	34	-30	56	0,001	2,736	130	1040

	Frontal Sup L	-20	-10	64	0,003	2,617	128	1024
	Postcentral L	-24	-36	72	0,001	2,722	76	608
	Paracentral Lobule L	-8	-14	76	0,002	3,020	75	600
ROCF copy	* Cerebellum	6	-64	-46	0,000	3,299	3376	27008
	Frontal Mid R	36	50	14	0,000	3,536	1005	8040
	* Frontal Inf Tri L	-40	42	-2	0,000	4,410	534	4272
	Frontal Sup R	16	14	56	0,000	3,245	294	2352
	Cingulum Ant R	16	28	26	0,001	2,865	238	1904
	Frontal Sup Medial R	12	26	48	0,000	3,051	208	1664
	Frontal Mid Orb L	-38	44	-2	0,000	3,736	179	1432
	Cingulum Mid R	16	26	30	0,001	3,125	135	1080
	* Insula L	-30	8	16	0,000	3,742	122	976
	Frontal Inf Tri R	30	26	26	0,001	3,115	98	784
	Frontal Mid L	-38	42	-2	0,000	4,014	95	760
	* Frontal Inf Orb L	-38	42	-4	0,000	4,352	90	720
	Supp Motor Area R	14	14	56	0,000	2,807	66	528

ROCF copy[¶]	Cingulum Mid R	8	-26	36	0,000	2,917	539	4312
	Cingulum Mid L	-6	0	38	0,000	2,909	255	2040
	Postcentral R	60	-20	44	0,000	3,030	243	1944
	Frontal Mid R	44	22	40	0,001	2,820	201	1608
	Temporal Sup R	44	-34	14	0,000	3,591	144	1152
	Frontal Med Orb L	-2	40	-10	0,001	2,927	90	720
	Frontal Inf Oper R	40	6	22	0,002	2,692	88	704
	Rolandic Oper R	36	-24	20	0,000	3,149	87	696
	SupraMarginal R	60	-22	42	0,001	2,944	87	696
	Insula R	36	-26	20	0,000	3,611	67	536
	* Caudate R	20	-4	24	0,010	2,472	66	528
	Frontal Med Orb R	2	44	-10	0,010	2,462	62	496
	Heschl's R	36	-26	16	0,001	3,532	60	480
	Precentral R	42	4	36	0,006	2,668	51	408
WCST categories completed	Cuneus L	-12	-82	26	0,000	3,274	627	5016

* Putamen L	-32	-18	-2	0,002	2,770	576	4608
Temporal Sup L	-42	-18	0	0,000	3,228	523	4184
* Putamen R	28	-14	4	0,001	2,813	519	4152
Occipital Sup L	-22	-68	36	0,000	3,138	513	4104
Precuneus L	-4	-62	28	0,000	3,554	503	4024
Frontal Sup Orb R	14	60	-14	0,000	3,204	399	3192
* Insula L	-40	-18	2	0,000	2,951	357	2856
* Precuneus R	4	-58	28	0,000	3,077	339	2712
Cuneus R	8	-88	20	0,000	3,007	293	2344
* Frontal Inf Orb L	-42	20	-4	0,001	2,826	277	2216
SupraMarginal L	-48	-28	22	0,000	3,257	266	2128
Postcentral L	-60	-18	26	0,000	3,850	262	2096
Rectus L	-8	46	-18	0,001	2,746	255	2040
Calcarine L	-10	-70	22	0,001	2,851	240	1920
Rolandic Oper L	-46	-28	22	0,000	3,390	207	1656
* Fusiform R	40	-24	-18	0,000	2,990	204	1632

Rectus R	22	14	-16	0,002	2,804	202	1616
Calcarine R	14	-56	12	0,002	2,755	196	1568
Temporal Inf R	48	-24	-26	0,002	3,030	189	1512
* Hippocampus R	28	-36	8	0,001	3,190	159	1272
* Frontal Inf Tri L	-42	20	-2	0,001	3,040	151	1208
Temporal Sup R	54	-18	2	0,002	2,647	134	1072
* Pallidum R	28	-10	0	0,000	2,849	125	1000
* Caudate R	14	2	12	0,019	2,482	122	976
* Olfactory R	24	14	-16	0,000	3,534	118	944
Hippocampus L	-14	-10	-18	0,000	3,134	113	904
Occipital Mid L	-24	-68	36	0,001	2,984	113	904
Temporal Pole Sup R	28	8	-20	0,000	3,153	110	880
Frontal Med Orb R	12	60	-14	0,000	2,928	106	848
Cingulum Post L	-8	-52	28	0,001	2,957	106	848
* ParaHippocampal R	30	0	-30	0,001	2,893	105	840
* Lingual R	12	-44	0	0,002	2,748	102	816

	Thalamus R	20	-16	2	0,000	3,331	87	696
	* Olfactory L	-2	12	-10	0,000	3,635	84	672
	* Caudate L	-6	18	-6	0,000	3,343	76	608
	Frontal Mid Orb R	18	58	-18	0,001	3,238	72	576
	* Amygdala R	32	2	-30	0,000	2,749	71	568
	Parietal Sup L	-20	-70	40	0,000	3,046	68	544
	Heschl's L	-40	-20	2	0,000	3,084	67	536
	Cingulum Ant R	2	24	-8	0,006	2,675	58	464
	Insula R	26	14	-18	0,000	3,290	52	416
CALCAP Simple RT[¶]	Temporal Sup R	56	-22	6	0,000	3,327	909	7272
	Temporal Mid R	64	-30	0	0,000	3,014	226	1808
	Rolandic Oper R	54	-24	22	0,000	3,222	193	1544
	Insula R	40	-16	-4	0,000	3,291	187	1496
	* Amygdala R	32	-6	-12	0,000	3,703	167	1336
	SupraMarginal R	56	-26	24	0,000	3,546	114	912
	* Putamen R	34	-10	-6	0,000	3,372	83	664

	* Hippocampus R	40	-26	-12	0,006	2,855	53	424
CALCAP Sequential 1 RT¶	* Cerebellum	12	-50	-24	0,000	3,168	1225	9800
	Postcentral R	60	-6	30	0,001	2,909	533	4264
	Rolandic Oper R	40	-14	18	0,000	3,246	510	4080
	Temporal Mid R	68	-46	10	0,000	3,182	487	3896
	Temporal Sup R	60	-10	4	0,000	3,199	397	3176
	Insula R	40	-14	16	0,000	3,758	386	3088
	* Hippocampus R	24	-26	-12	0,000	4,088	362	2896
	Rolandic Oper L	-56	-6	8	0,000	3,244	279	2232
	* ParaHippocampal R	24	-26	-14	0,000	3,681	233	1864
	Temporal Sup L	-56	-6	6	0,000	3,462	231	1848
	Postcentral L	-58	-22	32	0,000	2,869	224	1792
	Thalamus R	8	-6	0	0,000	3,037	215	1720
	Precentral R	62	2	36	0,001	2,881	211	1688
	* Fusiform R	22	-40	-18	0,001	2,912	174	1392
	Amygdala L	-22	-2	-14	0,001	3,634	151	1208

Frontal Inf Oper L	-52	10	0	0,002	2,821	133	1064
* Lingual R	18	-30	-8	0,000	3,042	120	960
Temporal Pole Sup R	64	6	-4	0,000	3,572	120	960
* Caudate L	-16	-4	18	0,002	2,923	106	848
* Caudate R	20	-6	22	0,004	3,003	104	832
* Putamen R	32	8	10	0,001	3,058	87	696
* Putamen L	-22	-2	6	0,006	2,576	86	688
* Thalamus L	-4	-8	14	0,001	2,924	82	656
* Insula L	-36	8	4	0,003	2,781	81	648
SupraMarginal L	-58	-24	32	0,001	3,232	81	648
Heschl's R	46	-22	14	0,000	3,549	73	584
Temporal Pole Sup L	-52	8	0	0,000	3,279	64	512
SupraMarginal R	58	-26	52	0,002	2,784	53	424

Note. DM1: Myotonic Dystrophy Type 1; MNI: Montreal Neurosciences Institute; ROCF: Rey-Osterrieth Complex Figure; CALCAP: California Computerized Assessment Package; RT: Reaction Time. Only results surviving multiple comparisons are shown, correcting for age, head size and time from baseline neuropsychological assessment to follow-up MRI scan. For each neuropsychological measure the table shows the MNI coordinates for the peak area of the region. Mean T representing the magnitude of the association between the mean gray

matter measure and the neuropsychological test and minimum p values of this association are given for each region (higher T indicates a stronger association).

*. Areas that fall within the mask where DM1 patients showed a significant decrease in volume compared with healthy controls at follow-up.¶ Negative association

Supplementary Table 6. Gray matter volume at follow-up associated with baseline MIRS score in adult/late DM1 patients

Region	MNI coord..			*p*	T	Num. voxels	Volume ($mm^{3)}$
	X	Y	Z				
* Putamen R	28	-14	10	0,006	2,523	433	3464
*Putamen L	-24	-2	-8	0,013	2,476	420	3360
Temporal Sup L	-42	-24	6	0,004	2,578	234	1872
Rolandic Oper L	-46	-20	22	0,007	2,639	231	1848
* Thalamus L	-20	-22	8	0,003	2,727	230	1840
* Insula L	-28	16	-16	0,005	2,640	182	1456
Postcentral L	-66	-20	28	0,002	2,615	166	1328
* Thalamus R	22	-22	6	0,004	2,733	154	1232
Frontal Med Orb R	2	48	-2	0,014	2,521	143	1144
Heschl's L	-40	-20	8	0,003	2,761	105	840
SupraMarginal L	-66	-20	30	0,007	2,597	104	832
Rectus L	0	52	-22	0,014	2,564	94	752
Frontal Sup Orb R	14	64	-18	0,003	2,653	80	640

* Olfactory R	4	18	-2	0,004	2,746	77	616
* Caudate L	-4	18	-4	0,010	2,466	62	496
Cingulum Ant R	0	28	-4	0,009	2,678	58	464
Rectus R	2	44	-16	0,014	2,483	56	448
* Hippocampus R	36	-8	-16	0,010	2,561	56	448
* Amygdala R	18	-2	-16	0,013	2,541	55	440
* Olfactory L	-24	6	-16	0,008	2,642	53	424

Note. DM1: Myotonic Dystrophy Type 1; MNI: Montreal Neurosciences Institute; MIRS: Muscular Impairment Rating Scale. Only results surviving multiple comparisons are shown, correcting for age, head size, and time from baseline to follow-up MRI scan. Table shows the MNI coordinates for the peak area of the region. Mean T representing the magnitude of the association between the mean gray matter measure and the MIRS score and minimum p values of this association are given for each region (higher T indicates a stronger association).

* Areas that fall within the mask where DM1 patients showed a significant decrease in volume compared with healthy controls at follow-up.